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(54) **Titre :Processing of biomass materials.**

(57) **Abrégé :**

Provided are methods of inducing enzymes, and for treating cellulosic and lignocellulosic biomass with the enzyme.

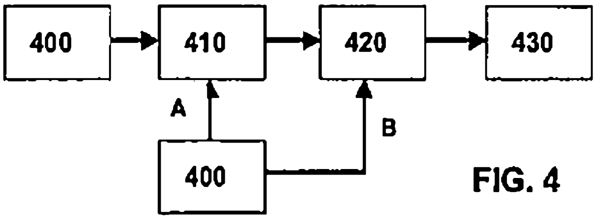

FIG. 4

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PROCESSING OF BIOMASS MATERIALS

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CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 61/579,550 and 61/579,562, both filed on December 22, 2011. The entire disclosures of the above applications are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention pertains to the preparation enzymes useful in the processing of biomass materials. For example, the invention relates to producing cellulase enzymes or other enzyme types.

BACKGROUND

[0003] As demand for petroleum increases, so too does interest in renewable feedstocks for manufacturing biofuels and biochemicals. The use of lignocellulosic biomass as a feedstock for such manufacturing processes has been studied since the 1970s. Lignocellulosic biomass is attractive because it is abundant, renewable, domestically produced, and does not compete with food industry uses.

[0004] Many potential lignocellulosic feedstocks are available today, including agricultural residues, woody biomass, municipal waste, oilseeds/cakes and sea weeds, to name a few. At present these materials are either used as animal feed, biocompost materials, are burned in a cogeneration facility or are landfilled.

[0005] Lignocellulosic biomass is recalcitrant to degradation as the plant cell walls have a structure that is rigid and compact. The structure comprises crystalline cellulose fibrils embedded in a hemicellulose matrix, surrounded by lignin. This compact matrix is difficult to access by enzymes and other chemical, biochemical and biological processes. Cellulosic biomass materials (*e.g.*, biomass material from which substantially all the lignin has been removed) can be 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.

[0006] While a number of methods have been tried to extract structural carbohydrates from lignocellulosic biomass, they are either are too expensive, produce too low a yield, leave undesirable chemicals in the resulting product, or simply degrade the sugars.

[0007] Saccharides from renewable biomass sources could become the basis of chemical and fuels industries by replacing, supplementing or substituting petroleum and other fossil feedstocks. However, techniques need to be developed that will make these monosaccharides available in large quantities and at acceptable purities and prices.

SUMMARY OF THE INVENTION

[0008] Provided herein are methods of inducing the production of one or more enzymes by a microorganism, through the use of an inductant.

[0009] In one aspect, a method is provided that includes combining a cellulosic or lignocellulosic biomass, which has been treated to reduce its recalcitrance, with a microorganism, to induce the production of one or more enzyme(s) by the microorganism by maintaining the microorganism-biomass combination under conditions that allow for the production of the enzyme(s) by the microorganism. In some implementations, the enzyme(s) are then used to saccharify cellulosic or lignocellulosic biomass.

[0010] Also provided herein is a method for inducing the production of an enzyme by a microorganism, where the method includes: providing a first cellulosic or lignocellulosic biomass; treating the first biomass with a treatment method to reduce its recalcitrance, thereby producing a first treated biomass; providing a microorganism; providing a liquid medium; combining the first treated biomass, the microorganism, and the liquid medium, thereby producing a microorganism-biomass combination; and maintaining the microorganism-biomass combination under conditions allowing for the production of an enzyme by the microorganism, thereby producing an inductant-enzyme combination; thereby inducing the production of the enzyme by the microorganism.

[0011] Also provided herein is a composition that includes a liquid medium, a cellulosic or lignocellulosic biomass treated to reduce its recalcitrance, a microorganism, and one or more enzymes made by the microorganism.

[0012] In any of the methods or compositions provided herein, the treatment for reducing the recalcitrance of the biomass material(s) can be any of: bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, and freeze grinding. Preferably, the treatment method is bombardment with electrons.

[0013] The methods and compositions can also include mechanically treating the first or the second cellulosic or lignocellulosic biomass to reduce its bulk density and/or increase its surface area. The biomass material(s) can be comminuted before being combined with the microorganism and liquid medium. The comminution can be dry milling or wet miling. The biomass material can have a particle size of about 30 to 1400 μm.

[0014] In any of the methods and compositions described herein, any of the cellulosic or lignocellulosic biomasses can be: 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, or mixtures of any of these. Alternatively, the cellulosic or lignocellulosic biomass can include material that was remaining after a prior cellulosic or lignocellulosic biomass was previously converted to a product by an enzyme of a microorganism.

[0015] In these methods and compositions, the microorganism can be any of a fungus, a bacterium, or a yeast. The microorganism can actually be a population of different microorganisms. The microorganism can be a strain that produces high levels of cellulase, and/or it can be genetically engineered. The microorganism can be *Trichoderma reesei*, or it can be *Clostridium thermocellum*, for example. The microorganism can be a *T. reesei* strain such as RUT-NG14, PC3-7, QM9414 or RUT-C30.

[0016] In any of these methods and compositions, the cellulosic or lignocellulosic biomass can be combined with the microorganism at a time when the microorganism is in lag phase.

[0017] The methods and compositions can also include removing all or a portion of the liquid from the microorganism-inductant-enzyme combination, to produce an enzyme extract. The methods and compositions can also include concentrating one or more of the enzymes, and/or isolating one or more of the enzymes.

[0018] The methods and compositions can also include allowing saccharification of the second cellulosic or lignocellulosic biomass to occur, so that one or more sugars are produced. The one or more sugars can be isolated and/or concentrated.

