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(54) Title: COMPOSITIONS FOR ENHANCED ENZYME PRODUCTION

(57) Abstract: The present invention relates to compositions to induce production of proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes in a host cell, and methods for increasing the yield of the proteins, e.g., enzymes produced. Such compositions comprise a caramelized sugar product. The methods described herein can also be used to enhance processing of biomass materials, e.g., to produce sugar products.

WO 2016/160956 A1

COMPOSITIONS FOR ENHANCED ENZYME PRODUCTION

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/140,785, filed March 31, 2015. The entire contents of this application are hereby incorporated by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 17, 2016, is named X2002-7002WO_SL.txt and is 63,369 bytes in size.

FIELD OF THE INVENTION

The present invention relates generally to compositions for enhanced production of a protein, e.g., an enzyme, e.g., an amylase or a biomass degrading enzyme, e.g., a cellulase or a hemicellulase, and methods for enhanced biomass degrading enzyme production. Such compositions comprise a caramelized sugar product. The methods described herein can be used to process biomass materials.

BACKGROUND OF THE INVENTION

Biomass degrading enzymes, such as cellulases, xylanases, and ligninases, are important for the degradation of biomass, such as feedstock. Cellulosic and lignocellulosic materials are produced, processed, and used in large quantities in a number of applications. Often such materials are used once, and then discarded as waste, or are simply considered to be wasted materials, e.g., sewage, bagasse, sawdust, and stover. Microorganisms that produce biomass degrading enzymes, endogenously or heterologously, can be used to process biomass materials, e.g., to produce sugar products. However, there exists a need for compositions

and/or methods that enhance biomass degrading enzyme production to increase the efficiency of biomass processing.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the surprising discovery that caramelized sugar products can induce production of biomass degrading enzymes when introduced to microorganisms capable of producing a biomass degrading enzyme, e.g., *T. reesei*. In addition, the yield of biomass degrading enzymes produced was higher than that produced from conventional induction methods, e.g., using cellulose containing biomass materials, e.g., corncob. Thus, provided herein are compositions and methods for enhancing the production of a biomass degrading enzyme from a microorganism.

Accordingly, in one aspect, the present invention features a method for inducing production of a protein comprising contacting a microorganism that produces the protein with a composition comprising a caramelized sugar product under conditions sufficient for production of a protein. In embodiments, the protein is an enzyme. In some embodiments, the enzyme is an amylase or a biomass degrading enzyme.

In embodiments, the microorganism is in a cell culture. In some embodiments, sugar is added to the cell culture prior to contacting the microorganism with the composition comprising a caramelized sugar product. In some embodiments, the microorganism is contacted with the composition comprising a caramelized sugar product when the cell culture is substantially free from sugar.

In embodiments, the caramelized sugar product is produced by caramelizing glucose, xylose, maltose, lactose, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose. In some embodiments, the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof. In some embodiments, the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof. In one embodiment, the caramelized sugar product is produced by caramelizing glucose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose. In one embodiment, the

caramelized sugar product is produced by caramelizing maltose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising maltose. In one embodiment, the caramelized sugar product is produced by caramelizing lactose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose. In one embodiment, the caramelized sugar product is produced by caramelizing xylose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose. In some embodiments, when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

In some embodiments, the biomass degrading enzyme comprises an amylase, e.g., an alpha, beta or gamma amylase, an endoglucanase, an exoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof.

In some embodiments, the composition further comprises an inducer biomass. In some embodiments, the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component. In some embodiments, the inducer biomass, e.g., starchy material or starchy material that includes a cellulosic component, comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof. In some embodiments, the agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof. In some embodiments, the agricultural product or waste comprises corn cobs, corn stover, corn fiber, or beeswing. In some embodiments, the agricultural product or waste comprises corn cobs. In some embodiments, the agricultural product or waste comprises beeswing. In some embodiments, the paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof. In some embodiments, the forestry product comprises aspen

wood, particle board, wood chips, or sawdust, or a combination thereof. In some embodiments, the general waste comprises manure, sewage, or offal, or a combination thereof.

In some embodiments, the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass. In some embodiments, the pre-treatment of the inducer biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding. In some embodiments, the pre-treatment of the inducer biomass comprises exposure to an electron beam or bombardment with electrons.

In one embodiment, the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

In some embodiments, the microorganism that produces a biomass degrading enzyme is from a species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma.* In some embodiments, the microorganism is a fungal cell. In some embodiments, the microorganism that produces a biomass degrading enzyme is selected from *Aspergillus*, *Humicola insolens (Scytalidium thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum*, *Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei,* or *Trichoderma koningii.* In some embodiments, the microorganism is *T. reesei.* In certain embodiments, the microorganism is *T. reesei*, or a variant thereof, e.g., RUT-NG14, PC3-7, QM9414, and RUT-C30.

In some embodiments, the amount of biomass degrading enzyme produced is increased by at least 1-fold, e.g., at least 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, or more, compared the amount of biomass degrading enzyme produced by the microorganism without contacting with a caramelized sugar product. In some embodiments, the amount of biomass degrading enzyme produced is increased by at least 1-fold, e.g., at least 1.2-fold, 1.5 fold, 1.8-fold, 2-fold, compared the amount of biomass degrading enzyme produced by contacting the microorganism

with a inducer biomass. In some embodiments, the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

In some embodiments, the method further comprises separating the biomass degrading enzyme from a component of the cell culture. In some embodiments, the method further comprises separating the biomass degrading enzyme from the microorganism or remaining inducer biomass. In some embodiments, the method further comprises separating the biomass degrading enzyme from the microorganism or remaining inducer biomass by chromatography or filtration. In some embodiments, the biomass degrading enzyme is purified from the cell culture.

In some embodiments, the method further comprises a step comprising: a) contacting the microorganism with a sugar in a first container under conditions such that the microorganism proliferates; and b) transferring the microorganism to a second container, wherein the second container is larger, e.g., by volume, than the first container; and wherein said step is performed prior to contacting the microorganism with the composition. In some embodiments, the step is repeated 1 or more times, e.g., 2, 3, 4, 5 times.

In another aspect, the present invention features a method for producing a product from a biomass, comprising: a) inducing the production of a biomass degrading enzyme using a method comprising contacting a microorganism that produces the biomass degrading enzyme with a composition comprising a caramelized sugar product under conditions sufficient for production of a biomass degrading enzyme; b) providing a biomass; and c) contacting the biomass with the microorganism of step a) or the biomass degrading enzyme that has been separated or purified from the microorganism of step a), under conditions suitable for production of the product. In some embodiments, the product is a sugar product. In some embodiments, the product is glucose and/or xylose. In some embodiments, the method further comprises isolating the product. In some embodiments, the isolating of the product comprises precipitation, crystallization, chromatography, centrifugation, and/or extraction.

In some embodiments, the biomass degrading enzyme is an endoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof. In some embodiments, the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

In some embodiments, the method further comprises a step of treating the biomass prior to step c) to reduce the recalcitrance of the biomass. In some embodiments, the treating

comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze-grinding. In some embodiments, the treating comprises exposure to an electron beam or bombardment with electrons.

In some embodiments, the biomass comprises a starchy material or a starchy material that includes a cellulosic component. In some embodiments, the biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein: a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof; b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof; c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and d) a general waste comprises manure, sewage, or offal, or a combination thereof.

In some embodiments, the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose. In some embodiments, the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof. In some embodiments, the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing glucose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose. In some embodiments, the caramelized sugar product is produced by caramelizing maltose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or

a combination thereof, comprising maltose. In some embodiments, the caramelized sugar product is produced by caramelizing lactose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose. In some embodiments, the caramelized sugar product is produced by caramelizing xylose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose. In some embodiments, when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

In some embodiments, the composition further comprises an inducer biomass. In some embodiments, the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component. In some embodiments, the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof; b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof; c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and d) a general waste comprises manure, sewage, or offal, or a combination thereof.

In some embodiments, the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass. In some embodiments, the pre-treatment of the biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding. In some embodiments, the pre-treatment of the biomass comprises exposure to an electron beam or bombardment with electrons. In some embodiments, the inducer biomass of the present invention is the same as the biomass provided in step (b).

In one embodiment, the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

In some embodiments, the microorganism that produces a biomass degrading enzyme is from a species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma.* In some embodiments, the microorganism is a fungal cell. In some embodiments, the microorganism that produces a biomass degrading enzyme is selected from *Aspergillus*, *Humicola insolens (Scytalidium thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum*, *Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei,* or *Trichoderma koningii.* In some embodiments, the microorganism is *T. reesei.* In certain embodiments, the microorganism is *T. reesei*, or a variant thereof, e.g., RUT-NG14, PC3-7, QM9414, and RUT-C30.

In yet another aspect, the present invention features a composition comprising a caramelized sugar product for use in the methods disclosed herein. In some embodiments, the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose. In some embodiments, the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof. In some embodiments, the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing glucose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose. In some embodiments, the caramelized sugar product is produced by caramelizing maltose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof,

comprising maltose. In some embodiments, the caramelized sugar product is produced by caramelizing lactose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose. In some embodiments, the caramelized sugar product is produced by caramelizing xylose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose. In some embodiments, when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

In some embodiments, the composition further comprises an inducer biomass. In some embodiments, the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component. In some embodiments, the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof. An agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof. A paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof. A forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof. A general waste comprises manure, sewage, or offal, or a combination thereof.

In some embodiments, the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass. In some embodiments, the pre-treatment of the biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding. In some embodiments, the pre-treatment of the biomass comprises exposure to an electron beam or bombardment with electrons.

In one embodiment, the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

In still another aspect, the present invention features a cell culture comprising a microorganism capable of producing a biomass degrading enzyme and a caramelized sugar product. In some embodiments, the cell culture further comprises cell culture media. In some embodiments, the biomass degrading enzyme is an endoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof. In some embodiments, the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1. In some embodiments, the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose. In some embodiments, the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof. In some embodiments, the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof. In some embodiments, the caramelized sugar product is produced by caramelizing glucose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose. In some embodiments, the caramelized sugar product is produced by caramelizing maltose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising maltose. In some embodiments, the caramelized sugar product is produced by caramelizing lactose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose. In some embodiments, the caramelized sugar product is produced by caramelizing xylose and the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose.

In some embodiments, the microorganism is from a species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma.*

In some embodiments, the microorganism is a fungal cell. In some embodiments, the microorganism is selected from *Aspergillus*, *Humicola insolens (Scytalidium thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum, Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei,* or *Trichoderma koningii.* In some embodiments, the microorganism is *T. reesei.* In certain embodiments, the microorganism is *T. reesei*, or a variant thereof, e.g., RUT-NG14, PC3-7, QM9414, and RUT-C30.

In some embodiments, the cell culture further comprises an inducer biomass. In some embodiments, the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component. In some embodiments, the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof; b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof; c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and d) a general waste comprises manure, sewage, or offal, or a combination thereof.

In some embodiments, the cell culture further comprises a biomass degrading enzyme produced by the microorganism. In some embodiments, the biomass degrading enzyme is an endoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof. In some embodiments, the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the induction of cellulase production by caramelized sugars without cellulose inducer corncob (No CC). Caramelized glucose, caramelized maltose, caramelized lactose or no caramelized sugar (no feeding) was fed to cultures of *T. reesei* at 5g/L/day. Cellulase production was measured by protein titer (g/L) at days 4, 6, 8 and 11, and represented on the y-axis.

Figure 2 is a graph showing the oligosaccharide composition of caramelized glucose, maltose, and lactose as determined by mass spectrometry. The number of saccharide units (2-6) is represented on the x-axis.

Figure 3 is a graph showing the effect of the cellulose inducer corncob (CC) and caramelized sugar on the induction of cellulase production in *T. reesei*. Corncob alone (CC only), glucose that was not caramelized (glucose), caramelized glucose (caramel glucose), and combinations of corncob and glucose that was not caramelized (CC + glucose), and corncob and caramelized glucose (CC+ caramel glucose) was fed to *T. reesei*. Cellulase production was measured by protein titer (g/L) at days 4, 6, 8 and 11, and represented on the y-axis.

Figure 4 is a graph showing the effect of the cellulose inducer beeswing (BW) and caramelized sugar on the induction of cellulase production in *T. reesei*. Beeswing only (BW) and the combination of beeswing and caramelized glucose (BW + Base Caramel glu) was fed to *T. reesei*. Cellulase production was measured by protein titer (g/L) at days 4, 6, 8 and 12, and represented on the y-axis.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

The term "a" and "an" refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "biomass", as used herein, refers to any non-fossilized, organic matter. Biomass can be a starchy material and/or a cellulosic, hemicellulosic, or lignocellulosic

material. For example, the biomass can be an agricultural product, a paper product, forestry product, or any intermediate, byproduct, residue or waste thereof, or a general waste. The biomass may be a combination of such materials. In an embodiment, the biomass is processed, e.g., by a saccharification and/or a fermentation reaction described herein, to produce products, such as sugars, alcohols, organic acids, or biofuels.

The term "biomass degrading enzyme", as used herein, refers to an enzyme that breaks down components of the biomass matter described herein into intermediates or final products. For example, a biomass degrading enzyme includes at least amylases, e.g., alpha, beta or gamma amylases, cellulases, hemicellulases, ligninases, endoglucancases, cellobiases, xylanases, and cellobiohydrolases. Biomass degrading enzymes are produced by a wide variety of microorganisms, and can be isolated from the microorganisms, such as *T. reesei*. The biomass degrading enzyme can be endogenously expressed or heterologously expressed.

The term "biomass degrading activity", as used herein, refers to enzymatic activity that breaks down components of the biomass matter described herein into intermediates or final products. Biomass degrading activity includes at least cellulase activity, hemicellulase activity, ligninase activity, endoglucanase activity, cellobiase activity, cellobiohydrolase activity, and xylanase activity.

The term "caramelized sugar product", as used herein, refers to a small molecule or compound, or a mixture thereof, that is produced from heating (with or without a solvent, such as water present) a sugar molecule, e.g., to at least 110 °C, to cause browning of the sugar. Sugar molecules suitable for producing caramelized sugar products include, for example, glucose, xylose, maltose, lactose, fructose, sucrose, sugar blends from saccharified biomass, such as saccharified corn cob, or any combination thereof. A caramelized sugar product may comprise oligosaccharides, e.g., oligosaccharides of the sugar molecule starting material, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof.

