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David

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(54) **COMPOSITIONS AND METHODS FOR PRODUCING FERMENTATION PRODUCTS AND RESIDUALS**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides compositions and methods designed to increase value output of a fermentation reaction. In particular, the present invention provides a business method of increasing value output of a fermentation plant. The present invention also provides a modified fermentation residual of higher commercial value. Also provided in the present invention are complete animal feeds, nutritional supplements comprising the subject ferment residuals. Further provided by the present invention is a method of performing fermentation, a modified fermentative microorganism and a genetic vehicle for modifying such microorganism.

24 Claims, 2 Drawing Sheets

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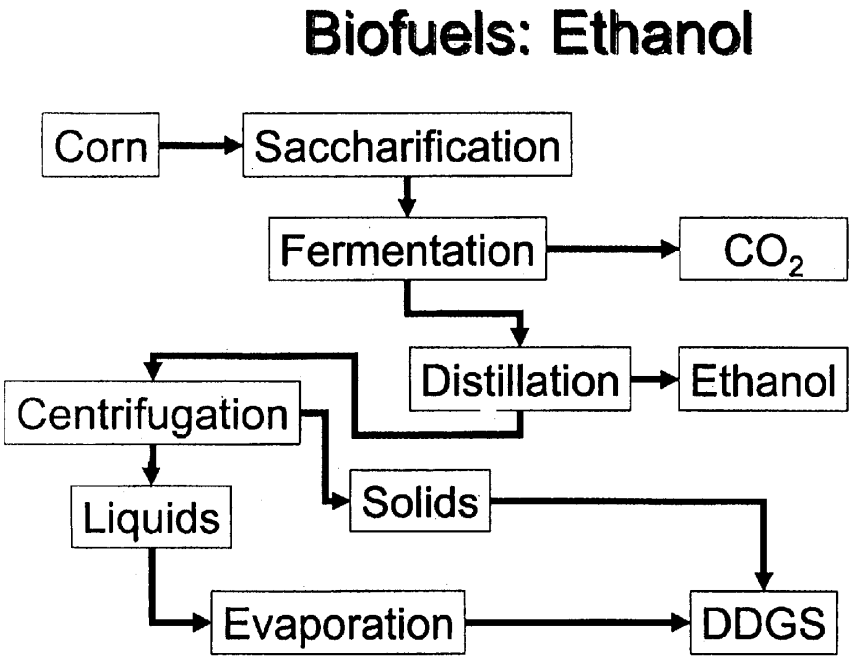
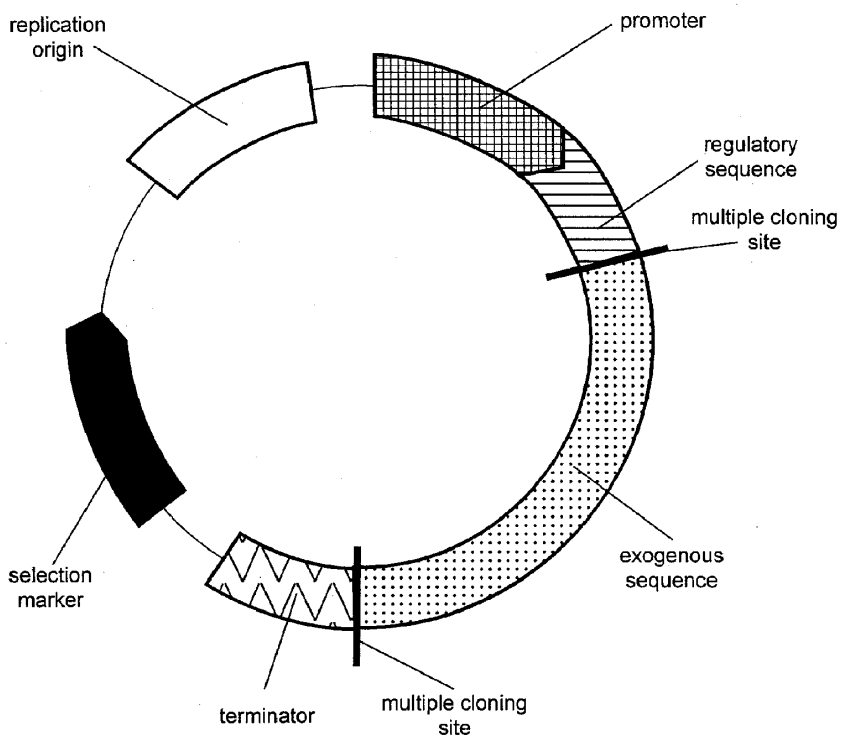


Figure 1

Vector for expressing exogenous sequence in yeast



Exemplary Selection Marker: Zeocin

Exemplary Regulatory Sequence:

- Glucose suppressor operon
- Regulatory sequence of a heat shock gene
- Regulatory sequence of a toxicity gene
- Regulatory sequence of a spore formation gene

Figure 2

COMPOSITIONS AND METHODS FOR PRODUCING FERMENTATION PRODUCTS AND RESIDUALS

CROSS REFERENCE

This application claims the benefit of U.S. Provisional Application No. 60/744,833 filed Apr. 13, 2006, U.S. Provisional Application No. 60/797,431 filed May 3, 2006, all of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

The ethanol fuel industry is growing at a rapid pace. Numerous federal and state incentives, such as clean burning fuel programs, have fostered the exponential growth of more than five times over the past two decades. In 2004, high oil prices, a bumper corn crop, and limited processing capacity created new market opportunities and resulted in record production of more than 3.4 billion gallons of fuel ethanol. Today, ethanol represents the third largest market for U.S. corn. At this pace, fuel ethanol production is positioning itself as an integral part of rural economic development and environmental improvement.

