measurement (**205**) can be performed at various stages of the process, as described in U.S. Pat. App. Pub. 2010/0203495 A1 by Medoff and Masterman, published August 12, 2010, ~~the complete disclosure of which is incorporated herein by reference~~. Saccharifying the feedstock (**220**) can also be modified by mixing the feedstock with medium and the enzyme (**221**).

[0050] The manufacture of the enzyme complexes will now be described first, followed by a description of the method steps discussed above with reference to FIG. 2, and the materials used in the process.

[0051] A number of different conditions were tested, and the results are as follows. In one embodiment, the enzyme induction biomass is corn cob. In this embodiment, the biomass is treated by electron bombardment with a 35 Mrad electron beam. Preferably, the biomass is comminuted to a particle size of 10-1400 um, more preferably less than 200 um, most preferably less than 50 um. The treated biomass (in either wet or dry form) is added in a total amount of about 25 to about 133 g/L of inoculated medium, more preferably 100 g/L. The inductant biomass can be added at any point in the growth of the microorganisms up through the third day after inoculation, but is preferably added 1-3 days after inoculation. The total amount of biomass to be added as an inductant can be added all at once, or in aliquots, for instance, in two parts, or in five parts. Preferably the corncob biomass is added all at once.

[0052] The enzyme induction biomass can be presented to the microorganisms as a solid, or as a slurry. Preferably it is added as a slurry.

ENZYME PRODUCTION

[0053] Filamentous fungi, or bacteria that produce cellulase, typically require a carbon source and an inducer for production of cellulase. Without being bound by any theory, it is believed that the enzymes of this disclosure are particularly suited for saccharification of the substrate used for inducing its production.

[0054] Lignocellulosic materials comprise different combinations of cellulose, hemicellulose and lignin. Cellulose is a linear polymer of glucose forming a fairly stiff linear structure without significant coiling. Due to this structure and the disposition of hydroxyl groups that can hydrogen bond, cellulose contains crystalline and non-crystalline portions. The crystalline portions can also be of different types, noted as I(alpha) and I(beta) for example, depending on the location of hydrogen bonds between strands. The polymer lengths themselves can vary

5% glucose) as a carbon source. Additionally the inducer media can contain, in addition to the biomass discussed previously, other inducers. For example, some known inducers are lactose, pure cellulose and sophorose. Various components can be added and removed during the processing to optimize the desired production of useful products.

[0063] The concentration of the biomass typically used for inducing enzyme production is greater than or equal to 0.1 wt.% and less than or equal to 50 wt.%, greater than or equal to 0.5 wt.% and less than or equal to 25 wt.%, greater than or equal to 1 wt. %, and less than or equal to 15 wt.%, and greater than or equal to 1 wt.% and less than or equal to 10 wt. %.

[0064] Any of the processes described above may be performed as a batch, a fed-batch or a continuous process. The processes are useful for especially industrial scale production, *e.g.*, having a culture medium of at least 50 liters, preferably at least 100 liters, more preferably at least 500 liters, even more preferably at least 1,000 liters, in particular at least 5,000 liters 100,000 liters or 500,000 liters. The process may be carried out aerobically or anaerobically. Some enzymes are produced by submerged cultivation and some by surface cultivation.

[0065] In any of the process described above, the enzyme can be manufactured and stored and then used to saccharify at a later date and/or different location.

[0066] Any of the processes described above may be conducted with agitation. In some cases, agitation may be performed using jet mixing as described in U.S. Pat. App. Pub. 2010/0297705 A1 by Medoff and Masterman, published November 25, 2010, U.S. Pat. App. Pub. 2012/0091035 A1 to Medoff and Masterman, published April 19, 2012, and U.S. Pat. App. Pub. 2012/0100572 A1 by Medoff and Masterman, published April 26, 2012~~, the full disclosures of which are incorporated by reference herein~~.

[0067] Temperatures for the growth of enzyme producing organisms are chosen to enhance organism growth. For example for *Trichoderma reesei* the optimal temperature is generally between 20 and 40°C (*e.g.*, 30°C), The temperature for enzyme production is optimized for that part of the process. For example for *Trichoderma reesei* the optimal temperature for enzyme production is between 20 and 40°C (*e.g.*, 27°C).