The term "cellobiase", as used herein, refers to an enzyme that catalyzes the hydrolysis of a dimer, trimer, tetramer, pentamer, hexamer, heptamer, octamer, or an oligomer of glucose, or an oligomer of glucose and xylose, to glucose and/or xylose. For example, the cellobiase is beta-glucosidase, which catalyzes beta-1,4 bonds in cellobiose to release two glucose molecules.

The term "cellobiohydrolase" as used herein, refers to an enzyme that hydrolyzes glycosidic bonds in cellulose. For example, the cellobiohydrolase is 1,4-beta-D-glucan cellobiohydrolase, which catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages, e.g., 1,4-beta-D-glucosidic linkages of the terminal units, in cellulose, cellulooligosaccharides, or any beta- 1,4-linked glucose containing polymer, releasing oligosaccharides from the polymer chain.

The term "cellulase", as used herein, refers to an enzyme that catalyzes the break down, e.g., hydrolysis, of cellulose and related polysaccharides into shorter polysaccharides, oligosaccharides, or monosaccharides. Examples of cellulases include endoglucanases, cellobiases, and cellobiohydrolases (or exoglucanases). Cellulase activity refers to the activity of a cellulose, e.g., cellulolysis, and can include the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicelluloses, lichenin, and cereal beta-D-glucans into shorter polysaccharides, oligosaccharides, or monosaccharides.

The term "endoglucanase" as used herein, refers to an enzyme that catalyzes the hydrolysis of internal β-1,4 glycosidic bonds. For example, the endoglucanase is endo- 1 ,4-(1 ,3; 1 ,4)-beta-D-glucan 4-glucanohydrolase, which catalyses endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenan, beta- 1,4 bonds in mixed beta- 1, 3 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components.

The term "inducer biomass", as used herein, refers to any non-fossilized, organic matter that is introduced to a microorganism to induce the production of a protein, such as an enzyme, such as a biomass degrading enzyme. The inducer biomass can be a starchy material and/or a cellulosic material comprising cellulose. The inducer biomass can also be referred to as a cellulose inducer. The inducer biomass can comprise the same component(s) as a biomass that is used for production of a sugar product. In an embodiment, the inducer biomass can be introduced with a caramelized sugar product to induce production of a biomass degrading enzyme.

The term "ligninase", as used herein, refers to an enzyme that catalyzes the breakdown of lignin, commonly found in the cell walls of plants, such as by an oxidation reaction. Ligninases include lignin-modifying enzymes, lignin peroxidases and laccases.

The terms "nucleic acid" or "polynucleotide" are used interchangeable, and refer to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof. A "plurality of polypeptides" refers to two or more polypeptides, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 100, 200, or 500 or more polypeptides.

The term "xylanase" as used herein, refers to enzymes that hydrolyze xylan-containing material. Xylan is polysaccharide comprising units of xylose. A xylanase can be an endoxylanase, a beta-xylosidase, an arabinofuranosidase, an alpha-glucuronidase, an acetylxylan esterase, a feruloyl esterase, or an alpha-glucuronyl esterase.

DESCRIPTION

Materials comprising cellulose or hemicellulose, e.g., corncob, wheat straw, sawdust, etc., can be used for production of cellulases and hemicellulases in the filamentous fungus, *Trichoderma reesei* (*T. reesei*) (Lynd et al., 2002, Microbiol & Mol Biol Rev, 66:506-577). Sugars, such as glucose, xylose and fructose, are typically regarded as a soluble carbon source for *T. reesei*. Some studies have shown that the presence of easily metabolisable carbon sources such as glucose and fructose, represses the expression of cellulolytic genes (Chambergo et al., 2002, J Biol Chem. 7:1383-13988).

The present invention is based, at least in part, on the surprising discovery that caramelized sugar products produced by heating sugar molecules, e.g., glucose and/or xylose, can induce production of proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes when introduced to microorganisms, e.g., *T. reesei*. Furthermore, the yield of proteins, e.g., biomass degrading enzymes produced was higher than that produced from conventional induction methods, e.g., using a cellulose-containing inducer, e.g., corncob. Accordingly, the present invention provides compositions comprising a caramelized sugar product for inducing production of proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes in a microorganism, and methods of use thereof.

Caramelized Sugar Product

In embodiments, a composition comprising a caramelized sugar product is introduced to a microorganism e.g., to induce production of a biomass degrading enzyme. Caramelization is a non-enzymatic process commonly used in cooking by which sugar molecules are heated to a sufficient temperature to brown the sugar and produce a caramel. Generally, the sugar can be a 3, 4, 5, 6, or 7-carbon carbohydrate, e.g., glyceraldehyde, dihidroxyacetone, erythrose, threose, arabinose, ribose, ribulose, xylose, xylulose, lyxose, allose, altrose, fructose, galactose, glucose, gulose, idose, mannose, sorbose, talose, tagatose, sedoheptulose and mannoheptulose or mixtures of any of these. For example, suitable sugar molecules that can be used as the starting material for caramelization include glucose, maltose, lactose, xylose, fructose, and sucrose. In an embodiment, glucose is caramelized. In an embodiment, maltose is caramelized. In an embodiment, lactose is caramelized. In an embodiment, xylose is caramelized. In an embodiment, sugars from saccharified biomass, e.g., saccharified corn cob, wheat straw and/or

a starchy material are caramelized. Any combination of glucose, maltose, lactose, xylose, fructose, and/or sucrose can be caramelized. For example, in one embodiment, a mixture comprising xylose and glucose is caramelized.

Sugar molecules are caramelized by heating to a sufficient temperature to brown the sugar. In embodiments, the sugar molecules are heated to at least 110 ºC, 120 ºC, 130 ºC, 140 ºC, 150 ºC, 160 ºC, 170 ºC, 180 ºC, 190 ºC, or 200 ºC. The temperature used can depend on the sugar starting material, as different sugar molecules can be caramelized at different temperatures, e.g., fructose caramelizes at 110 ºC while glucose caramelizes at 160 ºC. The sugar molecules can be heated at a caramelization temperature described herein for a duration of time until a desired level of caramelization is achieved. For example, the sugar molecules are heated at a caramelization temperature described herein for at least 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, or 48 hours. For example, sugar molecules can be caramelized by heating to 170 ºC for 17 hours. Heating can be performed by heating neat sugars, e.g., solid sugars, or sugars in solution, e.g., in water or another solvent.

The caramelization process produces a mixture of caramelized sugar products. The process includes reactions involving the oxidation of the sugar, the removal of water, and the break down of the sugar and results in hundreds of caramelized sugar products. A recent study has attempted to characterize the chemical composition of caramel, e.g., *see* Golon and Kuhnert, 2012, J. Agric. Food Chem., 60:3266-3274. Caramelized sugar products include one or more of: 1) oligosaccharides comprising the starting sugar molecule; 2) dehydration products of the starting sugar molecule and the oligosaccharides comprising the starting sugar molecule; 3) hydration products of the oligosaccharides comprising the starting sugar molecule; 4) fragmentation products arising from a redox disproportionation reaction of the oligosaccharides comprising the starting sugar molecule; and 5) and aromatic compounds. The caramelized sugar product utilized in the methods disclosed herein for inducing production of proteins e.g., biomass degrading enzymes in a host cell can be any of the caramelized sugar products described herein, or a combination thereof. Without wishing to be bound by theory, it is believed that oligosaccharides produced by the caramelization process plays a role in inducing the production of proteins, e.g., biomass degrading enzymes in microorganisms. It is believed that the described oligosaccharides perform two roles. First, it is believed that the

oligosaccharides can partially be utilized by the organism, thus growing cell mass. Second, the oligosaccharides may trick the organism into producing enzymes so that the organism can break down the oligosaccharides to release more monosaccharides that will enhance cell growth. Enhanced cell growth gives more protein.

In an embodiment, the caramelized sugar product comprises one or more oligosaccharides comprising the starting sugar molecule, where the oligosaccharides comprise two, three, four, five, or six units of the starting sugar molecule, or a portion thereof. The oligosaccharides comprising the starting sugar molecules are formed through unselective glycosidic bond formation. For example, caramelized glucose comprises oligosaccharides comprising 2, 3, 4, 5, or 6 glucose molecules, or a combination thereof, e.g., disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, or hexasaccharides comprising glucose, or a combination thereof. In another example, caramelized maltose comprises oligosaccharides comprising 2, 3, 4, 5, or 6 maltose molecules, e.g., disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, or hexasaccharides comprising maltose, or a combination thereof. In another example, caramelized xylose comprises oligosaccharides comprising 2, 3, 4, 5, or 6 xylose molecules, e.g., disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, or hexasaccharides comprising xylose, or a combination thereof. In yet another example, caramelized lactose comprises oligosaccharides comprising 2, 3, 4, 5, or 6 lactose molecules, or a combination thereof, e.g., disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, or hexasaccharides comprising lactose repeat units, or a combination thereof.

In an embodiment, the caramelized sugar product comprises a mixture of oligosaccharides comprising the starting sugar molecules, e.g., more than one, two, three, four, or five species of oligosaccharides. In embodiments where the caramelized sugar product comprises more than one species of oligosaccharides, each species of oligosaccharides may be present at different ratios compared to the remaining oligosaccharide species. In such embodiments wherein the caramelized sugar product comprises more than one species of oligosaccharides, the trisaccharides most abundant species of oligosaccharides. In embodiments wherein the caramelized sugar product comprises disaccharides and trisaccharides, the disaccharides are the second most abundant species of oligosaccharides. In embodiments wherein the caramelized sugar product comprises disaccharides, trisaccharides, and tetrasaccharides, tetrasaccharides are the third most abundant species of oligosaccharides.

In an embodiment, the caramelized sugar product comprises one or more dehydration products of the starting sugar molecule and/or the oligosaccharides comprising the starting sugar molecule. Dehydration products can comprise the loss of 1, 2, 3, 4, 5, 6, 7, or 8 water molecules, depending on the number of monosaccharide units. Loss of water molecules may occur at the same saccharide moiety or can be distributed over two or more different saccharide moieties, e.g., of an oligosaccharide comprising the starting sugar molecule. For example, for glucose, up to 7 dehydration products can be obtained by caramelization as a result of a loss of a single water molecule.

In an embodiment, the caramelized sugar product comprises one or more hydration products of the oligosaccharides comprising the starting sugar molecule. Hydration products can comprise one or two additional water molecules added to an oligosaccharide comprising the starting sugar molecule.

In an embodiment, the caramelized sugar product comprises one or more fragmentation products, or redox disproportionation products, of the oligosaccharides comprising the starting sugar molecule. The oligosaccharides comprising the starting sugar molecule may be oxidized and/or reduced. In an embodiment, an oligosaccharide comprising the starting sugar molecule is simultaneously reduced and oxidized.

In an embodiment, the caramelized sugar product comprises one or more aromatic products. Aromatic products include aromatic dye molecules that confer the brown color of caramel. Analysis of caramelized glucose and fructose indicated that the aromatic dye molecules may differ between different starting sugar molecules (Golon et al., 2012). Aromatic products can also include the aromatic flavor molecules that confer the flavor of caramel, e.g., the "butterscotch" flavor. Examples of aromatic flavor molecules include diacetyl furans, e.g., hydroxymethylfurfural (HMF) and hydroxyacetylfuran (HAF), furanones, e.g., hydroxydimethylfuranone (HDF), dihydroxydimethylfuranone (DDF), and maltol from disaccharides and hydroxymaltol from monosaccharides.

The chemical composition of a caramelized sugar can be determined by mass spectrometry. For example, a caramelized sugar can be prepared by heating a starting sugar molecule, e.g., glucose, at 170 °C for 17 hours or 180 ° for 2 hours. The caramelized sugar is then dissolved in water or a mixture of methanol/water (1:1, v/v, 1 ml), and analyzed by mass spectrometry. Examples of mass spectrometry techniques that can be used to analyze the caramelized sugar samples include MALDI-TOF, micrOTOF and direct infusion ion trap mass

spectrometry (ESI-TOF-MS), liquid chromatography – mass spectrometry (LC-MS or HPLC-MS), or liquid chromatography – time of flight mass spectrometry (LC-TOF or HPLC-TOF).

The composition comprising a caramelized sugar product for inducing production of a protein, e.g., a biomass degrading enzyme in a host cell or microorganism can further comprise one or more agents known in the art that can induce production of a protein, e.g., a biomass degrading enzyme. Examples of such agents include, but are not limited to, cellobiose (β-D-glucopyranosyl-(1→4)-β-D-glucopyranose), β-cellobiono-1, 5-lactone (β-D-glucopyranosyl-(1→4)-D-glucono-1,5-lactone), lactose (β-D-galactopyranosyl -(1→4)-D-glucose), sophorose (2-O-β-D-glucopyranosyl-α-D-glucose), D-xylose, xylobiose, galactose, l-arabitol and l-sorbose (Shmoll and Kubicek, 2003, Acta Microbiol Immunol Hung. 7:125-145; El-Gogary et al, 1989, Proc Natl Acad Sci USA, 7:6138-6141; Aro et al., 2005, FEMS Microbiol. Rev. 29:719-739; and Nogawa et al., 2001, Curr Genet. 7:329-334).

Also provided herein are compositions comprising a caramelized sugar product for inducing production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme for use in any of the methods described herein. The composition can further comprise an inducer biomass described herein, and/or additional agents known in the art that induce production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme.

In embodiments, the composition comprising a caramelized sugar product for inducing production of proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes in a host cell or microorganism may further comprise an inducer biomass described herein. In some embodiments, the inducer biomass material may be the same material that can be processed to generate various products, such as hydrogen, sugars, and alcohols. An inducer biomass can be a starchy material comprising cellulose, and is also referred to herein as a cellulose inducer. Suitable examples of inducer biomass are described further herein. The inducer biomass may be pretreated to reduce recalcitrance by any of the treatment methods described herein, e.g., exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, and/or freeze grinding.

Use of a composition comprising a caramelized sugar product to induce production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme can result in an increase in the amount of protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme produced. In an embodiment, the increase in the amount of biomass degrading enzyme

produced by contacting a host cell or microorganism with a composition comprising a caramelized sugar product is at least 1 fold, 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1. 8 fold 1.9 fold, 2 fold, 2.1 fold, 2.2 fold, 2.3 fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, or 3 fold more, as compared to the amount of biomass degrading enzyme produced without use of a composition comprising the caramelized sugar product. In an embodiment, the amount of biomass degrading enzyme produced by contacting a host cell or microorganism with a composition comprising a caramelized sugar product is at least 1%, 2%, 5%, 10%, 25%, 50%, 75%, 100%, 200%, 300%, 400%, or 500% more than the amount of biomass degrading enzyme produced without use of a composition comprising the caramelized sugar product or compared to the amount of biomass degrading enzyme produced by using an inducer biomass.

