# METHODS AND COMPOSITIONS FOR DEGRADATION OF LIGNOCELLULOSIC MATERIAL

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Application No. 60/806,876, filed on Jul. 10, 2006, the entire disclosure of which is incorporated herein by reference.

## FIELD OF THE INVENTION

The invention relates to a method to convert lignocellulosic biomass to fermentable sugars with enzymes that degrade the lignocellulosic material. The invention also relates to novel combinations of enzymes, including those that provide a synergistic release of sugars from plant biomass.

## BACKGROUND OF THE INVENTION

Large amounts of carbohydrates in plant biomass provide a plentiful source of potential energy in the form of sugars (both five carbon and six carbon sugars) that could be utilized for numerous industrial and agricultural processes. However, the enormous energy potential of these carbohydrates is currently under-utilized because the sugars are locked in complex polymers, and hence are not readily accessible for fermentation. These complex polymers are often referred to collectively as lignocellulose. Sugars generated from degradation of plant biomass could provide plentiful, economically competitive feedstocks for fermentation into chemicals, plastics, and fuels, including ethanol as a substitute for petroleum.

For example, distillers' dried grains (DDG) are lignocellulosic byproducts of the corn dry milling process. Milled whole corn kernels are treated with amylases to liquefy the starch within the kernels and hydrolyze it to glucose. The glucose so produced is then fermented in a second step to ethanol. The residual solids after the ethanol fermentation and distillation are centrifuged and dried, and the resulting product is DDG, which is used as an animal feed stock. Although DDG composition can vary, a typical composition for DDG is: 32% hemicellulose, 22% cellulose, 30% protein, 10% lipids, 4% residual starch, and 4% inorganics. In theory, the cellulose and hemicellulose fractions, comprising about 54% of the weight of the DDG, can be efficiently hydrolyzed to fermentable sugars by enzymes; however, it has been found that the carbohydrates comprising lignocellulosic materials in DDG are more difficult to digest. To date, the efficiency of hydrolysis of these (hemi) cellulosic polymers by enzymes is much lower than the hydrolytic efficiency of starch, due to the more complex and recalcitrant nature of these substrates. Accordingly, the cost of producing the requisite enzymes is higher than the cost of producing amylases for starch hydrolysis. It is therefore desirable to produce inexpensive enzyme mixtures that efficiently degrade cellulose and hemicellulose.

## SUMMARY OF THE INVENTION

One embodiment of the present invention provides a multi-enzyme product comprising a product of a *Chrysosporium* including *Chrysosporium lucknowense* and exhibiting a glucoamylase activity of at least about 0.05 U per milligram of dry matter, a 0-glucosidase activity of at least about 22 nmol/min/mg of dry matter, and an α-arabinofuranosidase activity of at least about 9 nmol/min/mg of dry matter



In some embodiments, the *Chrysosporium lucknowense* is selected from the group consisting of Strain C1 (Accession No. VKM F-3500-D); Strain UV13-6 (Accession No. VKM F-3632 D); Strain NG7C-19 (Accession No. VKM F-3633 D); and/or Strain UV18-25 (Accession No. VKM F-3631 D) and mutants or derivatives thereof.

The present invention also provides a multi-enzyme product comprising a product of a *Trichoderma* including *Trichoderma reesei* and exhibiting a glucoamylase activity of at least about 0.001 U per milligram of dry matter, a β-xylosidase activity of at least about 280 nmol/min/mg of dry matter, a β-glucosidase activity of at least about 454 nmol/min/mg of dry matter, and an α-arabinofuranosidase activity of at least about 100 nmol/min/mg of dry matter.

The present invention also provides a multi-enzyme product comprising a product of a *Aspergillus* including *Aspergillus japonicus* and exhibiting a glucoamylase activity of at least about 0.65 U per milligram of dry matter, a β-xylosidase activity of at least about 553 nmol/min/mg of dry matter, a β-glucosidase activity of at least about 167 nmol/min/mg of dry matter, and an α-arabinofuranosidase activity of at least about 62 nmol/min/mg of dry matter.

The present invention also provides a multi-enzyme product comprising a product of a *Penicillium* including *Penicillium funiculosum* (*Talaromyces* including *Talaromyces flavus*) and exhibiting a glucoamylase activity of at least about 0.18 U per milligram of dry matter, a xylosidase activity of at least about 8 nmol/min/mg of dry matter, a β-glucosidase activity of at least about 691 nmol/min/mg of dry matter, and an α-arabinofuranosidase activity of at least about 29 nmol/min/mg of dry matter.

The present invention also provides a multi-enzyme product comprising a product of a *Penicillium* including *Penicillium canescens* (*P. solitum*) and exhibiting a glucoamylase activity of at least about 0.08 U per milligram of dry matter, a β-xylosidase activity of at least about 15 nmol/min/mg of dry matter, a β-glucosidase activity of at least about 40 nmol/min/mg of dry matter, and an α-arabinofuranosidase activity of at least about 956 nmol/min/mg of dry matter.

In some embodiments, any of the above-described multi-enzyme products is a crude fermentation product.

In some embodiments, any of the above-described multi-enzyme products has been subjected to a purification step.

The present invention further provides a method for degrading a lignocellulosic material to fermentable sugars, comprising contacting the lignocellulosic material with an effective amount of a multi-enzyme product derived from one microorganism, whereby at least one fermentable sugar is produced, and wherein the multi-enzyme product comprises at least one enzyme in the multi-enzyme products of the present invention.

In some embodiments, at least one enzyme in the multi-enzyme product is derived from *Trichoderma* including *Trichoderma reesei, Chrysosporium* including *Chrysosporium lucknowense, Aspergillus* including *Aspergillus japonicus, Penicillium* including *Penicillium canescens* or *solitum, Penicillium* including *Penicillium funiculosum* (*Talaromyces* including *Talaromyces flavus*), or mutants of any of the foregoing.

In some embodiments, the lignocellulosic material is selected from the group consisting of herbaceous material, agricultural residue, forestry residue, municipal solid waste, waste paper, and pulp and paper mill residue.

In some embodiments, the lignocellulosic material is distiller's dried grains or distiller's dried grains with solubles. In some embodiments, the distiller's dried grains or distiller's dried grains with solubles are derived from corn.