CLAIMS

What is claimed is:

1. A method of producing enzymes, the method comprising:

treating a cellulosic or lignocellulosic biomass inducer with a treatment comprising bombardment with electrons to reduce its recalcitrance, the treatment having a level of treatment;

combining the treated cellulosic or lignocellulosic biomass inducer, with a cellulolytic microorganism;

maintaining the cellulolytic microorganism-biomass combination under conditions to induce the cellulolytic microorganism to produce a cellulase complex comprising one or more enzymes capable of saccharifying a recalcitrant-reduced cellulosic or lignocellulosic biomass, the induction having a level of enzyme induction; and

wherein the level of treatment of the cellulosic or lignocellulosic biomass inducer affects the level of enzyme induction.

2. The method of claim 1 further comprising saccharifying the recalcitrant-reduced cellulosic or lignocellulosic biomass with the cellulase complex, wherein the cellulase complex acts synergistically to degrade crystalline cellulose or lignin portions of the recalcitrant-reduced cellulosic or lignocellulosic biomass.

3. The method of claim 2 wherein the recalcitrant-reduced cellulosic or lignocellulosic biomass is lignocellulosic.

4. The method of claims 1, 2 or 3, wherein the cellulosic or lignocellulosic biomass inducer and/or the recalcitrant-reduced cellulosic or lignocellulosic biomass has been treated to reduce its recalcitrance by a treatment method selected from the group consisting of: sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, freeze grinding.

5. The method of claim 1, wherein the bombardment with electrons applied to the cellulosic or lignocellulosic biomass inducer is selected from a dose of 2 to 50 Mrad.

6. The method of any of the above claims, further comprising mechanically treating the cellulosic or lignocellulosic biomass inducer to reduce its bulk density and/or increase its surface area.

7. The method of any of the above claims, wherein the cellulosic or lignocellulosic biomass inducer 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 inducer and/or the recalcitrant-reduced 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 inducer and/or the recalcitrant-reduced 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 inducer and/or the recalcitrant-reduced 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 inducer and/or the recalcitrant-reduced 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.

16. The method of any one of the above claims, wherein the cellulolytic 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.

20. The method of any one of the above claims, wherein the cellulosic or lignocellulosic biomass inducer 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 recalcitrant-reduced 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 inducer having been treated with a dose of bombardment electrons to reduce its recalcitrance, the treatment having a level of treatment

a cellulolytic microorganism,

and a cellulase complex comprising one or more enzymes produced by the cellulolytic microorganism that is capable of saccharifying a recalcitrant-reduced cellulosic or lignocellulosic biomass, the production having a level of enzyme production; and wherein the level of treatment of the cellulosic or lignocellulosic biomass inducer affects the level of enzyme production.

CLAIMS

What is claimed is:

1. A method of producing enzymes tailored to saccharify a particular cellulosic or lignocellulosic biomass, the 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

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.

2. The method of claim 1, further comprising saccharifying the second quantity of the particular 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.

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

4. The method of claim 1, 2 or 3, wherein 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: 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 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.

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, 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 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.

22. A composition comprising: a liquid medium, a first quantity of a particular cellulosic or lignocellulosic biomass having been treated with a particular dose of bombardment electrons to reduce its recalcitrance, a cellulolytic microorganism, and a cellulase complex comprising one or more enzymes made by the cellulolytic microorganism, the relative concentrations of the one or more enzymes having been modulated by selection of the particular dose of bombardment with electrons and thereby being tailored to saccharify a second quantity of the particular cellulosic or lignocellulosic biomass.