Use of a composition comprising a caramelized sugar product and an inducer biomass can result in an increase in the amount of protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme produced. In an embodiment, the increase in the amount of biomass degrading enzyme produced by contacting a host cell or microorganism with a composition comprising a caramelized sugar product and an inducer biomass is at least 1 fold, 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1. 8 fold 1.9 fold, 2 fold, 2.1 fold, 2.2 fold, 2.3 fold, 2.4 fold, 2.5 fold, 2.6 fold, 2.7 fold, 2.8 fold, 2.9 fold, or 3 fold more, as compared to the amount of biomass degrading enzyme produced by using an inducer biomass alone or a caramelized sugar product alone. In an embodiment, the amount of biomass degrading enzyme produced by contacting a host cell or microorganism with a composition comprising a caramelized sugar product and an inducer biomass is at least 1%, 2%, 5%, 10%, 25%, 50%, 75%, 100%, 200%, 300%, 400%, or 500% more than the amount of biomass degrading enzyme produced by using an inducer biomass alone or a caramelized sugar product alone.

In some embodiments, the combination of a caramelized sugar product and an inducer biomass results in a synergistic increase in the amount of protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme produced. In a synergistic effect, the observed effect from using a combination of two or more induction compositions is greater than the sum of the effect from using each individual induction composition separately. In an embodiment, a composition comprising a caramelized sugar product and an inducer biomass described herein can induce a synergistic effect in the biomass degrading enzyme production of a microorganism, and results in a yield of biomass degrading enzyme that is greater than the sum of the yields produced from

inducing the microorganism with caramelized sugar product alone and the inducer biomass alone. In an embodiment, the amount of biomass degrading enzyme produced by contacting a host cell or microorganism with a composition comprising a caramelized sugar product and an inducer biomass is at least 1%, 2%, 5%, 10%, 25%, 50%, 75%, 100%, 200%, 300%, 400%, or 500% more than the sum of the amount of biomass degrading enzyme produced by using a caramelized sugar product alone and an inducer biomass alone.

The amount or titer of a protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme produced by a microorganism can be determined using assays described in the examples described herein. For example, the amount of biomass degrading enzyme produced can be determined by using a Bradford assay and/or a nanodrop apparatus. For nanodrop protein quantification, the molar extinction coefficient can be estimated by inserting the amino acid sequence of the biomass degrading enzyme into the ExPASy ProtParam online tool. Activity assays known in the art can also be performed to determine the activity of the biomass degrading enzyme produced.

Inducing Production of Biomass Degrading Enzymes

The present disclosure provides compositions and methods for inducing the production of a protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme. In embodiments, a composition comprising a caramelized sugar product described herein can be introduced to a microorganism or host cell under sufficient conditions for production of the biomass degrading enzyme. As used herein, producing or production of a biomass degrading enzyme by a microorganism includes the expression, translation, and/or secretion of the biomass degrading enzyme.

Microorganisms and host cells

The microorganism, or host cell, suitable for use in the present disclosure is capable of producing a protein, e.g., an enzyme, e.g., an amylase or a biomass degrading enzyme described herein. In an embodiment, the microorganism naturally produces a biomass degrading enzyme, e.g., expresses an endogenous biomass degrading enzyme. In an embodiment, the microorganism is genetically modified to produce a biomass degrading enzyme, e.g., to express a heterologous biomass degrading enzyme. In such embodiments, a nucleic acid encoding a heterologous biomass degrading enzyme is introduced to the

microorganism using standard methods known in the art, e.g., by electroporation, transfection, or transduction. The heterologous biomass degrading enzyme may be a biomass degrading enzyme that is naturally produced in a different microorganism, or may be a modified biomass degrading enzyme comprising a different amino acid sequence or different function and/or activity, e.g., increased or decreased activity, from that of the corresponding naturally occurring biomass degrading enzyme.

The microorganism can be a fungus, a bacterium, a protozoan, a yeast, a synthetic organism or a semi-synthetic organism that produces one or more proteins, e.g., one or more enzymes, such as one or more amylases or biomass degrading enzymes. In an embodiment, the microorganism is from a species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma*.

In an embodiment, the microorganism is selected from *Aspergillus*, *Humicola insolens (Scytalidium thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum, Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei,* or *Trichoderma koningii.*

In embodiments, the microorganism is a fungus, e.g., a filamentous fungus. In an embodiment, the microorganism is *Trichoderma reesei* or any industrial strain or variant thereof. For example, the microorganism can be *T. reesei* QM6a, *T. reesei* RL-P37, *T. reesei* MCG-80, *T. reesei* RUTC30, *T. reesei* RUT-NG14, *T. reesei* PC3-7, or *T. reesei* QM9414.

Biomass Degrading Enzymes

Provided herein are compositions and methods for inducing production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme in a microorganism. The biomass degrading enzyme can be naturally expressed by the microorganism. The biomass degrading enzyme can be a cellulase (e.g., a cellobiase, a cellobiohydrolase, or an endoglucanase); a hemicellulase (e.g., a xylanase), or a ligninase, or any combination thereof.

In an embodiment, the biomass degrading enzyme is a cellulase. Cellulase collectively refers to enzymes that catalyze cellulolysis, or the decomposition of cellulose and related

polysaccharides into monosaccharides, e.g., glucose or beta-glucose, or shorter polysaccharides and oligosaccharides. Cellulases are commonly produced by fungi, bacteria, and other protozoans. Examples of cellulases include cellobiases, cellobiohydrolases (exoglucanases), and endoglucanases.

In an embodiment, the biomass degrading enzyme is a cellobiase. A cellobiase is an enzyme that hydrolyzes beta-1,4 bonds in its substrate, e.g., cellobiose, to release two glucose molecules. Cellobiose is a water soluble 1,4-linked dimer of glucose. In an embodiment, the biomass degrading enzyme is Cel3a. Cel3a (also known as BglI) is a cellobiase that was identified in *Trichoderma reesei*.

In an embodiment, the biomass degrading enzyme is a cellobiohydrolase, also known as exoglucanase or avicelase. A cellobiohydrolase catalyzes the hydrolysis of 1-4-beta-D-glucosidic linkages in oligosaccharides containing that linkage, e.g., cellulose and cellotetraose, thereby releasing cellobiose from the non-reducing ends of the chains. Examples of cellobiohydrolases include cellobiohydrolase I (CBHI) and cellobiohydrolase II (CBHII) from *Trichoderma reesei*.

In an embodiment, the biomass degrading enzyme is an endoglucanase. An endoglucanase is an enzyme that catalyzes the hydrolysis of cellulose. Specifically, the endoglucanases cleave the internal bonds of the cellulose chain. Endoglucanases are produced by fungi, bacteria, and protozoans. Endoglucanases are also known as beta-1-4 endoglucanase, 4-beta-D-glucan cellobiohydrolase, exo-cellobiohydrolase, beta-1,4-glucan cellobiohydrolase, beta-1,4-glucan cellobiosylhydrolase, 1,4-beta-glucan cellobiosidase, C1 cellulase, cellobiohydrolase I, cellobiohydrolase, exo-beta-1,4-glucan cellobiohydrolase, 1,4-beta-D-glucan cellobiohydrolase, or cellobiosidase. Examples of endoglucanases include Cel5A, Cel5B, Cel7B, Cel12A, Cel45A, Cel61A, Cel61B, and Cel74A from *Trichoderma reesei.*

In an embodiment, the biomass degrading enzyme is a hemicellulase. A hemicellulase collectively refers to enzymes that hydrolyze hemicelluloses, e.g., various components of cell walls in plants with the exception of cellulose, e.g., xylans, glucans, galactans, mannans, and pentosans. Hemicellulases include xylanases and galactanases.

In an embodiment, the biomass degrading enzyme is a xylanase. Xylanases are also known as endo-(1-4)-beta-xylan 4-xylanohydrolase, endo-1,4-xylanase, endo-1,4-beta-xylanase, beta-1,4-xylanase, endo-1,4-beta-D-xylanase, 1,4-beta-xylan xylanohydrolase, beta-xylanase, beta-1,4-xylan xylanohydrolase, beta-D-xylanase. A xylanase breaks down a

component of plant cell walls called hemicellulose, e.g., degrades polysaccharides, such as xylan, e.g., beta-1,4-xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan, to release xylose. Examples of xylanases include Xyn1, Xyn2, and Xyn3 from *Trichoderma reesei*; and TERTU_1599, TERTU_3603, TERTU_2546, and TERTU_4506 from *Terendinibacter turnerae* T7901.

In an embodiment, the biomass degrading enzyme is a ligninase. A ligninase is an enzyme that breaks down lignin, which is a complex polymer of aromatic alcohols known as monolignols and plays an integral part of the secondary cell walls of plants and some algae. Ligninases include lignin peroxidases, 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen-peroxide oxidoreductase, diarylpropane oxygenase, ligninase I, diarylpropane peroxidase, LiP, hydrogen-peroxide oxidoreductase (C-C-bond-cleaving), and some laccases. Examples of ligninases include CIP2 from *Trichoderma reesei*; LPOA, GLG2, GLG4, LIPA, GLG5, GLG3, GLG6, and LIPB from *Phanerochaete chrysosporium*; ligninase-3 from *Phelbia radiate*; Ligninase A and B from *Coriolus versicolor*; and LPG I and LPGIV *Coriolus versicolor*.

In embodiments, the methods described herein are used to induce production of one or more cellulases or hemicellulases, e.g., one or more of a cellobiase, a cellobiohydrolase, an endoglucanase, a xylanase, and/or a ligninase. In an embodiment, the methods described herein are used to induce production of a mixture of biomass degrading enzymes comprising any combination of one or more of a cellobiase, a cellobiohydrolase, an endoglucanase, a xylanase, and/or a ligninase. In embodiments, the mixture of biomass degrading enzymes comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 15, or at least 20 biomass degrading enzymes, e.g., biomass degrading enzymes described herein.

Exemplary biomass degrading enzymes are listed in Table 1.

Table 1. Examples of Biomass Degrading Enzymes

Protein	MW, kDa	no AA's	th. pI	no. Cysteines	Organism
B2AF03	87.1	800	5.94	10	*Podospora anserina*
CIP1	32.9	316	4.93	8	*Trichoderma reesei*
CIP2	48.2	460	7.0	12	*Trichoderma reesei*

Cel1a	52.2	466	5.3	5	*Trichoderma reesei*
Cel3a	78.4	744	6.3	6	*Trichoderma reesei*
Cel5a	44.1	418	4.9	12	*Trichoderma reesei*
Cel6a	49.6	471	5.1	12	*Trichoderma reesei*
Cel7a	54.1	514	4.6	24	*Trichoderma reesei*
Cel7b	48.2	459	4.7	22	*Trichoderma reesei*
Cel12a	25.1	234	6.6	2	*Trichoderma reesei*
Cel45a	24.4	242	4.2	16	*Trichoderma reesei*
Cel74a	87.1	838	5.4	4	*Trichoderma reesei*
paMan5a	41.1	373	7.0	6	*Podospora anserina*
paMan26a	51.7	469	4.7	1	*Podospora anserina*
Swollenin	51.5	493	4.8	28	*Trichoderma reesei*

In an embodiment, the biomass degrading enzyme produced by the methods described herein is Cel3a, e.g., a Cel3a from *T. reesei*. In an embodiment, the mixture of biomass degrading enzymes comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the biomass degrading enzymes listed in Table 1.

The amino acid sequences for the biomass degrading enzymes listed in Table 1 are provided below.

B2AF03 (Podospora anserina)

MKSSVFWGASLTSAVVRAIDLPFQFYPNCVDDLLSTNQVCNTTLSPPERAAALVAALTPEEKL
QNIVSKSLGAPRIGLPAYNWWSEALHGVAYAPGTQFWQGDGPFNSSTSFPMPLLMAATFDDEL
LEKIAEVIGIEGRAFGNAGFSGLDYWTPNVNPFKDPRWGRGSETPGEDVLLVKRYAAAMIKGL
EGPVPEKERRVVATCKHYAANDFEDWNGATRHNFNAKISLQDMAEYYFMPFQQCVRDSRVGSI
MCAYNAVNGVPSCASPYLLQTILREHWNWTEHNNYITSDCEAVLDVSLNHKYAATNAEGTAIS
FEAGMDTSCEYEGSSDIPGAWSQGLLKESTVDRALLRLYEGIVRAGYFDGKQSLYSSLGWADV
NKPSAQKLSLQAAVDGTVLLKNDGTLPLSDLLDKSRPKKVAMIGFWSDAKDKLRGGYSGTAAY
LHTPAYAASQLGIPFSTASGPILHSDLASNQSWTDNAMAAAKDADYILYFGGIDTSAAGETKD
RYDLDWPGAQLSLINLLTTLSKPLIVLQMGDQLDNTPLLSNPKINAILWANWPGQDGGTAVME
LVTGLKSPAGRLPVTQYPSNFTELVPMTDMALRPSAGNSQLGRTYRWYKTPVQAFGFGLHYTT
FSPKFGKKFPAVIDVDEVLEGCDDKYLDTCPLPDLPVVVENRGNRTSDYVALAFVSAPGVGPG

PWPIKTLGAFTRLRGVKGGEKREGGLKWNLGNLARHDEEGNTVVYPGKYEVSLDEPPKARLRF
EIVRGGKGKGKVKGKGKAAQKGGVVLDRWPKPPKGQEPPAIERV (SEQ ID NO: 1)

CIP1 (Trichoderma reesei)

MVRRTALLALGALSTLSMAQISDDFESGWDQTKWPISAPDCNQGGTVSLDTTVAHSGSNSMKV
VGGPNGYCGHIFFGTTQVPTGDVYVRAWIRLQTALGSNHVTFIIMPDTAQGGKHLRIGGQSQV
LDYNRESDDATLPDLSPNGIASTVTLPTGAFQCFEYHLGTDGTIETWLNGSLIPGMTVGPGVD
NPNDAGWTRASYIPEITGVNFGWEAYSGDVNTVWFDDISIASTRVGCGPGSPGGPGSSTTGRS
STSGPTSTSRPSTTIPPPTSRTTTATGPTQTHYGQCGGIGYSGPTVCASGTTCQVLNPYYSQC
L (SEQ ID NO: 2)

CIP2 (Trichoderma reesei)