[0019] It should be understood that this invention is not limited to the embodiments disclosed in this Summary, and it is intended to cover modifications that are within the spirit and scope of the invention, as defined by the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

[0021] FIG. 1 is a diagram illustrating the enzymatic hydrolysis of cellulose to glucose. Cellulosic substrate (A) is converted by endocellulase (I) to cellulose (B), which is converted by exocellulase (II) to cellobiose (C), which is converted to glucose (D) by cellobiase (beta-glucosidase) (III).

[0022] FIG. 2 is a flow diagram illustrating conversion of a biomass feedstock to one or more products. Feedstock is physically pretreated (*e.g.*, to reduce its size) (200), optionally treated to reduce its recalcitrance (210), saccharified to form a sugar solution (220), the solution is transported (230) to a manufacturing plant (*e.g.*, by pipeline, railcar) (or if saccharification is performed en route, the feedstock, enzyme and water is transported), the saccharified feedstock is bio-processed to produce a desired product (*e.g.*, alcohol) (240), and the product can be

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processed further, *e.g.*, by distillation, to produce a final product (250). Treatment for recalcitrance can be modified by measuring lignin content (201) and setting or adjusting process parameters (205). Saccharifying the feedstock (220) can be modified by mixing the feedstock with medium and the enzyme (221).

[0023] FIG. 3 is a flow diagram illustrating the treatment of a first biomass (300), addition of a cellulase producing organism (310), addition of a second biomass (320), and processing the resulting sugars to make products (*e.g.*, alcohol(s), pure sugars) (330). The first treated biomass can optionally be split, and a portion added as the second biomass (A).

[0024] FIG. 4 is a flow diagram illustrating the production of enzymes. A cellulase-producing organism is added to growth medium (400), a treated first biomass (405) is added (A) to make a mixture (410), a second biomass is added (420), and the resulting sugars are processed to make products (*e.g.*, alcohol(s), pure sugars) (430). Portions of the first biomass (405) can also be added (B) to the second biomass (420).

[0025] FIG. 5 shows results of protein analysis using SDS PAGE.

DETAILED DESCRIPTION

[0026] Provided herein are methods of inducing the production of one or more enzymes by a microorganism, through the use of an inductant. The inductant is made from biomass (cellulosic or lignocellulosic) that has been treated to reduce its recalcitrance. The treatment method can include subjecting the biomass to bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding. As disclosed herein, biomass that has been treated with such a method can be combined with a microorganism in a medium (such as a liquid medium), to induce the microorganism to produce one or more enzymes.

[0027] In one aspect, the invention features a method that includes contacting an inducer comprising a lignocellulosic material with a microorganism to produce an enzyme.

[0028] Specifically, the processes described herein include saccharifying cellulosic and/or lignocellulosic materials using enzymes that have been produced by *Trichoderma reesei* fungi, as will be discussed in further detail below.

[0029] In general, the invention relates to improvements in processing biomass materials (*e.g.*, biomass materials or biomass-derived materials) to produce intermediates and products,

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such as fuels and/or other products. For example, the processes may be used to produce sugars, alcohols (such as ethanol, isobutanol, or n-butanol), sugar alcohols (such as erythritol), or organic acids.

[0030] The invention also relates to the preparation of enzymes useful in the processing of biomass materials. For example the invention relates to producing cellulase enzymes or other enzyme types.

[0031] A typical biomass resource contains cellulose, hemicellulose, and lignin plus lesser amounts of proteins, extractables and minerals. The complex carbohydrates contained in the cellulose and hemicellulose fractions can be converted into sugars, *e.g.*, fermentable sugars, by saccharification, and the sugars can then be used as an end product or an intermediate, or converted by further processing, *e.g.*, fermentation or hydrogenation, into a variety of products, such as alcohols or organic acids. The product obtained depends upon the method or microorganism utilized and the conditions under which the bioprocessing occurs.

[0032] In one embodiment, for instance, the invention includes a method of inducing the production of an enzyme. A cellulosic or lignocellulosic biomass is provided, treated to reduce its recalcitrance, and then combined with a microorganism in a liquid medium. The resulting microorganism-biomass combination is then maintained under conditions allowing for the growth of the organism and production of enzymes capable of degrading the biomass. The treated biomass acts as an inductant, causing the microorganism to produce enzymes. The method produces an inductant-enzyme combination.

[0033] Without wishing to be bound by any theory, it is believed that the treatment used to reduce the recalcitrance of the biomass is important in enzyme induction. The inventors have found that low levels of treatment result in either low levels of enzyme induction, or extremely long lag times, presumably because it is difficult for the microorganisms to extract sugars from the treated biomass material. Similarly, very high levels of treatment also cause the microorganisms to produce low levels of enzymes, possibly because relatively easy extraction of sugars from the treated biomass lessens the need for the microorganisms to produce large amounts of enzymes.

[0034] On a related matter, the recalcitrance treatment also serves to sterilize the material. Biomass material, by its nature, contains contaminating microbes, which are often embedded deep within the material itself. Because the enzyme inductions as disclosed herein tend to be

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long fermentations (up to a week or more), sterilization is important. It would therefore be advantageous to treat the material as heavily as possible to sterilize it. However, such high levels of treatment would likely be counterproductive because high levels of treatment lessen the enzyme production by the microorganisms.

[0035] As disclosed herein, there is therefore a large benefit to be gained from carefully balancing the level of treatment to sterilize the material, yet not over-treat the material so that the microorganisms fail to produce large amounts of enzyme.