Ethanol can be made through fermentation and distillation of starch found in crops such as corn, sorghum, potatoes, sugar cane, as well as in cornstalks. Ethanol is usually produced in either dry grind or wet mill facilities. The primary co-products generated from the wet mills or "corn refineries" include high fructose corn syrup, corn oil, gluten feed, and gluten meal. Co-products from the dry grind process include distillers grains and carbon dioxide. While both types of facilities have similar operating costs, the dry grind facilities are usually smaller and require a lower initial investment, making their capital costs two to four times less per gallon. The dry mill types of ethanol production process the starch portion of corn, which is about 60% of the kernel. All the remaining nutrients—protein, fat, minerals, and vitamins—are concentrated into distillers grain which is a valuable feed for livestock. A bushel of corn weighing nearly 56 pounds may produce approximately 2.8 gallons of ethanol and 18 pounds of distillers grain.

Distillers grain can provide a high quality feedstuff ration for dairy cattle, beef cattle, swine, poultry, pets, and aquaculture. The feed is an economical partial replacement for corn, soybean meal, and dicalcium phosphate in livestock and poultry feeds. Distillers grain continues to be an excellent, economical feed ingredient for use in ruminant diets. DDGS (distillers dried grains with solubles) production has been expected to double from 3.5 million metric tons in 2002 to over 7 million metric tons by 2006. The sale of distillers grain is an important part of the total profitability and growth of the ethanol industry. If dried distillers grain sales lag behind the increasing production of ethanol, the current ethanol industry could be significantly affected. An effective marketing of distillers grain as animal feed will undoubtedly contribute to the efficiency and overall profitability of an ethanol facility.

Current ethanol production schemes by fermentation are far from being optimized. While efforts have been directed to improve ethanol production, little research has been focused on enhancing the value output of the fermentation residuals including the distillers grain that contributes to a significant portion of the animal feed market.

Thus, there remains a considerable need for compositions and methods that are designed to increase the value output of a fermentation facility. An ideal fermentation scheme would maintain the high ethanol production, and at the same time yield fermentation residuals of higher commercial value. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods designed to increase value output of a fermentation reaction. In one embodiment, the present invention provides a business method of increasing value output of a fermentation plant. The method comprises the steps of (a) performing a fermentation reaction with the use of a modified microorganism; and (b) marketing or selling one or more of the products of the fermentation reaction comprising said modified microorganism. In a related embodiment, the method of increasing value output of a fermentation plant comprises performing a fermentation reaction using carbon-containing material in the presence of a modified microorganism to yield fermentation residual that has a higher commercial value than if the fermentation reaction were performed in the absence of the modified microorganism. In one aspect, the fermentation reaction can be performed under either aerobic or anaerobic conditions. The fermentation reaction typically produces products such as alcohol, including but not limited to methanol, ethanol, propanol, and butanol, as well as gaseous co-products such as carbon dioxide. In addition, the fermentation reaction also yields residuals that are of higher commercial value than conventional fermentation residuals. In another aspect, the fermentation reaction may utilize any carbon-containing starting material, e.g., carbohydrates that are present in a wide variety of substances, including but not limited to cellulose, wood chips, vegetables, biomass, excreta, animal wastes, oat, wheat, corn, barley, milo, millet, rice, rye, sorghum, potato, sugar beets, taro, cassaya, fruits, fruit juices, and sugar cane. The modified microorganism employed in the subject methods can be eukaryotic (e.g., yeast) or prokaryotic (e.g., bacteria or archaebacteria). In a preferred embodiment, the fermentation reaction yields fermentation residuals that have an enhanced nutritional content. In one aspect of this embodiment, the fermentation residuals are enriched in one or more types of cofactors, hormones, proteins, preservatives, stabilization agents, nutraceuticals, vitamins, essential amino acids, and/or lipids. In some aspects, the reaction is performed with the subject microorganisms to increase the value output of the entire fermentation reaction by enhancing the process to yield more valuable products and/or fermentation residuals. In some other aspects, the reaction is performed with the subject microorganisms to increase the value output without substantially decreasing the amount of fermentation products produced by the fermentation reaction, and/or without substantially decreasing the total values of fermentation products produced by the fermentation reaction.

The present invention also provides a fermentation residual comprising a genetically modified microorganism, wherein the fermentation residual has a commercial value (e.g. due to increase in nutritional content) higher than that of a fermentation residual that is deficient in said modified microorganism. In one aspect, the subject fermentation residual has a shelf life that is longer than that of a fermentation residual that is deficient in said modified microorganism. In another aspect, the residual is enriched in at least one essential amino acid, a significant faction of which (e.g. the majority of which) is encapsulated in a cell

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