MASRFFALLLLAIPIQAQSPVWGQCGGIGWSGPTTCVGGATCVSYNPYYSQCIPSTQASSSIA
STTLVTSFTTTTATRTSASTPPASSTGAGGATCSALPGSITLRSNAKLNDLFTMFNGDKVTTK
DKFSCRQAEMSELIQRYELGTLPGRPSTLTASFSGNTLTINCGEAGKSISFTVTITYPSSGTA
PYPAIIGYGGGSLPAPAGVAMINFNNDNIAAQVNTGSRGQGKFYDLYGSSHSAGAMTAWAWGV
SRVIDALELVPGARIDTTKIGVTGCSRNGKGAMVAGAFEKRIVLTLPQESGAGGSACWRISDY
LKSQGANIQTASEIIGEDPWFSTTFNSYVNQVPVLPFDHHSLAALIAPRGLFVIDNNIDWLGP
QSCFGCMTAAHMAWQALGVSDHMGYSQIGAHAHCAFPSNQQSQLTAFVQKFLLGQSTNTAIFQ
SDFSANQSQWIDWTTPTLS (SEQ ID NO: 3)

Cel1a (Trichoderma reesei)

MLPKDFQWGFATAAYQIEGAVDQDGRGPSIWDTFCAQPGKIADGSSGVTACDSYNRTAEDIAL
LKSLGAKSYRFSISWSRIIPEGGRGDAVNQAGIDHYVKFVDDLLDAGITPFITLFHWDLPEGL
HQRYGGLLNRTEFPLDFENYARVMFRALPKVRNWITFNEPLCSAIPGYGSGTFAPGRQSTSEP
WTVGHNILVAHGRAVKAYRDDFKPASGDGQIGIVLNGDFTYPWDAADPADKEAAERRLEFFTA
WFADPIYLGDYPASMRKQLGDRLPTFTPEERALVHGSNDFYGMNHYTSNYIRHRSSPASADDT
VGNVDVLFTNKQGNCIGPETQSPWLRPCAAGFRDFLVWISKRYGYPPIYVTENGTSIKGESDL
PKEKILEDDFRVKYYNEYIRAMVTAVELDGVNVKGYFAWSLMDNFEWADGYVTRFGVTYVDYE
NGQKRFPKKSAKSLKPLFDELIAAA (SEQ ID NO: 4)

Cel3a (Trichoderma reesei)

MRYRTAAALALATGPFARADSHSTSGASAEAVVPPAGTPWGTAYDKAKAALAKLNLQDKVGIV
SGVGWNGGPCVGNTSPASKISYPSLCLQDGPLGVRYSTGSTAFTPGVQAASTWDVNLIRERGQ
FIGEEVKASGIHVILGPVAGPLGKTPQGGRNWEGFGVDPYLTGIAMGQTINGIQSVGVQATAK
HYILNEQELNRETISSNPDDRTLHELYTWPFADAVQANVASVMCSYNKVNTTWACEDQYTLQT
VLKDQLGFPGYVMTDWNAQHTTVQSANSGLDMSMPGTDFNGNNRLWGPALTNAVNSNQVPTSR
VDDMVTRILAAWYLTGQDQAGYPSFNISRNVQGNHKTNVRAIARDGIVLLKNDANILPLKKPA
SIAVVGSAAIIGNHARNSPSCNDKGCDDGALGMGWGSGAVNYPYFVAPYDAINTRASSQGTQV
TLSNTDNTSSGASAARGKDVAIVFITADSGEGYITVEGNAGDRNNLDPWHNGNALVQAVAGAN
SNVIVVVHSVGAIILEQILALPQVKAVVWAGLPSQESGNALVDVLWGDVSPSGKLVYTIAKSP
NDYNTRIVSGGSDSFSEGLFIDYKHFDDANITPRYEFGYGLSYTKFNYSRLSVLSTAKSGPAT
GAVVPGGPSDLFQNVATVTVDIANSGQVTGAEVAQLYITYPSSAPRTPPKQLRGFAKLNLTPG
QSGTATFNIRRRDLSYWDTASQKWVVPSGSFGISVGASSRDIRLTSTLSVA (SEQ ID
NO: 5)

Cel5a (Trichoderma reesei)

MNKSVAPLLLAASILYGGAAAQQTVWGQCGGIGWSGPTNCAPGSACSTLNPYYAQCIPGATTI
TTSTRPPSGPTTTTRATSTSSSTPPTSSGVRFAGVNIAGFDFGCTTDGTCVTSKVYPPLKNFT
GSNNYPDGIGQMQHFVNDDGMTIFRLPVGWQYLVNNNLGGNLDSTSISKYDQLVQGCLSLGAY
CIVDIHNYARWNGGIIGQGGPTNAQFTSLWSQLASKYASQSRVWFGIMNEPHDVNINTWAATV
QEVVTAIRNAGATSQFISLPGNDWQSAGAFISDGSAAALSQVTNPDGSTTNLIFDVHKYLDSD
NSGTHAECTTNNIDGAFSPLATWLRQNNRQAILTETGGGNVQSCIQDMCQQIQYLNQNSDVYL
GYVGWGAGSFDSTYVLTETPTGSGNSWTDTSLVSSCLARK (SEQ ID NO: 6)

Cel6a (Trichoderma reesei)

MIVGILTTLATLATLAASVPLEERQACSSVWGQCGGQNWSGPTCCASGSTCVYSNDYYSQCLP
GAASSSSSTRAASTTSRVSPTTSRSSSATPPPGSTTTRVPPVGSGTATYSGNPFVGVTPWANA
YYASEVSSLAIPSLTGAMATAAAAVAKVPSFMWLDTLDKTPLMEQTLADIRTANKNGGNYAGQ
FVVYDLPDRDCAALASNGEYSIADGGVAKYKNYIDTIRQIVVEYSDIRTLLVIEPDSLANLVT
NLGTPKCANAQSAYLECINYAVTQLNLPNVAMYLDAGHAGWLGWPANQDPAAQLFANVYKNAS
SPRALRGLATNVANYNGWNITSPPSYTQGNAVYNEKLYIHAIGPLLANHGWSNAFFITDQGRS
GKQPTGQQQWGDWCNVIGTGFGIRPSANTGDSLLDSFVWVKPGGECDGTSDSSAPRFDSHCAL
PDALQPAPQAGAWFQAYFVQLLTNANPSFL (SEQ ID NO: 7)

Cel7a (Trichoderma reesei)

MYRKLAVISAFLATARAQSACTLQSETHPPLTWQKCSSGGTCTQQTGSVVIDANWRWTHATNS
STNCYDGNTWSSTLCPDNETCAKNCCLDGAAYASTYGVTTSGNSLSIGFVTQSAQKNVGARLY
LMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGALYFVSMDADGGVSKYPTNTAGAKYGTGYC
DSQCPRDLKFINGQANVEGWEPSSNNANTGIGGHGSCCSEMDIWEANSISEALTPHPCTTVGQ
EICEGDGCGGTYSDNRYGGTCDPDGCDWNPYRLGNTSFYGPGSSFTLDTTKKLTVVTQFETSG
AINRYYVQNGVTFQQPNAELGSYSGNELNDDYCTAEEAEFGGSSFSDKGGLTQFKKATSGGMV
LVMSLWDDYYANMLWLDSTYPTNETSSTPGAVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFG
PIGSTGNPSGGNPPGGNPPGTTTTRRPATTTGSSPGPTQSHYGQCGGIGYSGPTVCASGTTCQ
VLNPYYSQCL (SEQ ID NO: 8)

Cel7b (Trichoderma reesei)

MAPSVTLPLTTAILAIARLVAAQQPGTSTPEVHPKLTTYKCTKSGGCVAQDTSVVLDWNYRWM
HDANYNSCTVNGGVNTTLCPDEATCGKNCFIEGVDYAASGVTTSGSSLTMNQYMPSSSGGYSS
VSPRLYLLDSDGEYVMLKLNGQELSFDVDLSALPCGENGSLYLSQMDENGGANQYNTAGANYG
SGYCDAQCPVQTWRNGTLNTSHQGFCCNEMDILEGNSRANALTPHSCTATACDSAGCGFNPYG
SGYKSYYGPGDTVDTSKTFTIITQFNTDNGSPSGNLVSITRKYQQNGVDIPSAQPGGDTISSC
PSASAYGGLATMGKALSSGMVLVFSIWNDNSQYMNWLDSGNAGPCSSTEGNPSNILANNPNTH
VVFSNIRWGDIGSTTNSTAPPPPPASSTTFSTTRRSSTTSSSPSCTQTHWGQCGGIGYSGCKT
CTSGTTCQYSNDYYSQCL (SEQ ID NO: 9)

Cel12a (Trichoderma reesei)

MKFLQVLPALIPAALAQTSCDQWATFTGNGYTVSNNLWGASAGSGFGCVTAVSLSGGASWHAD
WQWSGGQNNVKSYQNSQIAIPQKRTVNSISSMPTTASWSYSGSNIRANVAYDLFTAANPNHVT
YSGDYELMIWLGKYGDIGPIGSSQGTVNVGGQSWTLYYGYNGAMQVYSFVAQTNTTNYSGDVK
NFFNYLRDNKGYNAAGQYVLSYQFGTEPFTGSGTLNVASWTASIN (SEQ ID NO: 10)

Cel45a (Trichoderma reesei)

MKATLVLGSLIVGAVSAYKATTTRYYDGQEGACGCGSSSGAFPWQLGIGNGVYTAAGSQALFD
TAGASWCGAGCGKCYQLTSTGQAPCSSCGTGGAAGQSIIVMVTNLCPNNGNAQWCPVVGGTNQ

YGYSYHFDIMAQNEIFGDNVVVDFEPIACPGQAASDWGTCLCVGQQETDPTPVLGNDTGSTPP
GSSPPATSSSPPSGGGQQTLYGQCGGAGWTGPTTCQAPGTCKVQNQWYSQCLP (SEQ ID
NO: 11)

Cel74a (Trichoderma reesei)

MKVSRVLALVLGAVIPAHAAFSWKNVKLGGGGGFVPGIIFHPKTKGVAYARTDIGGLYRLNAD
DSWTAVTDGIADNAGWHNWGIDAVALDPQDDQKVYAAVGMYTNSWDPSNGAIIRSSDRGATWS
FTNLPFKVGGNMPGRGAGERLAVDPANSNIIYFGARSGNGLWKSTDGGVTFSKVSSFTATGTY
IPDPSDSNGYNSDKQGLMWVTFDSTSSTTGGATSRIFVGTADNITASVYVSTNAGSTWSAVPG
QPGKYFPHKAKLQPAEKALYLTYSDGTGPYDGTLGSVWRYDIAGGTWKDITPVSGSDLYFGFG
GLGLDLQKPGTLVVASLNSWWPDAQLFRSTDSGTTWSPIWAWASYPTETYYYSISTPKAPWIK
NNFIDVTSESPSDGLIKRLGWMIESLEIDPTDSNHWLYGTGMTIFGGHDLTNWDTRHNVSIQS
LADGIEEFSVQDLASAPGGSELLAAVGDDNGFTFASRNDLGTSPQTVWATPTWATSTSVDYAG
NSVKSVVRVGNTAGTQQVAISSDGGATWSIDYAADTSMNGGTVAYSADGDTILWSTASSGVQR
SQFQGSFASVSSLPAGAVIASDKKTNSVFYAGSGSTFYVSKDTGSSFTRGPKLGSAGTIRDIA
AHPTTAGTLYVSTDVGIFRSTDSGTTFGQVSTALTNTYQIALGVGSGSNWNLYAFGTGPSGAR
LYASGDSGASWTDIQGSQGFGSIDSTKVAGSGSTAGQVYVGTNGRGVFYAQGTVGGGTGGTSS
STKQSSSSTSSASSSTTLRSSVVSTTRASTVTSSRTSSAAGPTGSGVAGHYAQCGGIGWTGPT
QCVAPYVCQKQNDYYYQCV (SEQ ID NO: 12)

paMan5a (Podospora anserina)

MKGLFAFGLGLLSLVNALPQAQGGGAAASAKVSGTRFVIDGKTGYFAGTNSYWIGFLTNNRDV
DTTLDHIASSGLKILRVWGFNDVNNQPSGNTVWFQRLASSGSQINTGPNGLQRLDYLVRSAET
RGIKLIIALVNYWDDFGGMKAYVNAFGGTKESWYTNARAQEQYKRYIQAVVSRYVNSPAIFAW
ELANEPRCKGCNTNVIFNWATQISDYIRSLDKDHLITLGDEGFGLPGQTTYPYQYGEGTDFVK
NLQIKNLDFGTFHMYPGHWGVPTSFGPGWIKDHAAACRAAGKPCLLEEYGYESDRCNVQKGWQ
QASRELSRDGMSGDLFWQWGDQLSTGQTHNDGFTIYYGSSLATCLVTDHVRAINALPA
(SEQ ID NO: 13)

paMan26a (Podospora anserina)

MVKLLDIGLFALALASSAVAKPCKPRDGPVTYEAEDAILTGTTVDTAQVGYTGRGYVTGFDEG
SDKITFQISSATTKLYDLSIRYAAIYGDKRTNVVLNNGAVSEVFFPAGDSFTSVAAGQVLLNA
GQNTIDIVNNWGWYLIDSITLTPSAPRPPHDINPNLNNPNADTNAKKLYSYLRSVYGNKIISG
QQELHHAEWIRQQTGKTPALVAVDLMDYSPSRVERGTTSHAVEDAIAHHNAGGIVSVLWHWNA
PVGLYDTEENKWWSGFYTRATDFDIAATLANPQGANYTLLIRDIDAIAVQLKRLEAAGVPVLW
RPLHEAEGGWFWWGAKGPEPAKQLWDILYERLTVHHGLDNLIWVWNSILEDWYPGDDTVDILS
ADVYAQGNGPMSTQYNELIALGRDKKMIAAAEVGAAPLPGLLQAYQANWLWFAVWGDDFINNP
SWNTVAVLNEIYNSDYVLTLDEIQGWRS (SEQ ID NO: 14)

Swollenin (Trichoderma reesei)

MAGKLILVALASLVSLSIQQNCAALFGQCGGIGWSGTTCCVAGAQCSFVNDWYSQCLASTGGN
PPNGTTSSSLVSRTSSASSSVGSSSPGGNSPTGSASTYTTTDTATVAPHSQSPYPSIAASSCG
SWTLVDNVCCPSYCANDDTSESCSGCGTCTTPPSADCKSGTMYPEVHHVSSNESWHYSRSTHF
GLTSGGACGFGLYGLCTKGSVTASWTDPMLGATCDAFCTAYPLLCKDPTGTTLRGNFAAPNGD
YYTQFWSSLPGALDNYLSCGECIELIQTKPDGTDYAVGEAGYTDPITLEIVDSCPCSANSKWC
CGPGADHCGEIDFKYGCPLPADSIHLDLSDIAMGRLQGNGSLTNGVIPTRYRRVQCPKVGNAY
IWLRNGGGPYYFALTAVNTNGPGSVTKIEIKGADTDNWVALVHDPNYTSSRPQERYGSWVIPQ

GSGPFNLPVGIRLTSPTGEQIVNEQAIKTFTPPATGDPNFYYIDIGVQFSQN (SEQ ID NO: 15)

In embodiments, the biomass degrading enzyme comprises an amino acid sequence with at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a biomass degrading enzyme described herein, e.g., listed in Table 1, or a functional fragment thereof, e.g., at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to a biomass degrading enzyme described herein, e.g., listed in Table 1, or a functional fragment thereof.