FEEDSTOCK, BIOMASS MATERIALS, AND/OR INDUCERS

[0068] The feedstock, which may also be the inducer for enzyme production, is preferably a lignocellulosic material, although the processes described herein may also be used with

newsprint), printer paper, polycoated paper, card stock, cardboard, paperboard, materials having a high alpha-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~~.

[0076] Cellulosic materials can also include lignocellulosic materials which have been de-lignified.

[0077] 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.

[0078] 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 alveolates, chlorarachniophytes, cryptomonads, euglenids, glaucophytes, haptophytes, red algae, stramenopiles, and viridaeplantae). Other examples include seaweed, plankton (*e.g.*, macroplankton, mesoplankton, microplankton, nanoplankton, picoplankton, and femptoplankton), 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.

[0079] The biomass material can also include offal, and similar sources of material.

[0080] 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~~.

[0081] Any of the methods described herein can be practiced with mixtures of any biomass materials described herein.

BIOMASS -- MECHANICAL PREPARATION

[0082] Mechanical treatments of the feedstock may include, for example, cutting, milling, *e.g.*, hammermilling, wet milling, grinding, pressing, shearing or chopping. The initial mechanical treatment step may, in some implementations, include reducing the size of the feedstock. In some cases, loose feedstock (*e.g.*, recycled paper or switchgrass) is initially prepared by cutting, shearing and/or shredding.

[0083] In addition to this size reduction, which can be performed initially and/or later during processing, mechanical treatment can also be advantageous for "opening up," "stressing," breaking or shattering the feedstock materials, making the cellulose of the materials more

susceptible to chain scission and/or disruption of crystalline structure during the structural modification treatment.

[0084] Methods of mechanically treating the feedstock 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 or grist mill. Grinding may be performed using, for example, a cutting/impact type grinder. Specific examples of 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.

[0085] Mechanical treatments that may be used, and the characteristics of the mechanically treated feedstocks, are described in further detail in U.S. Serial No. 13/276,192, filed October 18, 2011, and published on April 26, 2012 as U.S. Pat. App. Pub. 2012/0100577 A1~~, the full disclosure of which is hereby incorporated herein by reference~~.

BIOMASS TREATMENT -- ELECTRON BOMBARDMENT

[0086] In some cases, the feedstock may be treated with electron bombardment to modify its structure and thereby reduce its recalcitrance. Such treatment may, 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.

[0087] Electron bombardment via an electron beam is generally preferred, because it provides very high throughput and because the use of a relatively low voltage/high power electron beam device eliminates the need for expensive concrete vault shielding, as such devices are "self-shielded" and provide a safe, efficient process. While the "self-shielded" devices do include shielding (*e.g.*, metal plate shielding), they do not require the construction of a concrete vault, greatly reducing capital expenditure and often allowing an existing manufacturing facility to be used without expensive modification. Electron beam accelerators are available, for example, from IBA (Ion Beam Applications, Louvain-la-Neuve, Belgium), Titan Corporation (San Diego, California, USA), and NHV Corporation (Nippon High Voltage, Japan).

feedstock. 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

[0105] The 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.

[0106] 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.

[0107] 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. The enzymes can be made/induced according to the methods described herein.

[0108] Specifically, the enzymes can be supplied by organisms that are capable of breaking down biomass (such as the cellulose and/or the lignin portions of the biomass), or that 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).

[0109] 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

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] Many of the products obtained, such as ethanol or n-butanol, can be utilized as a fuel for powering cars, trucks, tractors, ships or trains, *e.g.*, as an internal combustion fuel or as a fuel cell feedstock. Many of the products obtained can also be utilized to power aircraft, such as planes, *e.g.*, having jet engines or helicopters. In addition, the products described herein can be utilized for electrical power generation, *e.g.*, in a conventional steam generating plant or in a fuel cell plant.

[0117] 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~~.

SACCHARIFICATION

[0118] The reduced-recalcitrance feedstock is treated with the enzymes discussed above, generally by combining the material and the enzyme in a fluid medium, *e.g.*, an aqueous solution. In some cases, the feedstock 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~~.

[0119] 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 biomass 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.

[0120] 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~~.

[0121] 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.

[0122] 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 weight. 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.

[0123] 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

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).