[0036] The term "inductant," as used herein, means a cellulosic or lignocellulosic biomass that encourages an organism to produce enzyme. An example would be biomass that has been treated to reduce its recalcitrance. The treated biomass is then used as an enzyme inductant, by being combined with one or more microorganisms in a liquid medium, and then being maintained under conditions that allow the microorganism to produce one or more enzymes.

[0037] The inductant-enzyme combination can then be combined with another biomass, and used to saccharify it.

[0038] Surprisingly, it has been found that treating the biomass before inoculating it with the microorganism causes an increased amount of enzymes to be produced by the microbes. In addition, different enzymes are produced on the treated biomass, relative to the use of untreated biomass.

[0039] As described herein, the cellulosic or lignocellulosic biomass can be sourced from a wide variety of materials. In one embodiment, the biomass can be lignin hulls. By "lignin hulls," as used herein, is meant material that is remaining after a biomass has been saccharified.

[0040] In certain embodiments, the invention relates to processes for saccharifying a cellulosic or lignocellulosic material using an enzyme that has been produced by a fungus, *e.g.*, by strains of the cellulolytic filamentous fungus *Trichoderma reesei*. In some implementations, high-yielding cellulase mutants of *Trichoderma reesei* are used, *e.g.*, RUT-NG14, PC3-7, QM9414 and/or RUT-C30. Such strains are described, for example, in "Selective Screening Methods for the Isolation of High Yielding Cellulase Mutants of *Trichoderma reesei*," Montenecourt, B.S. and Everleigh, D.E. *Adv. Chem. Ser.* 181, 289-301 (1979). These mutants are hyperproducing and are catabolite repression-resistant, allowing high yields of cellulases to be achieved.

[0041] In preferred embodiments, the enzyme production is conducted in the presence of a portion of the lignocellulosic material to be saccharified. The lignocellulosic material can act in the enzyme production process as an inducer for cellulase synthesis, producing a cellulase complex having an activity that is tailored to the particular lignocellulosic material. In some implementations, the recalcitrance of the lignocellulosic material is reduced prior to using it as an inducer. It is believed that this makes the cellulose within the lignocellulosic material more readily available to the fungus. Reducing the recalcitrance of the lignocellulosic material also facilitates saccharification.

[0042] In some cases, reducing the recalcitrance of the lignocellulosic material includes treating the lignocellulosic material with a physical treatment. The physical treatment can be, for example, radiation, *e.g.*, electron bombardment, sonication, pyrolysis, oxidation, steam explosion, chemical treatment, or combinations of any of these treatments. The treatments can also include any one or more of the treatments disclosed herein, applied alone or in any desired combination, and applied once or multiple times.

[0043] Enzymes and biomass-destroying 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-destroying 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).

[0044] As shown in FIG. 1, for example, during saccharification a cellulosic substrate (A) is initially hydrolyzed by endoglucanases (I) at random locations producing oligomeric intermediates (*e.g.*, cellulose) (B). These intermediates are then substrates for exo-splitting glucanases (II) 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 (III) cleaves cellobiose (C) to yield glucose (D). Therefore, the endoglucanases are particularly effective in attacking the crystalline portions of cellulose and increasing the effectiveness of exocellulases to produce cellobiose, which then requires the specificity of the cellobiose to produce glucose. Therefore, it is evident that depending on the nature and structure of the cellulosic substrate, the amount and type of the three different enzymes may need to be modified.

[0045] The enzymes produced and used in the processes described herein can be produced by a fungus, *e.g.*, by one or more strains of the fungus *Trichoderma reesei*. In preferred implementations, high-yielding cellulase mutants of *Trichoderma reesei*, *e.g.*, RUT-NG14, PC3-7, QM9414 and/or RUT-C30, are used.

[0046] It is preferred that enzyme production be conducted in the presence of a portion of the feedstock that will be saccharified, thereby producing a cellulase complex that is tailored to the particular feedstock. The feedstock may be treated prior to such use to reduce its recalcitrance, *e.g.*, using one or more of the recalcitrance-reducing processes described herein, so as to make the cellulose in the feedstock more readily available to the fungus.

[0047] In a preferred embodiment the enzyme-inducing biomass can be treated by electron bombardment. The biomass can be treated, for instance, by electron bombardment with a total dose of less than about 1 Mrad, less than about 2 Mrad, , less than about 5, about 10, about 20, about 50, about 100 or about 150 Mrad. Preferably, the enzyme-inducing biomass is treated with a total dose of about 0.1 Mrad to about 150 Mrad, about 1 to about 100 Mrad, preferably about 2 to about 50 Mrad, or about 5 to about 40 Mrad.

[0048] As will be discussed further below, once the enzyme has been produced, it is used to saccharify the remaining feedstock that has not been used to produce the enzyme. The process for converting the feedstock to a desired product or intermediate generally includes other steps in addition to this saccharification step.

[0049] For example, referring to FIG. 2, a process for manufacturing an alcohol can include, for example, optionally mechanically treating a feedstock, *e.g.*, to reduce its size (200), before and/or after this treatment, optionally treating the feedstock with another physical treatment to further reduce its recalcitrance (210), then saccharifying the feedstock, using the enzyme complex, to form a sugar solution (220). Optionally, the method may also include transporting, *e.g.*, by pipeline, railcar, truck or barge, the solution (or the feedstock, enzyme and water, if saccharification is performed en route) to a manufacturing plant (230). In some cases the saccharified feedstock is further bioprocessed (*e.g.*, fermented) to produce a desired product *e.g.*, alcohol (240). This resulting product may in some implementations be processed further, *e.g.*, by distillation (250), to produce a final product. One method of reducing the recalcitrance of the feedstock is by electron bombardment of the feedstock. If desired, the steps of measuring lignin content of the feedstock (201) and setting or adjusting process parameters based on this

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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 lending more variety to the form of the cellulose. Hemicellulose is any of several heteropolymers, such as xylan, glucuronoxylan, arabinoxylans, and xyloglucan. The primary sugar monomer present is xylose, although other monomers such as mannose, galactose, rhamnose, arabinose and glucose are present. Typically hemicellulose forms branched structures with lower molecular weights than cellulose. Hemicellulose is therefore an amorphous material that is generally susceptible to enzymatic hydrolysis. Lignin is a complex high molecular weight heteropolymer generally. 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 biomaterial depends on the source of the biomaterial. For example wood derived biomaterial 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.