Percent identity in the context of two or more amino acid or nucleic acid sequences, refers to two or more sequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (e.g., 60% identity, optionally 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides, 100 nucleotides, 150 nucleotides, in length. More preferably, the identity exists over a region that is at least about 200 or more amino acids, or at least about 500 or 1000 or more nucleotides, in length.

For sequence comparison, one sequence typically acts as a reference sequence, to which one or more test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, (1970) Adv. Appl.

Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman, (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Brent et al., (2003) Current Protocols in Molecular Biology).

Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977) Nuc. Acids Res. 25:3389-3402; and Altschul et al., (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

Functional variants may comprise one or more mutations, such that the variant retains some level of activity, e.g., biomass degrading activity, of an enzyme, e.g., a biomass degrading enzyme described herein produced by the microorganism from which the enzyme originates from. In an embodiment, the functional variant has at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% (e.g., at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) of the biomass degrading activity as the corresponding naturally occurring biomass degrading enzyme. In embodiments, the functional variant has at least 200%, at least 300%, at least 400%, at least 500%, at least 1000% or more of the biomass degrading activity as the corresponding naturally occurring biomass degrading enzyme. Biomass degrading activity can be tested using the functional assays known in the art. For example, if the biomass degrading enzyme is a cellulase, then functional assays that measure cellulase activity can be performed.

The mutations present in a functional variant include amino acid substitutions, additions, and deletions. Mutations can be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Mutagenesis can also be achieved through using CRISPR (Clustered regularly–interspaced short palindromic repeats)/Cas systems. The CRISPR/Cas system is naturally found in bacteria and archaea, and has been modified for use in gene editing (silencing, enhancing or mutating specific genes) in eukaryotes such as mice or primates. Wiedenheft *et al.* (2012) *Nature* 482: 331-8. This is

accomplished by introducing into the cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas.

The mutation may be a conservative amino acid substitution, in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the biomass degrading enzyme can be replaced with other amino acids from the same side chain family, and the resultant biomass degrading activity comparable (e.g., at least 80%, 85%, 90%, 95%, or 99% of the biomass degrading activity) to that of the wild-type biomass degrading enzyme. Alternatively, the mutation may be an amino acid substitution in which an amino acid residue is replaced with an amino acid residue having a different side chain.

Such mutations may alter or affect various enzymatic characteristics of the biomass degrading enzyme, e.g., cellobiase, ligninase, endoglucanase, or cellobiohydrolase. For example, such mutations may alter or affect the activity, e.g., the biomass degrading activity, thermostability, optimal pH for reaction, enzyme kinetics, or substrate recognition of the enzyme, e.g., the biomass degrading enzyme. In some embodiments, a mutation increases the biomass degrading activity of the variant in comparison to the biomass degrading enzyme, e.g., a cellulase produced by *T. reesei*. In some embodiments, a mutation increases or decreases the thermostability of the variant in comparison to a wild-type biomass degrading enzyme, e.g., a cellulase produced by *T. reesei*. In an embodiment, a mutation changes the pH range at which the variant optimally performs the biomass degrading reaction in comparison to wild-type biomass degrading enzyme, e.g., a cellulase produced by *T. reesei*. In an embodiment, a mutation increases or decreases the kinetics of the biomass degrading reaction (e.g., k_{cat}, K_M, k_{cat}/K_M, or K_D) in comparison to wild-type biomass degrading enzyme, e.g., a cellulase produced by *T. reesei*. In an embodiment, a mutation increases or decreases the ability of the cellobiase to recognize or bind to the substrate in comparison to wild-type biomass degrading enzyme, e.g., a cellulase produced by *T. reesei*.

Cell Culture and Induction

The microorganism that can produce a protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme can be in a cell culture. A cell culture comprises one or more cells in a cell culture medium. The cell culture medium can be an aqueous cell culture medium comprising components that support cell maintenance, cell viability, cell growth, and/or cell proliferation. Cell culture media can typically comprises physiological salts, e.g., ammonium salt, phosphate salt, potassium salt, magnesium salt, calcium salt, iron salt, manganese salt, zinc salt, or cobalt salt; amino acids; water, and optionally, a carbon source. In an embodiment, a cell culture media suitable for growing a microorganism described herein comprises an ammonium salt, e.g., ammonium sulfate and/or ammonium hydroxide; a potassium salt, e.g., potassium hydroxide; a calcium salt, e.g., calcium chloride; a magnesium salt, e.g., magnesium sulfate; a manganese salt, e.g., manganese sulfate; an iron salt, e.g., iron sulfate; a zinc salt, e.g., zinc sulfate, a cobalt salt, e.g., cobalt chloride, phthalic acid; lactose; antibiotics, e.g., ACETOBAN®; and a carbon source, e.g., glucose. An exemplary growth media is summarized in Table 2 and 3.

The microorganism or cell culture is contacted with, e.g., fed, a carbon source, such as a sugar, to support the growth or proliferation of the microorganism. In an embodiment, the microorganism or cell culture is contacted with, e.g., fed, glucose.

As the microorganism proliferates in culture, the cell culture can be transferred from one container, e.g., a cell culture container, to a larger container to allow and encourage the microorganism to continue to proliferate. For example, the microorganism is contacted with sugar in a first container under suitable conditions, as described herein, such that the microorganism proliferates. The proliferation can be monitored, and once a desired level of growth, e.g., a specific growth phase, or a desired level of proliferation, e.g., as measured by turbidity of the culture or by cell number, the microorganism can be transferred to a second container, where the second container is larger, e.g., by volume, than the first container. Transferring the microorganism to the larger second container allows and encourages the microorganism to continue to proliferate. In embodiments, the microorganism is transferred once, e.g., from a first container to a larger second container. In embodiments, the microorganism is transferred more than once, e.g., two, three, four, five, six, seven, eight, nine, or ten times, wherein for each transfer, the microorganism is transferred into a container that is larger than the container from which the microorganism was transferred from.

Containers suitable for transferring and culturing the microorganisms described herein include any cell culture container known in the art. Examples of suitable containers include, but are not limited to, a cell culture flask, a roller bottle, a bioreactor, or a tank.

Other cell culture conditions appropriate for maintaining cell viability or promoting cell proliferation are known in the art. Cell culture conditions for consideration include pH, temperature, oxygen levels, and movement. The pH of the cell culture, e.g., the media, is generally at physiological pH, e.g., between pH 4-8, or between pH 5-7, e.g., at pH 5, pH 6, or pH 7. The temperature for growth of a microorganism producing a biomass degrading enzyme is generally between 20 and 40°C, e.g., 30°C. In some embodiments, a particular strain of the microorganism may show enhanced proliferation of enzyme production at an elevated temperature, e.g., 32 or 37 °C, or at a lower temperature, e.g., 27 °C. Optimal oxygen levels for growth of a microorganism producing a biomass degrading enzyme is generally between 15 and 30%, e.g., 20%. The cell culture may be stationary or may use movement to promote maintenance or proliferation. For example, the cell culture may be rolled, shaken, or agitated to enhance cell proliferation. The cell culture conditions disclosed herein are merely exemplary, and should not be construed as limiting. Varying cell culture conditions from those explicitly listed herein may be envisioned or experimentally determined, and may depend on the species or strain of microorganism used. Cell culture conditions sufficient for proliferation of the microorganism that can produce a biomass degrading enzyme result in an increase in the cell number of a culture of the microorganism. Cell culture conditions sufficient for the production of a biomass degrading enzyme results in one or more cells of the microorganism producing a biomass degrading enzyme.

Once the cell culture has achieved a desired level of growth, e.g., a specific growth phase or culture volume size, or when the cell culture, e.g., the aqueous portion, is substantially free from the carbon source, e.g., sugar, utilized to stimulate proliferation, the cell culture can be induced to produce a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme described herein. A composition described herein comprising a caramelized sugar product is added e.g., fed, to the microorganism or cell culture that is capable of producing a biomass degrading enzyme, thereby inducing the microorganism to produce the biomass degrading enzyme. In an embodiment, the composition comprising a caramelized sugar product is added to the culture directly. In an embodiment, the composition comprising a caramelized sugar product is added to an enzyme production culture media, comprising

components that support and encourage the production of the protein, e.g., biomass degrading enzyme. The microorganism is then transferred or cultured in the enzyme production culture media. An enzyme production culture media can comprise physiological salts, e.g., ammonium salts, and a composition comprising a caramelized sugar product and/or an inducer biomass, and is adjusted to pH 4-7, e.g., pH 6. In an embodiment, an enzyme production culture media comprises ammonium sulfate, rice bran, and a composition comprising a caramelized sugar product and/or an inducer biomass, e.g., corncob or beeswing, and is adjusted to pH 6, e.g., with 6M ammonium hydroxide.

Production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme can be induced by contacting the microorganism with a combination of a caramelized sugar product and an inducer biomass described herein. The inducer biomass can be a starchy material comprising cellulose. The biomass may also comprise hemicellulose and/or lignin. The inducer biomass can comprise one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof. An agricultural product or waste comprises material that can be cultivated, harvested, or processed for use or consumption, e.g., by humans or animals, or any intermediate, byproduct, or waste that is generated from the cultivation, harvest, or processing methods. Agricultural products or waste include, but are not limited to, sugar cane, jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof. A paper product or waste comprises material that is used to make a paper product, any paper product, or any intermediate, byproduct or waste that is generated from making or breaking down the paper product. Paper products or waste include, but are not limited to, paper, pigmented papers, loaded papers, coated papers, corrugated paper, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof. A forestry product or waste comprises material that is produced by cultivating, harvesting, or processing of wood, or any intermediate, byproduct, or waste that is generated from the cultivation, harvest, or processing of the wood. Forestry products or waste include, but are not limited to, aspen wood, wood from any genus or species of tree, particle board, wood

chips, or sawdust, or a combination thereof. A general waste includes, but is not limited to, manure, sewage, or offal, or a combination thereof.

In an embodiment, a caramelized sugar product and an inducer biomass are added to the microorganism or cell culture simultaneously. The caramelized sugar product and the inducer biomass can be present in the same composition or can be in separate compositions. When the caramelized sugar product and inducer biomass are present in the same composition, the caramelized sugar product and inducer biomass can be components of an enzyme production culture media. In another embodiment, a caramelized sugar product and an inducer biomass are in separate compositions, and are added to the microorganism or cell culture sequentially. For example, a caramelized sugar product can be added to the microorganism or cell culture prior to or after an inducer biomass is added to the microorganism or cell culture. In such sequential induction processes, the duration between the addition of the caramelized sugar product and the addition of an inducer biomass can be hours, e.g., 1, 2, 3, 4, 5, 6, 12, 18, or more hours, or days, e.g., 1, 2, 3, 4, 5, 6, 7 or more days.

A caramelized sugar product can be introduced to the microorganism, e.g., by direct addition to the culture or by enzyme production culture media, twice a day, once a day, every other day, every three days or once a week. The caramelized sugar product can be added at a concentration range of 1-20 g/L, 1-15 g/L, 1-10 g/L, 1-5 g/L, 2-15 g/L, 2-10g/L, 2-5 g/L, 5-20 g/L, 5-15 g/L, 5-10 g/L, 4-5 g/L, 10-20 g/L or 10-15 g/L of microorganism cell culture. The caramelized sugar product can be added at a concentration of 0.5 g/L, 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 15 g/L or 20 g/L or more, of microorganism cell culture. In an embodiment, the caramelized sugar product is added to the microorganism at 4 g/L once per day, or 5 g/L once per day.

An inducer biomass can be introduced to the microorganism, e.g., by direct addition to the culture or by enzyme production culture media, twice a day, once a day, every other day, every 3 days, or once a week. The inducer biomass can be added at a concentration range of 1-20 g/L, 1-15 g/L, 1-10 g/L, 1-5 g/L, 2-15 g/L, 2-10g/L, 2-5 g/L, 5-20 g/L, 5-15 g/L, 5-10 g/L, 10-20 g/L, or 10-15 g/L of microorganism cell culture. The inducer biomass can be added at a concentration of 0.5 g/L, 1 g/L, 2 g/L, 3 g/L, 4 g/L, 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 15 g/L or 20 g/L or more, of microorganism cell culture. In an embodiment, the inducer biomass is added to the microorganism at 5 g/L, once per day.

In embodiments, the concentration of a caramelized sugar product or an inducer biomass used for inducing production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme is greater than or equal to 0.1% weight by volume (w/v), 0.5% w/v, 1% w/v, 2% w/v, or 5% w/v, and less than or equal to 25% w/v, 20% w/v, 15% w/v, and 10% w/v.

The microorganism can be induced to produce a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme for one or more days, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, or 30 or more days. The duration of the induction can depend on the size, e.g., volume or cell number, of the microorganism culture, the microorganism used, or the amount of the protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme needed. In an embodiment, the microorganism is induced to produce a biomass degrading enzyme for 11 or 12 days.

Production of the protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme can be measured from the cell culture by measuring the level of proteins, e.g. biomass degrading enzymes, present in the cell culture that were produced by the cells. For example, the aqueous portion of the culture can be isolated, e.g., by centrifuging the cell culture or an aliquot or sample of the cell culture. A protein assay known in the art, such as the Bradford assay or nanodrop protein quantification, can be used to determine the level or titer of protein, e.g., g/L, in the aqueous portion of the culture. The protein titer indicates the amount of biomass degrading enzyme produced by the microorganism or cell culture. A control sample can be used to normalize for the amount of proteins present in a cell culture that has not been induced to produce a biomass degrading enzyme.

The proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes produced by the microorganism as described herein can be used in biological or industrial processes, such as processing biomass materials described herein into products, e.g., sugar products or biofuels. Methods for process biomass materials into products, e.g., degrading or converting biomass materials into sugars or biofuels, are described further herein. The microorganism, or culture thereof, that has been induced to produce a protein, e.g., a biomass degrading enzyme, as described herein, can be added directly to the biomass to be processed. Alternatively, the biomass to be processed can be added directly to the microorganism or culture that has been induced to produce a biomass degrading enzyme.

A protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme produced by the microorganism as described herein can also be separated or purified prior use in biomass

processing. The protein, e.g., biomass degrading enzyme can be separated from one or more of the following components: the microorganism, e.g., the cells of the microorganism; the caramelized sugar product used to induce enzyme production, e.g., the caramelized sugar product that is remaining after enzyme induction; the inducer biomass used to induce enzyme production, e.g., the inducer biomass that is remaining after enzyme induction; components of the cell culture media, e.g., glucose, physiological salts; and one or more proteins present in the culture that do not have biomass degrading activity. The protein, e.g., biomass degrading enzyme can be purified, such that the biomass degrading enzyme is substantially free of other proteins that do not have biomass degrading activity, cell debris, nucleic acids, e.g., from the microorganism, caramelized sugar product, and/or inducer biomass. Methods for separation or purification of an enzyme are known in the art, and can include centrifugation, filtration, protein fractionation, size exclusion chromatography, affinity chromatography, or any combination thereof.

Converting Biomass into Products

The present invention provides methods and compositions for converting or processing a biomass into a product using a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme, wherein the protein, e.g., enzyme, e.g., amylase or biomass degrading enzyme is produced by contacting a microorganism with a composition comprising a caramelized sugar product, as described herein. Methods for converting a biomass to products, such as sugar products, are known in the art, for example, as described in US Patent Application 2014/0011258, the contents of which are incorporated by reference in its entirety. Briefly, a biomass is optimally pretreated, e.g., to reduce the recalcitrance, and saccharified by a saccharification process that involves incubating the treated biomass with biomass degrading or cellulolytic, enzymes to produce sugar products (e.g., glucose and/or xylose). The sugar products can then be further processed, e.g., by fermentation or distillation, to produce other products. Such products include alcohols (e.g., ethanol, isobutanol, or n-butanol), sugar alcohols (e.g., erythritol, xylitol, or sorbitol), or organic acids (e.g., lactic acid, pyruvic acid, succinic acid).

Products

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, cellobiose, 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 (e.g., lactic acid), 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.

In an embodiment, the product of the methods for converting a biomass provided herein, e.g., comprising using a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme produced as described herein, is a sugar product. In an embodiment, the sugar product is glucose. In an embodiment, the sugar product is xylose. In an embodiment, the sugar product is a mixture of glucose and xylose.

In an embodiment, the product of the methods for converting a biomass provided herein, e.g., comprising using a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme produced as described herein, is an organic acid product. In an embodiment, the organic acid product is lactic acid.

Biomass

The biomass to be processed using the methods described herein is a starchy material and/or a cellulosic material comprising cellulose, e.g., a lignocellulosic material. The biomass may also comprise hemicellulose and/or lignin. The biomass can comprise one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof. An agricultural product or waste comprises material that can be cultivated, harvested, or processed for use or consumption, e.g., by humans or animals, or any intermediate, byproduct, or waste that is generated from the cultivation, harvest, or processing methods. Agricultural products or waste include, but are not limited to, sugar cane, jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof. A paper product or waste comprises material that is used to make a paper product, any paper product, or any intermediate, byproduct or waste that is generated from making or breaking down the paper product. Paper products or waste include, but are not limited to, paper, pigmented papers, loaded papers, coated papers, corrugated paper, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof. A forestry product or waste comprises material that is produced by cultivating, harvesting, or processing of wood, or any intermediate, byproduct, or waste that is generated from the cultivation, harvest, or processing of the wood. Forestry products or waste include, but are not limited to, aspen wood, wood from any genus or species of tree, particle board, wood chips, or sawdust, or a combination thereof. A general waste includes, but is not limited to, manure, sewage, or offal, or a combination thereof.

The biomass to be converted into products can be the same as the inducer biomass. Alternatively, the biomass to be converted into products is different than the inducer biomass.

In one embodiment, the biomass is treated prior to use in the process described herein. For example, the biomass is treated to reduce the recalcitrance of the biomass, to reduce its bulk density, and/or increase its surface area. Suitable biomass treatment process may include, but are not limited to: bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, and freeze grinding. Preferably, the treatment method is bombardment with electrons.

In some embodiments, electron bombardment is performed until the biomass 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 biomass receives a dose a of from about 0.5 Mrad to about 150 Mrad, about 1 Mrad to about 100 Mrad, about 5 Mrad to about 75 Mrad, about 2 Mrad to about 75 Mrad, about 10 Mrad to about 50 Mrad, e.g., about 5 Mrad to about 50 Mrad, about 20 Mrad to about 40 Mrad, about 10 Mrad to about 35 Mrad, or from about 20 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 9 Mrad/pass can in some cases cause thermal degradation of the feedstock material.

The biomass material (e.g., agricultural product or waste, paper product or waste, forestry product or waste, or general waste) 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.

In order to convert the biomass to a form that can be readily processed, the glucan- or xylan-containing cellulose in the biomass can be hydrolyzed to low molecular weight carbohydrates, such as sugars, by a saccharifying agent in a process referred to as saccharification. The saccharifying agent can comprise one or more enzymes, e.g., a biomass degrading enzyme, or acid, or a mixture thereof. 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.

The biomass can be hydrolyzed using an enzyme, e.g., a biomass degrading enzyme, by combining the biomass material(s) and the enzyme in a solvent, e.g., in an aqueous solution. The enzymes can be induced and/or produced according to the methods described herein. In an embodiment, a biomass is hydrolyzed using a biomass degrading enzyme that has been produced by contacting a microorganism with a composition comprising a caramelized sugar product as described herein.

Specifically, the biomass degrading enzyme can be supplied by microorganisms 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).

During saccharification a cellulosic substrate, e.g., of the biomass, 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.

Saccharification

The reduced-recalcitrance biomass is treated with the biomass degrading enzymes discussed above, generally by combining the reduced-recalcitrance biomass and a saccharifying agent, e.g., comprising one or more biomass degrading enzymes, in a fluid medium, e.g., an aqueous solution. In some cases, the biomass 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 Apr. 26, 2012, the entire contents of which are incorporated herein.

Provided herein are methods and compositions for enhancing the production of a protein, e.g., an enzyme, e.g., an amylase or biomass degrading enzyme in a microorganism. The proteins, e.g., enzymes, e.g., amylases or biomass degrading enzyme(s) produced using the induction compositions and methods described herein can be used in the saccharification

process as the saccharifying agent. The saccharifying agent is added directly to a biomass, e.g., a treated biomass, to initiate and perform the saccharification process to produce sugar products.

The saccharification agent may comprise the proteins, e.g., biomass degrading enzyme(s) produced using the induction compositions and methods described herein. The biomass degrading enzyme produced using the induction compositions and methods described herein can be a cellulase, a hemicellulase, or a ligninase. In an embodiment, the biomass degrading enzyme produced using the induction compositions and methods described herein can be one or more of the enzymes listed in Table 1. The saccharification agent may further comprise one or more additional agents that participate in the saccharification process, e.g., other proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes that were not obtained using the induction compositions or methods described herein.

In embodiments, the biomass is added to a culture comprising the microorganisms that have been induced to produce the proteins, e.g., enzymes, e.g., amylases or biomass degrading enzymes as described herein. Other saccharifying agents, e.g., proteins (e.g., biomass degrading enzymes) or acids, can be added to biomass and culture mixture for the saccharification process.

The saccharification process can be partially or completely performed in a tank (e.g., a tank having a volume of at least 4000 L, 40,000 L, 500,000 L, 2,000,000 L, 4,000,000 L, or 6,000,000L or more) 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.

In a preferred embodiment, the saccharification reaction occurs at a pH optimal for the enzymatic reactions to occur, e.g., at the pH optimal for the activity of the biomass degrading enzymes. Preferably, the pH of the saccharification reaction is at pH 4-4.5. In a preferred embodiment, the saccharification reaction occurs at a temperature optimal for the enzymatic reactions to occur, e.g., at the temperature optimal for the activity of the biomass degrading enzymes. Preferably, the temperature of the saccharification reaction is at 42°C – 52°C.

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.

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.

It is generally preferred that the concentration of the sugar solution resulting from saccharification be relatively high, e.g., greater than 5%, 7.5%, 10%, 10.5%, or greater than 40%, or greater than 50, 60, 70, or even greater than 80% 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.

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 10,000 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.

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.

In the processes described herein, for example after saccharification, a sugar product (e.g., glucose and/or 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.

Further Processing

Further processing steps may be performed on the sugars produced by saccharification to produce alternative products. For example, the sugars can be hydrogenated, fermented, or treated with other chemicals to produce other products.

Glucose can be hydrogenated to sorbitol. Xylose can be hydrogenated to xylitol. 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). The sorbitol and/or xylitol products can be isolated and purified using methods known in the art.

Sugar products from saccharification can also be fermented to produce alcohols, sugar alcohols, such as erythritol, or organic acids, e.g., lactic lacid, glutamic or citric acids or amino acids.

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.

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 conditions can be achieved or maintained by carbon dioxide production during the fermentation and no additional inert gas is needed.

In some embodiments, all or a portion of the fermentation process can be interrupted before the low molecular weight sugar is completely converted to a product (e.g., ethanol). The intermediate fermentation products include sugar and carbohydrates in high concentrations. The sugars and carbohydrates can be isolated via any means known in the art. These

intermediate fermentation products can be used in preparation of food for human or animal consumption. Additionally or alternatively, the intermediate fermentation products can be ground to a fine particle size in a stainless-steel laboratory mill to produce a flour-like substance.

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

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 Jul. 15, 2011, the complete disclosure of which is incorporated herein by reference.

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

Mobile fermenters can be utilized, as described in International App. No. PCT/US2007/074028 (which was filed Jul. 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.

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 algae. When the organisms are compatible, mixtures of organisms can be utilized.

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. pseudotropicalis,* and *C. brassicae*), *Pichia stipitis* (a relative of *Candida shehatae*), the genus *Clavispora* (including, but not limited to, *C. lusitaniae* and *C. opuntiae*), the genus *Pachysolen* (including, but not limited to, *P.*

tannophilus), the genus *Bretannomyces* (including, but not limited to, e.g., *B. clausenii* (Philippidis, G. P., 1996, Cellulose bioconversion technology, in Handbook on Bioethanol: Production and Utilization, Wyman, C. E., ed., Taylor & Francis, Washington, D.C., 179-212)). Other suitable microorganisms include, for example, *Zymomonas mobilis, Clostridium* spp. (including, but not limited to, *C. thermocellum* (Philippidis, 1996, supra), *C. saccharobutylacetonicum, C. saccharobutylicum, C. Puniceum, C. beijerncki,* and *C. acetobutylicum*), *Moniliella pollinis, Moniliella megachiliensis, Lactobacillus* spp. *Yarrowia lipolytica, Aureobasidium* sp., *Trichosporonoides* sp., *Trigonopsis variabilis, Trichosporon* sp., *Moniliellaacetoabutans* sp., *Typhula variabilis, Candida magnoliae, Ustilaginomycetes* sp., *Pseudozyma tsukubaensis,* yeast species of genera *Zygosaccharomyces, Debaryomyces, Hansenula* and *Pichia,* and fungi of the dematioid genus *Torula.*

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

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

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).

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.

After fermentation, the resulting fluids can be distilled using, for example, a "beer column" to separate ethanol and other alcohols from the majority of water and residual solids. The vapor exiting the beer column can be, e.g., 35% by weight ethanol and can be fed to a rectification column. A mixture of nearly azeotropic (92.5%) ethanol and water from the rectification column can be purified to pure (99.5%) ethanol using vapor-phase molecular sieves. The beer column bottoms can be sent to the first effect of a three-effect evaporator. The rectification column reflux condenser can provide heat for this first effect. After the first effect,

solids can be separated using a centrifuge and dried in a rotary dryer. A portion (25%) of the centrifuge effluent can be recycled to fermentation and the rest sent to the second and third evaporator effects. Most of the evaporator condensate can be returned to the process as fairly clean condensate with a small portion split off to waste water treatment to prevent build-up of low-boiling compounds.

Other 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. Patent Publication No. 2014/0011248, filed July 3, 2013, the disclosure of which is incorporated herein by reference in its entirety.

EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: General Methods

General materials and methods used for the examples described herein are provided.

Microorganisms and culture conditions

T. reesei strain RUTC30 (ATCC 56765) was used to produce cellulases. Spores (>10^8/ml) of *T. reesei* were inoculated into the seed culture media (Table 2 and 3) with 0.25% inoculation ratio at 30ºC, shaken at 175 rpm.

Table 2. *T. reesei* seed culture media

Chemical	**Amount (gram or mL)**
Corn Steep	2
Ammonium Sulfate	1.4
Potassium Hydroxide	0.8
Phthalic Acid	5
Lactose	35
Ammonium Hydroxide (6M)	Adjust pH to 6
$CaCl_2$	0.3
$MgSO_4$-$7H_2O$	0.3
DI Water	886.7
Total (ml)	939
Autoclave	
After cooling	
100X Antibiotics (1 g/L Acetoban), mL	10
Metal Solution (1000x), mL	1
20X (60%) Glucose, mL	50
Total volume (mL)	1000

Table 3. 1000X metal solution

Component	**Amount (gram or mL)**
$Fe(SO_4)$ $7H_2O$ (g)	1
$MnSO_4$ $7H_2O$ (g)	0.32
$ZnSO_4$ 7H2O (g)	0.28
$CoCl_2$ $6H_2O$ (g)	0.4
6N HCl	0.2
DI Water	198
Total (mL)	200

Filter sterilize	

Seed culture was inoculated into the main culture media (Table 4) with 5% inoculation ratio. Main culture was conducted at 27 ºC, 700 rpm, 0.3 VVM in 3L bioreactor (New Brunswick). pH was maintained at 3.8 with 6M NH_4OH. Culture period was 11 days.

Table 4. *T. reesei* enzyme production culture media

Material	**Amount (g/L)**
Ammonium sulfate	8
Rice bran	4
Corncob or beeswing (35mrad treated)	80
6M NH_4OH	Adjust to pH 6

Caramelized sugar preparation

Reagent grade glucose, maltose, and lactose were each used for making caramelized sugars. The caramelization reaction was carried out on stirred hot plates. 50% (w/v) sugar solution was prepared and pH was adjusted to 2.5 or 12 with HCl or NaOH, respectively. Temperature was maintained at 170 ºC and the reaction time was 17 hours. Caramelized sugar (or sugar) was fed into the main culture in feeding rate of 4 to 5 g/L/day.