[0126] Many microorganisms that can be used to saccharify biomass material and produce sugars can also be used to ferment and convert those sugars to useful products.

SUGARS

[0127] 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.

HYDROGENATION AND OTHER CHEMICAL TRANSFORMATIONS

[0128] 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-Al_2O_3, Ru/C, Raney Nickel, or other catalysts know in the art) in combination with H_2 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 U.S. Prov. App. No. 61/667,481, filed July 3, 2012~~, the disclosure of which is incorporated herein by reference in its entirety~~.

FERMENTATION

[0129] The sugars produced by saccharification can be isolated as a final product, or can be fermented to produce other products, *e.g.*, alcohols, sugar alcohols, such as erythritol, or organic acids, *e.g.*, lactic, glutamic or citric acids or amino acids.

[0130] 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.

[0131] In some embodiments, *e.g.*, when anaerobic organisms are used, at least a portion of the fermentation is conducted in the absence of oxygen, *e.g.*, under a blanket of an inert gas such as N_2, Ar, He, CO_2 or mixtures thereof. Additionally, the mixture may have a constant purge of an inert gas flowing through the tank during part of or all of the fermentation. In some cases, anaerobic condition, can be achieved or maintained by carbon dioxide production during the fermentation and no additional inert gas is needed.

[0132] 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.

[0133] Jet mixing may be used during fermentation, and in some cases saccharification and fermentation are performed in the same tank.

[0134] 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~~.

[0135] "Fermentation" includes the methods and products that are disclosed in U.S. Prov. App. No. 61/579,559, filed December 22, 2012, and U.S. Prov. App. No. 61/579,576, filed December 22, 2012~~, the contents of both of which are incorporated by reference herein in their entirety~~.

[0136] 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)~~, the contents of which is 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.

[0171] Example 3. SDS-PAGE of Enzyme Produced With Treated Biomass

[0172] A bioreactor culture was prepared using the method described above except that the mixing was done at 50 rpm rather than 500 rpm. The protein assay showed that 3.4 g/L protein was produced.

[0173] The analysis of the protein using SDS PAGE is shown in FIG. 5. Lane 1 and 5 are molecular weight markers, Lane 2 is a 30 uL load of the protein, Lane 3 is a 40 uL load of the protein, Lane 4 is Duet™ Accelerase enzyme complex (Genencor).

[0174] Example 4. Range of Conditions Tested

Induction	Parameters Tested	Range Tested	Working Range	Best Range
Corn Cob	Particle Size	1400- <5mm	1400- <5mm	<50mm
	Amount Added	25-133g/L	25-133g/L	100g/L
	Timing of Addition	Day 0-3	1-3	Day 1-3
	Frequency of Addition	1, 2, and 5	1, 2, and 5	1
	Presentation	wet or dry	wet or dry	wet or dry
	Treatment Levels	35	35	35
Lactose	Timing of Addition	Day 3	Day 3	Day 3
	Amount Added	4.7-40g/L/d continuous	4.7-18.7g/L/d continuous	18.7g/L/d continuous
	Frequency of Addition	feed	feed	feed

~~**[0175]** 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.~~

CLAIMS

What is claimed is:

1. A method comprising:

selectively combining a cellulolytic microorganism with a first quantity of a particular cellulosic or lignocellulosic biomass, which has been treated by bombardment with electrons to reduce its recalcitrance; and

~~, with a microorganism, and inducing the production of one or more enzymes by the microorganism by~~ maintaining the cellulolytic microorganism-biomass combination under conditions that cause the first quantity of the particular cellulosic or lignocellulosic biomass to induce the cellulolytic microorganism to produce a cellulase complex comprising one or more enzymes tailored to saccharify a second quantity of the particular cellulosic or lignocellulosic biomass which has been treated by bombardment with electrons to reduce its recalcitrance, the relative concentration of the one or more enzymes being modulated by selection of the particular dose of bombardment with electrons, the particular dose effecting the crystallinity of polymers in the first quantity of the particular cellulosic or lignocellulosic biomass ~~allow for the production of the enzyme(s) by the microorganism~~.