[0055] The diversity of biomass materials may be further increased by pretreatment, for example, by changing the crystallinity and molecular weights of the polymers.

[0056] The cellulase producing organism when contacted with a biomass will tend to produce enzymes that release molecules advantageous to the organism's growth, such as glucose. This is done through the phenomenon of enzyme induction as described above. Since there are a variety of substrates in a particular biomaterial, there are a variety of cellulases, for example, the endoglucanase, exoglucanase and cellobiase discussed previously. By selecting a particular lignocellulosic material as the inducer the relative concentrations and/or activities of these enzymes can be modulated so that the resulting enzyme complex will work efficiently on the lignocellulosic material used as the inducer or a similar material. For example, a biomaterial with a higher portion of crystalline cellulose may induce a more effective or higher amount of endoglucanase than a biomaterial with little crystalline cellulose.

[0057] Therefore, there may be many methods for optimal formation and use of cellulases. Some details of these processes will be described with reference to the figures.

[0058] For example, referring to FIG. 3, a first biomass is optionally pre-treated (300), for example to reduce its recalcitrance, and is then mixed with an aqueous medium and a cellulase producing organism (310). After an adequate time has passed for the cells to grow to a desired stage and enough enzymes have been produced, a second biomass is added (320). The action of the enzyme on the second and any remaining first biomass produces a mix of sugars, which can be further processed to useful products (*e.g.*, alcohols, pure sugars) (330). The first and second biomass can be portions of the same biomass source material. For example, a portion of the biomass can be combined with the cellulase producing organism and then another portion added at a later stage (A) once some of the enzymes have been produced. Optionally, the first and second biomass may both be pretreated to reduce recalcitrance. The aqueous media will be discussed below.

[0059] Referring now to FIG. 4, the cellulase producing organism (400) can be grown in a growth medium for a time to reach a specific growth phase. For example, this growth period could extend over a period of days or even weeks. Pretreated first biomass (405) can then be contacted (A) with the enzyme producing cells (410) so that after a time enzymes are produced. Enzyme production may also take place over an extended period of time. The enzyme containing solution is then combined with a second biomass (420). The action of the enzyme on the second and remaining first biomass produces mixed sugars which can be further processed to useful products (430). The first and second biomass can be portions of the same biomass or could be similar but not identical (*e.g.*, pretreated and non-pretreated) material (B).

[0060] In addition to the methods discussed above in FIG. 4, the cellulase producing organism (400) may optionally be harvested prior to being combined with the first pretreated biomass (410). Harvesting may include partial or almost complete removal of the solvent and growth media components. For example the cells may be collected by centrifugation and then washed with water or another solution.

[0061] In another embodiment, after the enzyme(s) is produced (410), it can be concentrated (*e.g.*, between steps 410 and 420 of FIG. 4). Concentration may be by any useful method including chromatography, centrifugation, filtration, dialysis, extraction, evaporation of solvents, spray drying and adsorption onto a solid support. The concentrated enzyme can be stored for a time and then be used by addition of a second biomass (420) and production of useful products (430).

[0062] The aqueous media used in the above described methods can contain added yeast extract, corn steep, peptones, amino acids, ammonium salts, phosphate salts, potassium salts, magnesium salts, calcium salts, iron salts, manganese salts, zinc salts and cobalt salts. In addition to these components, the growth media typically contains 0 to 10% glucose (*e.g.*, 1 to

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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

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cellulosic materials, *e.g.*, paper, paper products, paper pulp, cotton, and mixtures of any of these, and other types of biomass. The processes described herein are particularly useful with lignocellulosic materials, because these processes are particularly effective in reducing the recalcitrance of lignocellulosic materials and allowing such materials to be processed into products and intermediates in an economically viable manner.

[0069] As used herein, the term "biomass materials" includes lignocellulosic, cellulosic, starchy, and microbial materials.

[0070] Preferably the enzyme-inducing biomass materials are agricultural waste such as corn cobs, more preferably corn stover. Most preferably, the enzyme-inducing biomass material comprises grasses.

[0071] 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.

[0072] In some cases, the lignocellulosic material includes corncobs. Ground or hammermilled 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.

[0073] 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.

[0074] 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.

[0075] 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 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 delignified.

[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

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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).

[0088] 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, from about 0.7 to 1 MeV, or from about 1 to about 3 MeV. In some implementations the nominal energy is about 500 to 800 keV.

[0089] 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.

[0090] 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.

[0091] In some implementations, it is desirable to cool the material during electron bombardment. For example, the material can be cooled while it is being conveyed, for example by a screw extruder or other conveying equipment.

[0092] To reduce the energy required by the recalcitrance-reducing process, 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 generally require higher line speeds, to avoid thermal decomposition of the material. In one implementation, the accelerator is set for 3 MeV, 50 mAmp 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/cm^3).