Analysis

Culture samples during the fermentation were taken from the main culture and analyzed by size exclusion chromatography (SEC)-HPLC for the determination of proteins. For cellulase activity assay, treated corncob was used for substrate of cellulase. Liberated glucose and xylose by cellulase produced in *T. reesei* culture were analyzed by HPLC.

Example 2: Caramelized sugars induce cellulase production

In this example, caramelized sugars are used to induce cellulose production in *T. reesei*. *T. reesei* was cultured and grown as described in the methods provided herein, e.g., Example 1.

Caramelized glucose, maltose, and lactose was prepared according to the methods provided herein, e.g., Example 1.

Caramelized sugars, e.g., caramelized glucose, caramelized maltose, and caramelized lactose, (without cellulose inducer) were fed to a *T. reesei* culture at 5g/L/day. Levels of protein produced (g/L) was measured at days 4, 6, 8, and 11 or 12. Without any sugar feeding, very little protein was produced (Figure 1). Caramelized glucose, caramelized maltose, and caramelized lactose all induced protein production. Specifically, caramelized glucose and caramelized maltose showed higher induction capability than caramelized lactose. These results show that caramelized sugar was capable of inducing cellulase production.

Example 3: Analysis of the composition of caramelized sugar

Reagent grade glucose, maltose, and lactose were each used for making caramelized sugars. The caramelization reaction was carried out on stirred hot plates. 50% (w/v) sugar solution was prepared and pH was adjusted to 2.5 or 12 with HCl or NaOH, respectively. Temperature was maintained at 170 °C and the reaction time was 17 hours.

Mass spectrometry analysis was performed to identify the components of caramelized glucose, caramelized maltose, and caramelized lactose. Caramelized sugar samples were diluted 1000-fold and analyzed by ESI-MS. Extracted ion chromatograms were generated from the TIC, stacked, and integrated.

The results show that the caramelized sugar samples contain oligosaccharides (Figure 2). Degree of polymerizations of oligosaccharides was in the range of 2 to 6, with trisaccharides being the most abundant species of oligosaccharides. In all three samples (caramelized glucose, caramelized maltose, and caramelized lactose), oligosaccharides up to at least pentasaccharides were observed, indicating that condensation reactions were taking place during the caramelization process. Some hydrolysis occurred as well, as indicated by the monosaccharide content in the caramelized maltose and caramelized lactose samples. The overall concentration of oligosaccharides in the caramelized lactose sample was the lowest compared to caramelized glucose and caramelized maltose. This data corresponds with the lower level of cellulase production measured after induction by caramelized lactose in *T. reesei* described in Example 2 (Figure 1).

Example 4: Synergistic effect of caramelized sugar with a cellulose inducer for cellulase production

To assess the combined effect of cellulose inducer and caramelized sugar, cellulose inducers, e.g., corncob and beeswing, was added with caramelized sugar to a *T. reesei* culture, and cellulase production was measured by determining the resulting protein titer. *T. reesei* were cultured and induced to produce cellulase as described in Example 2.

Corncob and caramelized sugar was added together to a *T. reesei* culture. Induction by both cellulose inducer (corncob, CC) and caramelized sugar showed synergistic effect for cellulase production, as measured by protein titer (Figure 3). Induction by corncob and caramelized glucose resulted in a 1.9 fold increase in protein titer, when compared to induction with corncob only (Figure 3). Induction by corncob and caramelized glucose resulted in a 1.9 fold increase in protein titer, when compared to induction with caramelized sugar only (Figure 3).

Adding glucose (not caramelized) to the corncob induction culture was also observed to help increase protein titer, but the effect was less than that observed from induction with caramelized glucose. As a negative control, glucose (not caramelized) was fed to the culture, and glucose-only feeding induced very little protein production, resulting in a low protein titer.

A second cellulose inducer, beeswing, was next tested in combination with caramelized sugar generated in basic (pH 12) conditions. Synergistic effect of caramelized sugar with beeswing on cellulase production was also observed (Figure 4). In this experiment, protein production was 2.3 fold higher when a combination of caramelized sugar and beeswing was used to induce production compared to the protein production observed using a single inducer, e.g., beeswing.

These results show that the combination of a caramelized sugar product and an inducer biomass, or cellulose inducer, further enhances the production of a biomass degrading enzyme in a microorganism.

EQUIVALENTS

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific aspects, it is apparent that other aspects and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such aspects and equivalent variations.

What is claimed is:

1. A method for inducing production of a biomass degrading enzyme comprising contacting a microorganism that produces the biomass degrading enzyme with a composition comprising a caramelized sugar product under conditions sufficient for production of a biomass degrading enzyme.

2. The method of claim 1, wherein the microorganism is in a cell culture.

3. The method of claim 2, wherein sugar is added to the cell culture prior to contacting the microorganism with the composition comprising a caramelized sugar product.

4. The method of claim 3, wherein the microorganism is contacted with the composition comprising a caramelized sugar product when the cell culture is substantially free from sugar.

5. The method of claims 1 or 4, wherein the caramelized sugar product is produced by caramelizing glucose, xylose, maltose, lactose, or a combination thereof.

6. The method of claim 5, wherein the caramelized sugar product produced by caramelizing saccharified biomass comprises xylose and glucose.

7. The method of claims 1, 5 or 6, wherein the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof.

8. The method of claim 7, wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof.

9. The method of claim 7, wherein the caramelized sugar product is produced by caramelizing glucose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose.

10. The method of claim 7, wherein the caramelized sugar product is produced by caramelizing maltose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising maltose.

11. The method of claim 7, wherein the caramelized sugar product is produced by caramelizing lactose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose.

12. The method of claim 7, wherein the caramelized sugar product is produced by caramelizing xylose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose.

13. The method of any of claims 7-12, wherein when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

14. The method of any of claims 1-13, wherein the biomass degrading enzyme comprises an amylase, e.g., an alpha, beta or gamma amylase, an endoglucanase, an exoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof.

15. The method of any of claims 1-14, wherein the composition further comprises an inducer biomass.

16. The method of claim 15, wherein the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component.

17. The method of claim 15 or 16, wherein the inducer biomass, e.g., starchy material or starchy material that includes a cellulosic component, comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof.

18. The method of claim 17, wherein an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava,

kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof.

19. The method of claim 17, wherein a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof.

20. The method of claim 17, wherein a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof.

21. The method of claim 17, wherein a general waste comprises manure, sewage, or offal, or a combination thereof.

22. The method of any of claims 15-21, wherein the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass, wherein the pre-treatment of the inducer biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding.

23. The method of claim 1, wherein the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

24. The method of any of claims 1-23, wherein the microorganism is a fungal cell.

25. The method of any of claims 1-23, wherein the microorganism that produces a biomass degrading enzyme is from a species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma*.

26. The method of any of claims 1-24, wherein the microorganism that produces a biomass degrading enzyme is selected from *Aspergillus*, *Humicola insolens (Scytalidium*

thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum, Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei, or *Trichoderma koningii.*

27. The method of any of claims 1-26, wherein the microorganism is *T. reesei* or a variant thereof, e.g., RUT-NG14, PC3-7, QM9414, and RUT-C30.

28. The method of any of claims 1-27, wherein the amount of biomass degrading enzyme produced is increased by at least 1-fold, e.g., at least 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, or more, compared the amount of biomass degrading enzyme produced by the microorganism without contacting with a caramelized sugar product.

29. The method of any of claims 1-27, wherein the amount of biomass degrading enzyme produced is increased by at least 1-fold, e.g., at least 1.2-fold, 1.5 fold, 1.8-fold, 2-fold, compared the amount of biomass degrading enzyme produced by contacting the microorganism with a inducer biomass.

30. The method of any of claims 1-29, wherein the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

31. The method of any of claims 2-30, further comprising separating the biomass degrading enzyme from a component of the cell culture, e.g., the microorganism or remaining inducer biomass, e.g., by chromatography or filtration.

32. The method of claim 31, wherein the biomass degrading enzyme is purified from the cell culture.

33. The method of any of claims 1-32, further comprising a step comprising:

a) contacting the microorganism with a sugar in a first container under conditions such that the microorganism proliferates; and

b) transferring the microorganism to a second container, wherein the second container is larger, e.g., by volume, than the first container;

and wherein said step is performed prior to contacting the microorganism with the composition.

34. The method of claim 33, wherein the step is repeated 1 or more times, e.g., 2, 3, 4, 5 times.

35. A method for producing a product (e.g., hydrogen, a sugar, an alcohol) from a biomass, comprising:
    a) inducing the production of a biomass degrading enzyme using a method according to claim 1;
    b) providing a biomass; and
    c) contacting the biomass with the microorganism of step (a) or the biomass degrading enzyme that has been separated or purified from the microorganism of step (a), under conditions suitable for production of the product.

36. The method of claim 35, wherein the product is a sugar product.

37. The method of claim 36, wherein the product is glucose and/or xylose.

38. The method of any of claims 35-37, further comprising isolating the product.

39. The method of claim 38, wherein the isolating of the product comprises precipitation, crystallization, chromatography, centrifugation, and/or extraction.

40. The method of any of claims 35-39, wherein the biomass degrading enzyme is an endoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof.

41. The method of any of claims 35-40, wherein the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

42. The method of any of claims 35-41, further comprises a step of treating the biomass prior to step (c) to reduce the recalcitrance of the biomass.

43. The method of claim 42, wherein the treating comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze-grinding.

44. The method of any of claims 35-43, wherein the biomass comprises a starchy material or a starchy material that includes a cellulosic component.

45. The method any of claims 35-44, wherein the biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein:

    a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof;

    b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof;

    c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and

    d) a general waste comprises manure, sewage, or offal, or a combination thereof.

46. The method of claim 35, wherein the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof.

47. The method of claim 46, wherein the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose.

48. The method of claims 35 or 46, wherein the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof.

49. The method of claim 48, wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof.

50. The method of claims 35-49 wherein the caramelized sugar product is produced by caramelizing glucose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose.

51. The method of claims 35-49, wherein the caramelized sugar product is produced by caramelizing maltose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising maltose.

52. The method of claims 35-49, wherein the caramelized sugar product is produced by caramelizing lactose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose.

53. The method of claims 35-49, wherein the caramelized sugar product is produced by caramelizing xylose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose.

54. The method of any of claims 48-53, wherein when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

55. The method of any of claims 47-54, wherein the composition further comprises an inducer biomass.

56. The method of claim 55, wherein the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component.

57. The method of claim 56, wherein the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein:

a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof;

b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof;

c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and

d) a general waste comprises manure, sewage, or offal, or a combination thereof.

58. The method of claims 55-57, wherein the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass, wherein the pre-treatment of the biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding.

59. The method of any of claims 35-58 wherein the inducer biomass is the same as the biomass provided in step (b).

60. The method of claim 35, wherein the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

61. The method of any of claims 35-60, wherein the microorganism is a fungal cell.

62. The method of any of claims 35-60, wherein the microorganism that produces a biomass degrading enzyme is from species in the genera selected from *Bacillus, Coprinus, Myceliophthora, Cephalosporium, Scytalidium, Penicillium, Aspergillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium, Chrysosporium* or *Trichoderma*.

63. The method any of claims 35-61, wherein the microorganism that produces a biomass degrading enzyme is selected from *Aspergillus*, *Humicola insolens (Scytalidium thermophilum), Coprinus cinereus, Fusarium oxysporum, Myceliophthora thermophila, Meripilus giganteus, Thielavia terrestris, Acremonium persicinum, Acremonium acremonium, Acremonium brachypenium, Acremonium dichromosporum, Acremonium obclavatum, Acremonium pinkertoniae, Acremonium roseogriseum, Acremonium incoloratum, Acremonium furatum*, *Chrysosporium lucknowense, Trichoderma viride, Trichoderma reesei,* or *Trichoderma koningii.*

64. The method of any of claims 35-63 wherein the microorganism is *T. reesei* or a variant thereof.

65. A composition comprising a caramelized sugar product for use in the method of any of claims 1-64.

66. The composition of claim 65, wherein the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof.

67. The composition of claim 66, wherein the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose.

68. The composition of claims 65 or 66, wherein the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof.

69. The composition of claim 68, wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasacchrides, hexasaccharides, or a combination thereof.

70. The composition of claim 68, wherein the caramelized sugar product is produced by caramelizing glucose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising glucose.

71. The composition of claim 68, wherein the caramelized sugar product is produced by caramelizing maltose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising maltose.

72. The composition of claim 68, wherein the caramelized sugar product is produced by caramelizing lactose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising lactose.

73. The composition of claim 68, wherein the caramelized sugar product is produced by caramelizing xylose and wherein the oligosaccharides comprise disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, or a combination thereof, comprising xylose.

74. The composition of any of claims 68-73, wherein when the oligosaccharides comprise more than one species of oligosaccharides, trisaccharides are the most abundant species.

75. The composition of any of claims 65-74 further comprising an inducer biomass.

76. The composition of claim 75, wherein the inducer biomass comprises a starchy material comprising cellulose.

77. The composition of claim 76, wherein the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein:

    a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof;

b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof;

c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and

d) a general waste comprises manure, sewage, or offal, or a combination thereof.

78. The composition of claims 75-77 wherein the inducer biomass is pre-treated to reduce the recalcitrance of the inducer biomass, wherein the pre-treatment of the biomass comprises exposure to an electron beam, bombardment with electrons, sonication, oxidation, pyrolysis, steam explosion, chemical treatment, mechanical treatment, or freeze grinding.

79. The composition of any of claims 75-77 wherein the composition further comprises cellobiose, β-cellobiono-1,5-lactone, lactose, D-xylose, xylobiose, galactose, and sophorose.

80. A cell culture comprising a microorganism capable of producing a biomass degrading enzyme and a caramelized sugar product.

81. The cell culture of claim 80, further comprising cell culture media.

82. The cell culture of claim 80 or 81, wherein the biomass degrading enzyme is an endoglucanase, a cellobiase, a cellobiohydrolase, a xylanase, a ligninase, or a hemicellulase, or a combination thereof.

83. The cell culture of any of claims 80-82, wherein the biomass degrading enzyme comprises one or more, or all, of the enzymes listed in Table 1.

84. The cell culture of any of claims 80-83, wherein the caramelized sugar product is produced by caramelizing glucose, maltose, xylose, lactose, or a combination thereof.