2. The method of claim 1, further comprising saccharifying the second quantity of the particular ~~combining a second~~ cellulosic or lignocellulosic biomass with the cellulase complex, wherein the cellulase complex exhibits an enhanced activity attributable, at least in part, to endoglucanase derived from the level of crystalline cellulose in the first quantity of the particular lignocellulosic material ~~microorganism-biomass combination~~.

3. The method of claim 2 wherein the ~~second~~ cellulosic or lignocellulosic biomass is lignocellulosic.

4. The method of claim 1, 2 or 3, wherein ~~where~~ the first quantity of the particular cellulosic or lignocellulosic biomass and/or the second quantity of the particular cellulosic or lignocellulosic biomass has been treated to reduce its recalcitrance by a treatment method selected from the group consisting of: ~~bombardment with electrons,~~ sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, and freeze grinding.

5. The method of claim 1, 2, 3, or 4, wherein the dose of ~~treatment method is~~ bombardment with electrons applied to the first quantity of the particular cellulosic or lignocellulosic biomass is selected from the range of 2 to 50 Mrad.

6. The method of any of the above claims, further comprising mechanically treating the first quantity of the particular cellulosic or lignocellulosic biomass and/or the second quantity of the

particular cellulosic or lignocellulosic biomass, to reduce its bulk density and/or increase its surface area.

7. The method of any of the above claims, wherein the first quantity of the particular cellulosic or lignocellulosic biomass and/or the second quantity of the particular cellulosic or lignocellulosic biomass is comminuted before being combined with the cellulolytic microorganism.

8. The method of claim 7, wherein the comminution comprises dry milling.

9. The method of claim 7, wherein the comminution comprises wet milling.

10. The method of any of the above claims, wherein the cellulosic or lignocellulosic biomass has a particle size of about 30 to 1400 μm.

11. The method of any of the above claims, wherein the cellulosic or lignocellulosic biomass is a lignocellulosic biomass selected from the group consisting of wood, grasses, and agricultural residues.

12. The method of any one of the above claims, wherein the cellulosic or lignocellulosic biomass is selected from the group consisting of: paper, paper products, paper waste, paper pulp, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, card stock, cardboard, paperboard, cotton, wood, particle board, forestry wastes, sawdust, aspen wood, wood chips, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, agricultural waste, 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, bagasse, beet pulp, agave bagasse, algae, seaweed, manure, sewage, offal, agricultural or industrial waste, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, and mixtures of any of these.

13. The method of any one of the above claims, wherein the cellulosic or lignocellulosic biomass comprises a residue of a saccharification or fermentation process.

14. The method of any one of the above claims, wherein the cellulolytic microorganism is selected from the group consisting of a fungus, a bacterium, and a yeast.

15. The method of any one of the above claims, wherein the cellulolytic microorganism is a strain of cellulolytic filamentous fungus ~~that produces high levels of cellulase~~.

16. The method of any one of the above claims, wherein the cellulolytic microorganism is genetically engineered.

17. The method of any one of the above claims, ~~claim 14~~ wherein the cellulolytic microorganism is a fungus comprising a catabolite repression-resistant mutant of *Trichoderma reesei*.

18. The method of any one of the above claims, wherein the cellulolytic microorganism is selected from the group consisting of *Trichoderma reesei*, and *Clostridium thermocellum*.

19. The method of ~~claim 18, wherein the~~ any one of the above claims, wherein the cellulolytic microorganism is a *T. reesei* strain is selected from the group consisting of: RUT-NG14, PC3-7, QM9414 and/or RUT-C30.

20. The method of any one of the above claims, wherein the cellulosic or lignocellulosic biomass is combined with the cellulolytic microorganism when the cellulolytic microorganism is in a lag phase.

21. The method of any one of the above claims, further comprising concentrating the one or more enzymes and storing the one or more enzymes for subsequent saccharification of the second quantity of the particular cellulosic or lignocellulosic biomass ~~saccharifying the cellulosic or lignocellulosic biomass, and/or additional cellulosic or lignocellulosic biomass, using the enzyme(s)~~.

22. A composition comprising: a liquid medium, a first quantity of a particular cellulosic or lignocellulosic biomass treated to reduce its recalcitrance, a cellulolytic microorganism, and one or more enzymes made by the cellulolytic microorganism, the relative concentrations of the one or more enzymes being tailored to saccharify a second quantity of the particular cellulosic or lignocellulosic biomass.