[0093] In some embodiments, electron bombardment is performed until the material receives a total dose of at least 0.5 Mrad, *e.g.*, at least 5, 10, 20, 30 or at least 40 Mrad. In some embodiments, the treatment is performed until the material receives a dose of from about 0.5 Mrad to about 150 Mrad, about 1 Mrad to about 100 Mrad, about 2 Mrad to about 75 Mrad, 10 Mrad to about 50 Mrad, *e.g.*, about 5 Mrad to about 50 Mrad, from about 20 Mrad to about 40 Mrad, about 10 Mrad to about 35 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 seconds, *e.g.*, at 5 Mrad/pass with each pass being applied for about one second. Applying a dose of greater than 7 to 8 Mrad/pass can in some cases cause thermal degradation of the feedstock material.

[0100] 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.*, 9 to 11 Mrad/pass. As discussed above, 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.

[0101] 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.

[0102] 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 five percent by weight retained water, measured at 25°C and at fifty percent relative humidity.

[0103] Electron bombardment can be applied while the cellulosic and/or lignocellulosic material is exposed to air, oxygen-enriched air, or even oxygen itself, or blanketed by an inert gas such as nitrogen, argon, or helium. When maximum oxidation is desired, an oxidizing environment is utilized, such as air or oxygen and the distance from the beam source is optimized to maximize reactive gas formation, *e.g.*, ozone and/or oxides of nitrogen.

BIOMASS TREATMENT – SONICATION, PYROLYSIS, OXIDATION, STEAM EXPLOSION

[0104] If desired, one or more sonication, pyrolysis, oxidative, or steam explosion processes can be used in addition to or instead of electron bombardment to reduce the recalcitrance of the

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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 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.

[0110]

INTERMEDIATES AND PRODUCTS

[0111] 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, 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 and polyols (*e.g.*, glycol, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, galactitol, iditol, inositol, volemitol, isomalt, maltitol, lactitol, maltotriitol, maltotetraitol, and polyglycitol and other polyols), and methyl or ethyl esters of any of these alcohols. Other products include methyl acrylate, methylmethacrylate, lactic acid, citric acid, formic acid, acetic acid, propionic 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.

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[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 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 of preservative properties may be used. Preferably the antimicrobial additive(s) are food-grade.

[0124] A relatively high concentration solution can be obtained by limiting the amount of water added to the biomass 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 biomass 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

[0125] 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. brachypenium*, *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 brachypenium* 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

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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.

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FERMENTATION AGENTS

[0137] 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.

[0138] 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 *Saccharomyces 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. pseudatropicalis*, and *C. brassicae*), *Pichia stipitis* (a relative of *Candida shehatae*), the genus *Clavispara* (including, but not limited to, *C. lusitaniae* and *C. opuntiae*), the genus *Pachysalen* (including, but not limited to, *P. tannophilus*), the genus *Bretannamyces* (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, *Zymamanas mobilis*, *Clostridium spp.* (including, but not limited to, *C. thermocellum* (Philippidis, 1996, *supra*), *C. saccharobutylacetanicum*, *C. saccharobutylicum*, *C. Puniceum*, *C. beijernckii*, and *C. acetobutylicum*), *Moniliella pollinis*, *Maniliella megachiliensis*, *Lactobacillus* spp. *Yarrawia lipalytica*, *Aureobasidium sp.*, *Trichasparonaides sp.*, *Trigonapsis variabilis*, *Trichosparan sp.*, *Maniliellaacetaabutans sp.*, *Typhula variabilis*, *Candida magnoliae*, *Ustilaginamycetes sp.*, *Pseudozyma tsukubaensis*, yeast species of genera *Zygosaccharomyces*, *Debaryomyces*, *Hansenula and Pichia*, and fungi of the dematioid genus *Tarula*.

[0139] For instance, *Clostridium* spp. can be used to produce ethanol, butanol, butyric acid, acetic acid, and acetone. *Lactobacillus* spp., can be used to produce lactice acid.

[0140] Many such microbial strains are publicly available, either commercially or through depositories such as the ATCC (American Type Culture Collection, Manassas, Virginia, USA), the NRRL (Agricultural Research Sevice Culture Collection, Peoria, Illinois, USA), or the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), to name a few.

[0141] Commercially available yeasts include, for example, Red Star®/Lesaffre Ethanol Red (available from Red Star/Lesaffre, USA), FALI® (available from Fleischmann's Yeast, a division of Burns Philip Food Inc., USA), SUPERSTART® (available from Alltech, now Lalemand), GERT STRAND® (available from Gert Strand AB, Sweden) and FERMOL® (available from DSM Specialties).

[0142] 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.

DISTILLATION

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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.

EXAMPLES

[0147] Materials & Methods

[0148] The following procedures and materials were used in the following examples.

[0149] *Cell banking:* The following *Trichiderma reesei* strains were banked: ATCC 66589, PC3-7; ATCC 56765, RUT-C30; ATCC 56767, NG-14; ATCC 26921, QM 9414.

[0150] Each cell was rehydrated and propagated in potato dextrose (PD) media at 25°C.

[0151] For production of master cell banks, each strain was rehydrated overnight in 0.5ml sterile water. To propagate cells, 40 ul of rehydrated cells were used to inoculate potato dextrose agar (PDA) solid medium. Rehydrated cells were also inoculated into 50 ml of PD liquid medium and incubated at 25°C and 200 rpm. After 2 weeks culture in PDA media, spores were resuspended in sterile NaCl (9g/L), 20% glycerol solution, and stored in -80°C freezer for use as a cell bank.

[0152] *Protein measurement and cellulase assay:* Protein concentration was measured by the Bradford method using bovine serum albumin as a standard.

[0153] Filter paper assay (FPU), cellobase activity and CMC activity was carried out using the IUPAC method (T.K. Ghose, *Pure Appl. Chem.* 59:257-68, 1987).