85. The cell culture of claim 84, wherein the caramelized sugar product is produced by caramelizing saccharified biomass comprising xylose and glucose.

86. The cell culture of any of claims 80-85, wherein the caramelized sugar product comprises oligosaccharides, dehydration products of the oligosaccharides, hydration products of the oligosaccharides, disproportionation products of the oligosaccharides, colored aromatic products, or any combination thereof.

87. The cell culture of any of claims 80-86, wherein the microorganism is *T. reesei* or a variant thereof, e.g., RUTC30.

88. The cell culture of any of claims 80-87, further comprising an inducer biomass.

89. The cell culture of claim 88, wherein the inducer biomass comprises a starchy material or a starchy material that includes a cellulosic component.

90. The cell culture of claim 89, wherein the inducer biomass comprises one or more of an agricultural product or waste, a paper product or waste, a forestry product, or a general waste, or any combination thereof; wherein:

    a) an agricultural product or waste comprises sugar cane jute, hemp, flax, bamboo, sisal, alfalfa, hay, arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, potato, sweet potato, taro, yams, beans, favas, lentils, peas, grasses, switchgrass, miscanthus, cord grass, reed canary grass, grain residues, canola straw, wheat straw, barley straw, oat straw, rice straw, corn cobs, corn stover, corn fiber, coconut hair, beet pulp, bagasse, soybean stover, grain residues, rice hulls, oat hulls, wheat chaff, barley hulls, or beeswing, or a combination thereof;

    b) a paper product or waste comprises paper, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter, printer paper, polycoated paper, cardstock, cardboard, paperboard, or paper pulp, or a combination thereof;

    c) a forestry product comprises aspen wood, particle board, wood chips, or sawdust, or a combination thereof; and

    d) a general waste comprises manure, sewage, or offal, or a combination thereof.

91. The cell culture of any of claims 80-90, further comprising a biomass degrading enzyme produced by the microorganism.

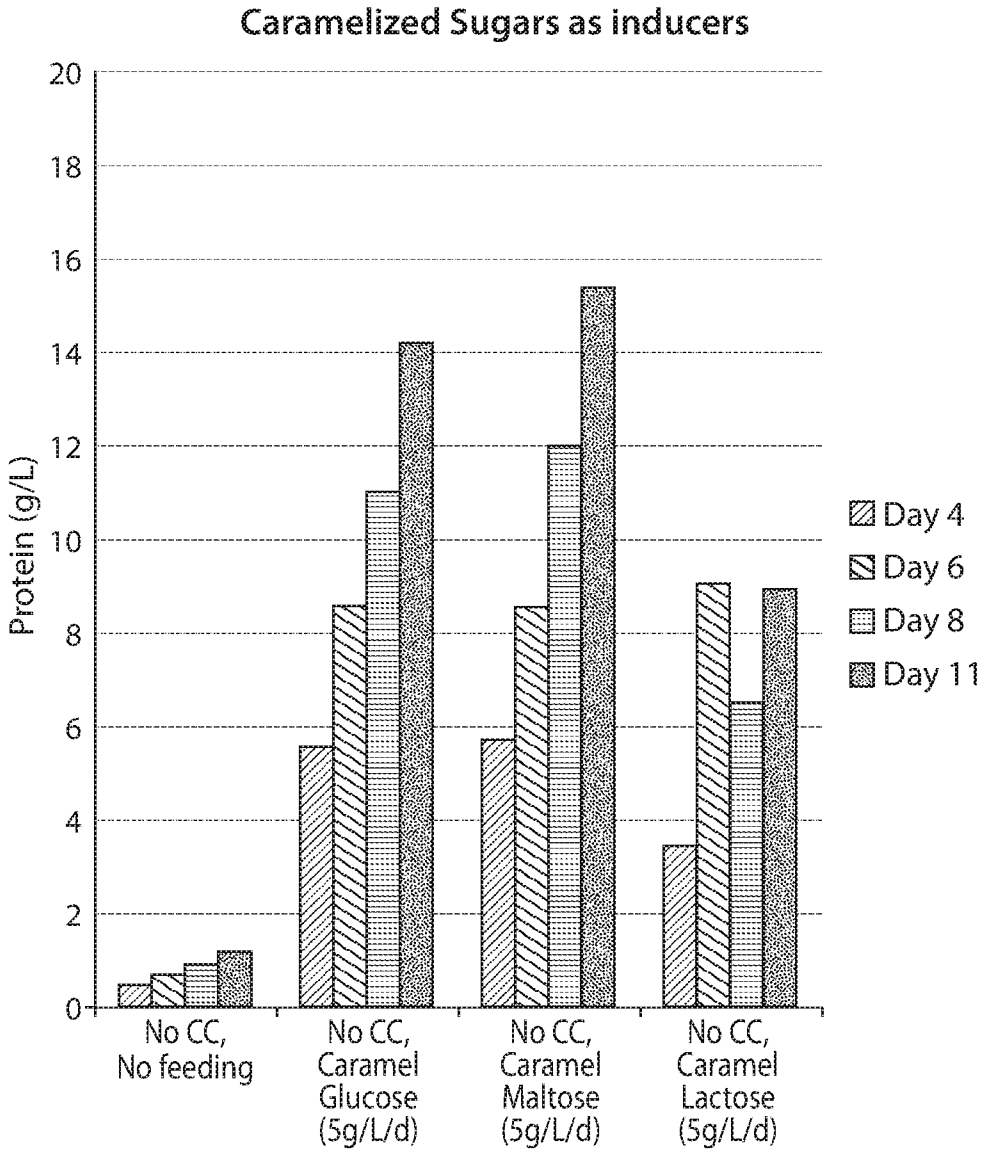
Caramelized Sugars as inducers
Protein (g/L)
20
18
16
14
12
10
8
6
4
2
0
No CC, No feeding
No CC, Caramel Glucose (5g/L/d)
No CC, Caramel Maltose (5g/L/d)
No CC, Caramel Lactose (5g/L/d)
Day 4
Day 6
Day 8
Day 11

FIGURE 1

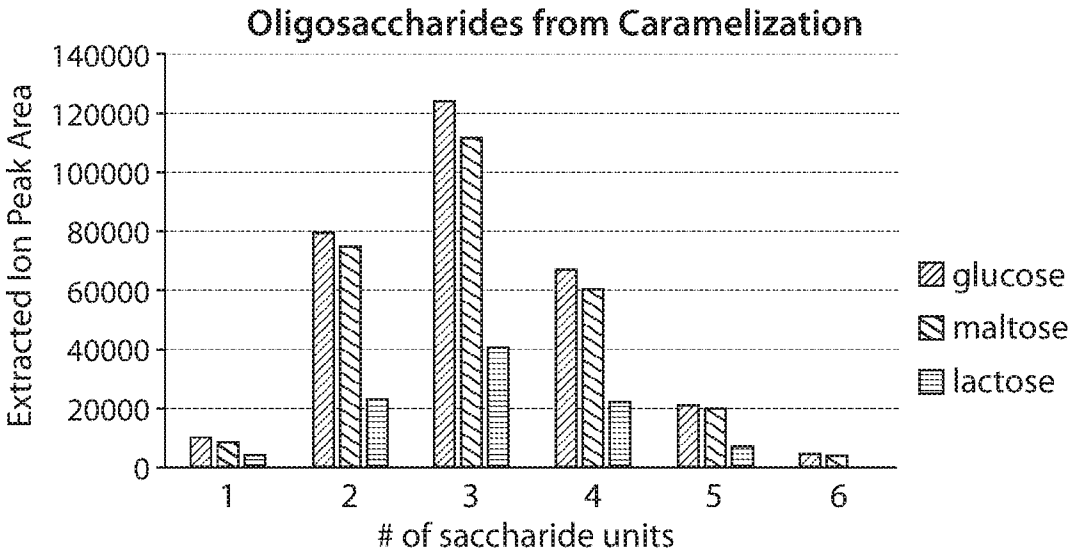
Oligosaccharides from Caramelization
Extracted Ion Peak Area
140000
120000
100000
80000
60000
40000
20000
0
1
2
3
4
5
6
# of saccharide units
glucose
maltose
lactose

FIGURE 2

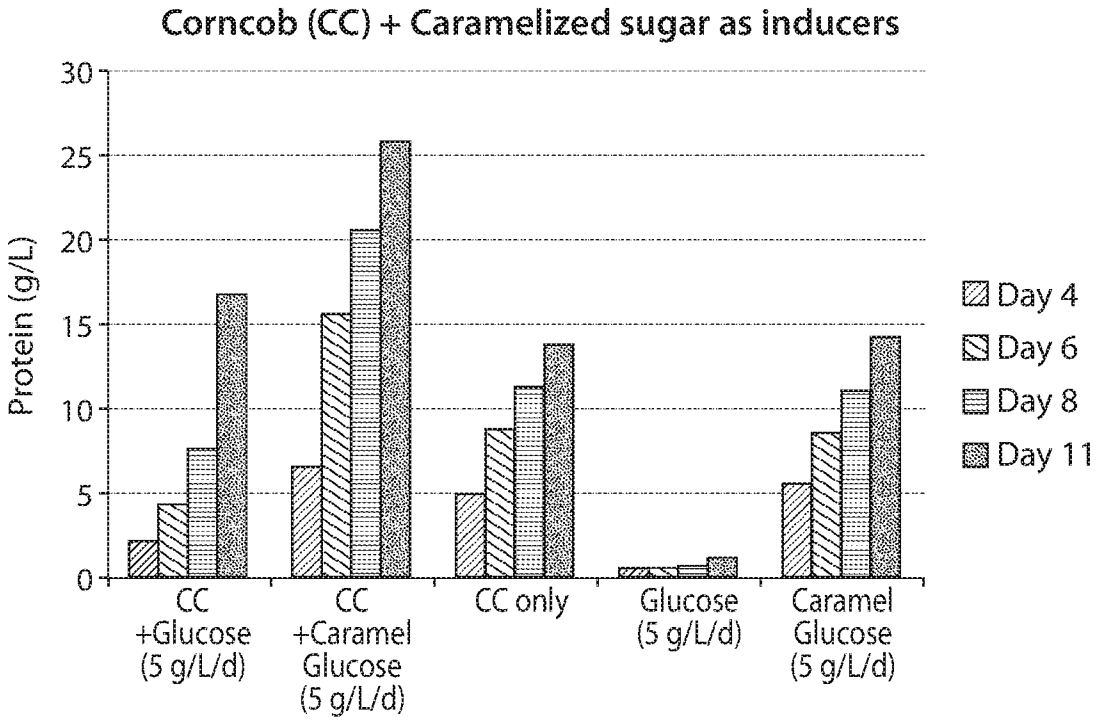
Corncob (CC) + Caramelized sugar as inducers
Protein (g/L)
30
25
20
15
10
5
0
CC +Glucose (5 g/L/d)
CC +Caramel Glucose (5 g/L/d)
CC only
Glucose (5 g/L/d)
Caramel Glucose (5 g/L/d)
Day 4
Day 6
Day 8
Day 11

FIGURE 3

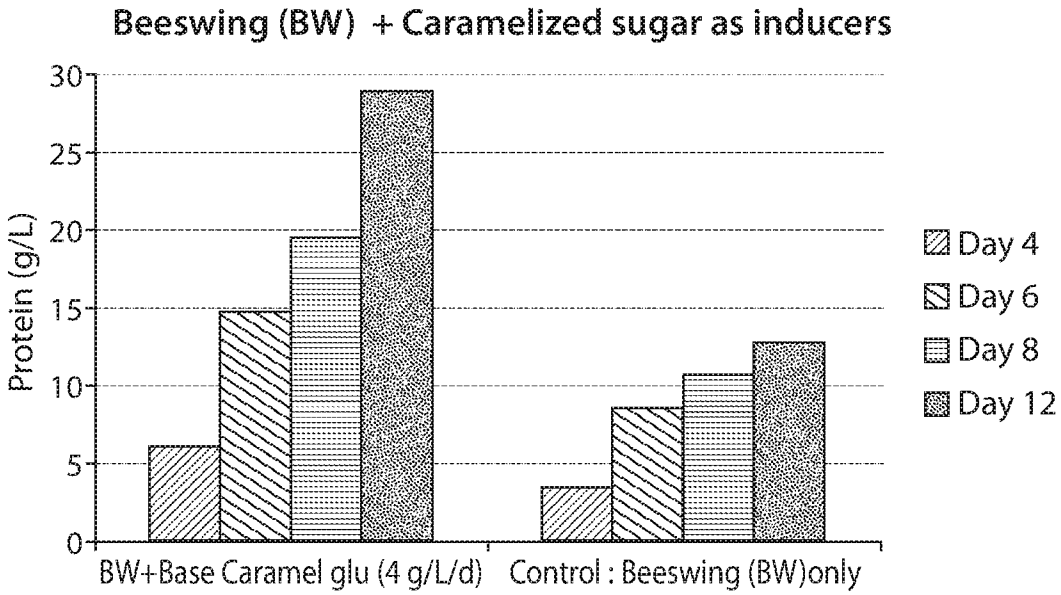
Beeswing (BW) + Caramelized sugar as inducers
Protein (g/L)
30
25
20
15
10
5
0
BW+Base Caramel glu (4 g/L/d)
Control : Beeswing (BW)only
Day 4
Day 6
Day 8
Day 12

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/24966

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 1/38; C12N 1/22; C12N 1/20 (2016.01)
CPC - C12N1/38; C12N1/22
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12N 1/38; C12N 1/22; C12N 1/20 (2016.01)
CPC: C12N1/38; C12N1/22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/252; 435/244; 435/252.1 (Keyword limited, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar (NPL); Keywords: microorganism, induce, enzyme production, biomass degrading enzyme, caramelized sugar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0011258 A1 (Medoff et al.) 09 January 2014 (09.01.2014) para [0002], [0009], [0010], [0011], [0020], [0024], [0028], [0033], [0041], [0056]	1-6, 23, 35-39, 46-49, 60 and 80-82
Y	US 2012/0201947 A1 (Stuart) 09 August 2012 (09.08.2012) abstract, para [0002], [0016], [0040]	1-6, 23, 35-39, 46-49, 60 and 80-82
A	WO 2014/176508 A2 (Xyleco, Inc.) 30 October 2014 (30.10.2014) entire document	1-6, 23, 35-39, 46-49, 60 and 80-82
A	US 2013/0344555 A1 (Angelidaki et al.) 26 December 2013 (26.12.2013) entire document	1-6, 23, 35-39, 46-49, 60 and 80-82

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2016	30 JUN 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/24966

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-22, 24-34, 40-45, 50-59, 61-79 and 83-91
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.