[0154] The reaction product (glucose) was analyzed on a YSI 7100 Multiparameter Bioanalytical System (YSI Life Sciences, Yellow Springs, Ohio, USA) or HPLC.

[0155] *Media:* The media included corn steep (2 g/L), ammonium sulfate (1.4 g/L), potassium hydroxide (0.8 g/L), Phosphoric acid (85%, 4mL/L), phthalic acid dipotassium salt (5 g/L), magnesium sulfate heptahydrate (0.3g/L), calcium chloride (0.3g/L), ferrous sulfate heptahydrate (5 mg/L), manganese sulfate mono hydrate (1.6 mg/L), zinc sulfate heptahydrate (5 mg/L) and cobalt chloride hexahydrate (2mg/L). The media is described in Herpoel-Gimbert *et al., Biotechnology for Biofuels*, 2008, 1:18.

[0156] *Bio-reactor:* The freezer stock from the cell banking was used to make the seed culture using the media described above, with 2.5% additional glucose. The seed culture was typically made in a flask using an incubator set at 30°C and 200rpm for 72hrs. Seed culture broth (50mL) was used as an inoculum in the 1L starting medium in a 3L fermenter. In growth phase, 35 g/L of lactose was added to the medium. The culture conditions were as follows: 27°C, pH 4.8 (with 6M ammonia), air flow 0.5 VVM, stirring 500 rpm, and dissolved oxygen (DO) was maintained above 40 % oxygen saturation. In the induction phase, the desired inducer (discussed below) was added. During fermentation, Antiform 204 (Sigma) was injected into the culture when the foam reached the fermentor head.

[0157] *Shake flask:* In addition to the media described above, for the flask culture, Tris buffer (12.1g/L), maleic acid (11.06g/L) and sodium hydroxide (2.08g/L) were added. A starter culture was prepared in the media with added glucose. After 3 days of growth, the cell mass was harvested by centrifugation. The cell mass was re-suspended in 50 ml of media with the desired inducer. The flasks were placed in a shaker incubator set at an agitation speed of 200 rpm and temperature of 30°C.

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[0158] Example 1. Cellulase Performance Test on Paper, Treated Corn Cob and Untreated Corn Cob

[0159] Various inducers (treated biomass (TBM), untreated biomass (UBM), paper (P) and carboxylmethylcellulose (CMC, Aldrich)) were used to produce enzymes. The biomass (TBM and UBM) was milled corn cob collected between mesh sizes of 15 and 40. Treatment of the biomass (UBM) to produce the TBM involved electron bombardment with an electron beam to a total dose of 35 Mrad. The paper was shredded and screened to have a nominal particle size smaller than 0.16 inch. The inducer experiments were conducted using shake flasks and PC3-7 and RUT-C30 strains. After 3 days of the growth culture, the harvested cell mass was added to a series of shake flasks each containing 50 ml of medium and 1 wt. % of one of the inducers.

[0160] The induction experiment was allowed to proceed for 11 days. The culture supernatant was then harvested by centrifugation at 14,500 rpm for 5 minutes, and stored at 4°C.

[0161] *Protein concentration of culture supernatant:* Using the cell culture grown in the shake flasks and derived from PC3-7, protein concentrations after 11 days were 1.39, 1.18, 1.06 and 0.26 mg/mL for TBM, UBM, P and CMC respectively. For RUT-C30, the protein concentrations were 1.26, 1.26, 1.00 and 0.26 mg/mL for TBM, P, UBM and CMC respectively.

[0162] *Cellulase activity:* The cellulase activities were assessed and are listed in the table below.

[0163] Table 1. Cellulase activity for different inducers and cell strains.

Inducer	Cell type	FPU (U/mL)	Cellobiase activity (U/mL)	FPU (U/mg)	Cellobiase activity (U/mg)
TBM	PC-3-7	0.57	0.47	1.04	0.86
UBM	PC-3-7	0.45	0.39	1.08	0.93
P	PC-3-7	0.57	0.39	0.96	0.66
CMC	PC-3-7	0.06	0.11	0.55	0.99
TBM	RUT-C30	1.02	0.53	1.97	1.03
UBM	RUT-C30	0.72	0.42	1.76	1.03
P	RUT-C30	0.71	0.40	1.31	0.74
CMC	RUT-C30	0.24	0.18	1.77	1.31

[0164] These results show that treated biomass serves to induce enzyme production at a greater rate than untreated biomass.

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[0165] Example 2. Enzyme Production in Different Concentrations of TBM Inducer

[0166] This Example was done using bioreactors. Cell strain RUT-C30 was propagated in the media with 2.5 % glucose. After 3 days of growth the culture was centrifuged and the cells was re-suspended in 50 ml of media with 1, 3, 5, 7 and 9 wt.% TBM. The protein concentrations and activities after 11 days of incubation at 27°C and 200 rpm are shown in the table below.

[0167] Table 2. Amounts of Protein and Enzyme Made With Differing Amounts of Inducer

Inducer amount (wt.%)	Protein (g/L)	FPU (U/mL)	CMC (U/mL)
1	0.7	1.4	1.3
2	1.4	3.1	1.7
5	3.4	6.2	2.6
7	2.9	2.5	1.5
9	1.5	0.6	1.0

[0168] These results show that higher levels of enzymes were produced when the treated biomass (TBM) was added at a rate of 5 %.

[0169] *Saccharification of biomass with enzymes:* Saccharification of biomass (TBM) using enzymes produced by addition of 2, 5 and 7 wt.% treated biomass inducer (TBM) versus a commercial enzyme (Duet^{TM} Accellerase, Genencor) was conducted. The biomass, 10wt. % TBM, was combined with either 0.25 ml/g of enzyme culture broth or commercial enzyme. The saccharification was carried out at 50°C and 200 rpm in a shaking incubator. After 24 hours the amount of generated glucose was measured by YSI. The amount of glucose produced per L of solution and mg of protein is shown in the table below.

[0170] Table 3. Amount of Glucose Produced From Varying Levels of Inducer

Enzyme produced from	Glucose (g/L)	Glucose (g/mg)
2% TBM inducer	4.04	2.31
5% TBM inducer	4.06	1.08
7% TBM inducer	3.02	0.83
Commercial enzyme	14.4	0.50

[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	4.7-18.7g/L/d	18.7g/L/d
	Frequency of Addition	continuous feed	continuous feed	continuous 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.

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[0176] 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.

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CLAIMS

What is claimed is:

1. A method comprising:
combining a cellulosic or lignocellulosic biomass, which has been treated to reduce its recalcitrance, with a microorganism, and inducing the production of one or more enzymes by the microorganism by maintaining the microorganism-biomass combination under conditions that allow for the production of the enzyme(s) by the microorganism.

2. The method of claim 1 further comprising combining a second cellulosic or lignocellulosic biomass with the miccroorganism-biomass combination.

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

4. The method of claim 1, 2 or 3, where the 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, freeze grinding.

5. The method of claim 4, wherein the treatment method is bombardment with electrons.

6. The method of any of the above claims, further comprising mechanically treating the first 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 cellulosic or lignocellulosic biomass is comminuted before being combined with the 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 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 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 microorganism is a strain that produces high levels of cellulase.

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

17. The method of claim 14 wherein the microorganism is a fungus.

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

19. The method of claim 18, wherein the *T. reesei* strain is selected from the group consisting of: RUT-NG14, PC3-7, QM9414 and/or RUT-C30.

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

21. The method of any one of the above claims, further comprising 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 cellulosic or lignocellulosic biomass treated to reduce its recalcitrance, a microorganism, and one or more enzymes made by the microorganism.

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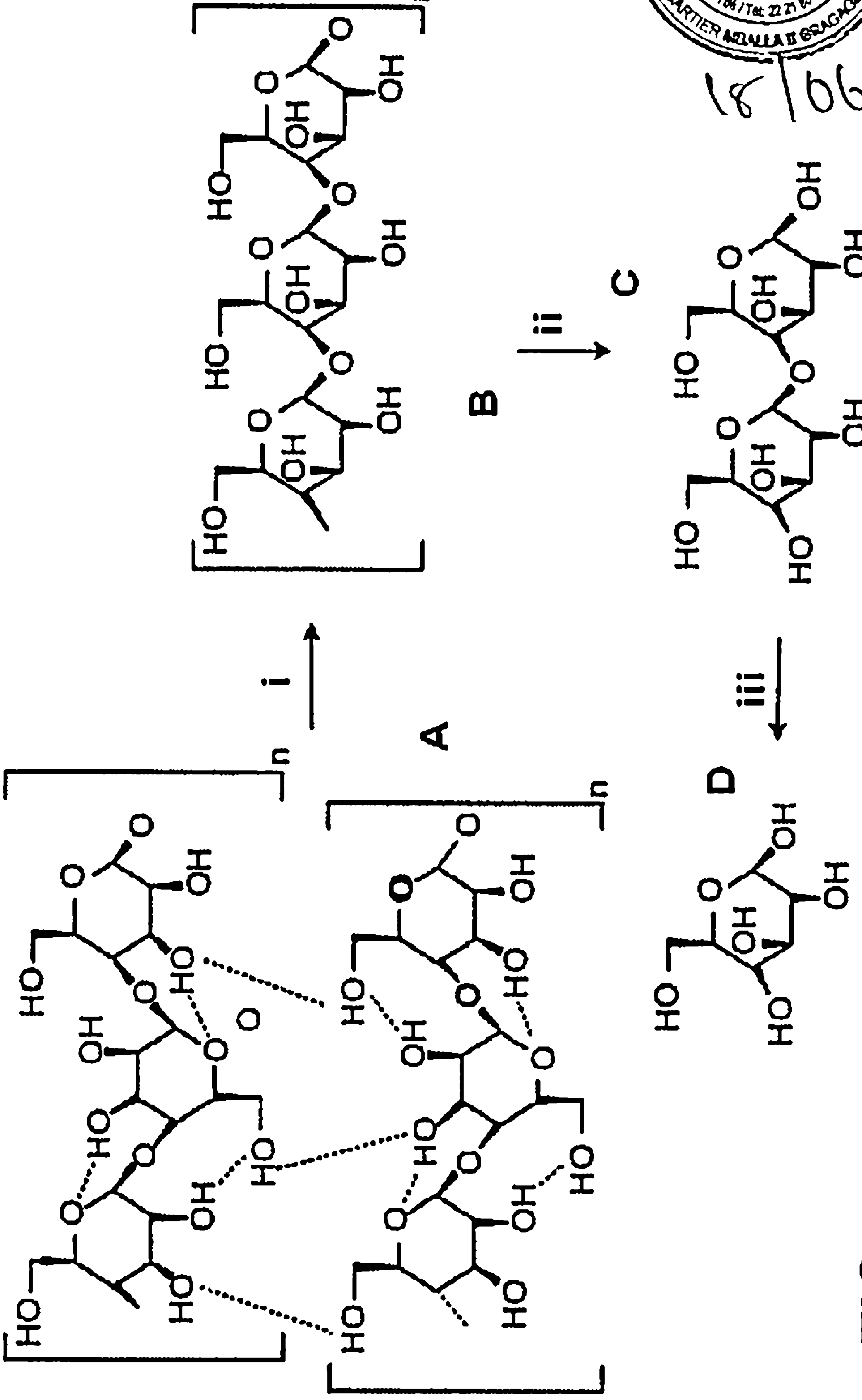

FIG. 1

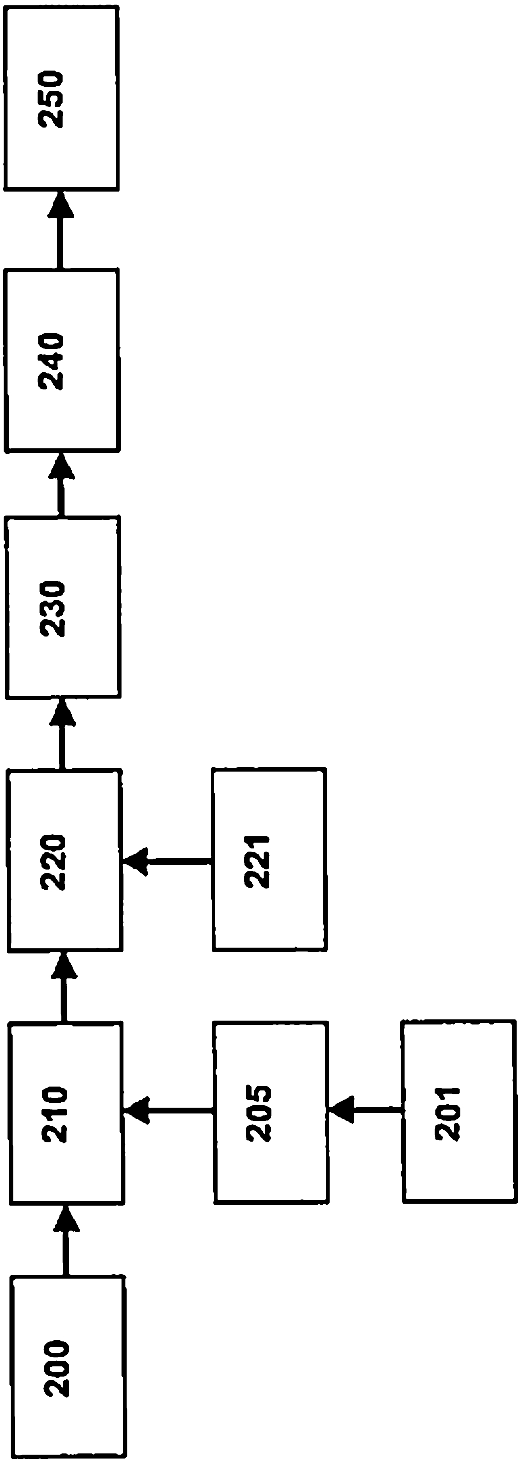

FIG. 2

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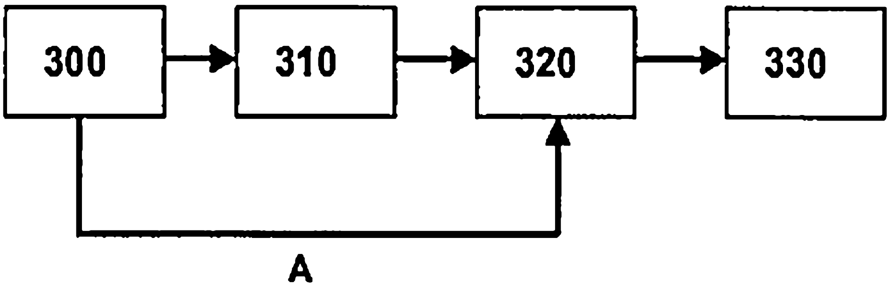

FIG. 3

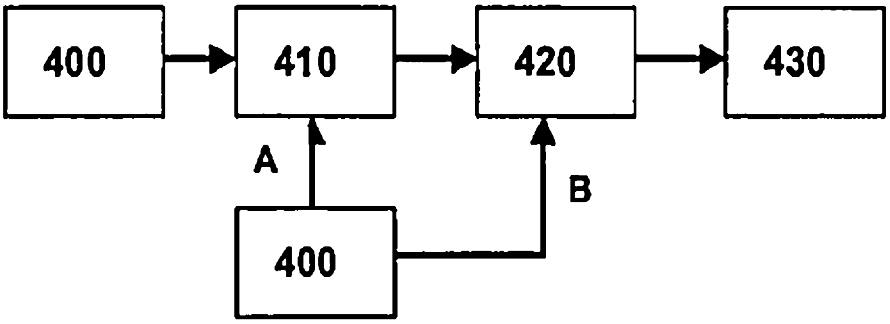

FIG. 4

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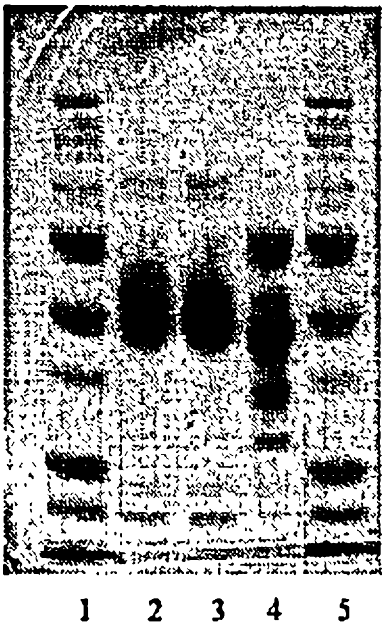

FIG. 5

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