PROCESSING BIOMASS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 61/365,493, filed July 19, 2010. ~~The complete disclosure of this provisional application is hereby incorporated by reference herein.~~

BACKGROUND

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 waste materials, e.g., sewage, bagasse, sawdust, and stover.

Various cellulosic and lignocellulosic materials, their uses, and applications have been described in U.S. Patent Nos. 7,074,918, 6,448,307, 6,258,876, 6,207,729, 5,973,035 and 5,952,105; and in various patent applications, including "FIBROUS MATERIALS AND COMPOSITES," PCT/US2006/010648, filed on March 23, 2006, AND "FIBROUS MATERIALS AND COMPOSITES," U.S. Patent Application Publication No. 2007/0045456.

SUMMARY

Generally, this invention relates to bioprocessing of carbon-containing materials, such as carbohydrate-containing materials (e.g., biomass materials, biomass-derived materials or chitin), in particular cellulosic and lignocellulosic materials, and saccharified carbohydrate-containing materials. The bioprocessing techniques disclosed herein include combining the carbohydrate-containing materials with a microorganism that utilizes the carbohydrate-containing material, or its saccharified derivative, to produce a product or intermediate. This process is generally performed in a fluid medium, and in some implementations comprises fermentation.

A typical biomass resource contains cellulose, hemicellulose, and lignin plus lesser amounts of proteins, extractables and minerals. The complex carbohydrates contained in the cellulose and hemicellulose fractions can be processed into fermentable

In some preferred implementations, the mixture further comprises an enzyme system selected to release nutrients, e.g., nitrogen, amino acids, and fats, from the food-based nutrient source. For example, the enzyme system may include one or more enzymes selected from the group consisting of amylases, proteases, and mixtures thereof. In some cases, the enzyme system comprises a protease and an amylase.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. ~~All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.~~ In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is a flow diagram illustrating conversion of a feedstock to ethanol via production of a glucose solution.

FIG. 2 is a schematic diagram of an ethanol manufacturing facility.

FIG. 3 is a diagram illustrating the enzymatic hydrolysis of cellulose to glucose.

DETAILED DESCRIPTION

Using the methods and nutrient packages described herein, carbon-containing materials such as cellulosic and lignocellulosic materials and their saccharified derivatives can be bioprocessed, e.g., using fermentation, to produce useful intermediates and products such as those described herein.

The inventors have found that by adding low levels of a food product and/or a food residue to the fermentation batch, efficient fermentation can be obtained, resulting in relatively high concentrations of ethanol, e.g., up to 10%, 15%, 20%, 25%, or even up to 30% or more. In some cases, concentrations can be, for example, from about 0.1 to 80

Referring to FIG. 1, a process for manufacturing an alcohol, e.g., ethanol, can include, for example, optionally mechanically treating the feedstock (step 110), before and/or after this treatment, optionally treating the feedstock with another physical treatment, for example irradiation (e.g., electron beam irradiation), to further reduce its recalcitrance (step 112), saccharifying the feedstock to form a sugar solution (step 114), optionally transporting, e.g., by pipeline, railcar, truck or barge, the solution (or the feedstock, enzyme and water, if saccharification is performed en route) to a manufacturing plant (step 116), and then bio-processing the treated feedstock to produce a desired product (step 118), which is then processed further, e.g., by distillation (step 120). If desired, lignin content can be measured (step 122) and process parameters can be set or adjusted based on this measurement (step 124), as described in U.S. Provisional Application Number 61/151,724, filed on February 11, 2009~~, the complete disclosure of which is incorporated herein by reference~~.

The food-based nutrient source or nutrient package is present during bio-processing (step 118), e.g., fermentation, and may in some preferred implementations also be present during the saccharification step (step 114). In some implementations, the food-based nutrient source or nutrient package is added at the beginning of step 114, along with an enzyme combination suitable for saccharification, fermentation, and release of nutrients from the food-based nutrient source. Saccharification is conducted under a first set of process conditions (e.g., temperature and pH), and then when saccharification has proceeded to a desired extent the process conditions are adjusted (e.g., by adjusting pH from 4 to 5) to allow fermentation to proceed.

The manufacturing plant used in steps 118-120 (and in some cases all of the steps described above) can be, for example, an existing starch-based or sugar-based ethanol plant or one that has been retrofitted by removing or decommissioning the equipment upstream from the bio-processing system (which in a typical ethanol plant generally includes grain receiving equipment, a hammermill, a slurry mixer, cooking equipment and liquefaction equipment). In some cases, the feedstock received by the plant can be input directly into the fermentation equipment. A retrofitted plant is shown schematically in FIG. 2.

Steps 110-112 are described, for example, in U.S. Serial No. 12/429,045, filed April 23, 2009, the complete disclosure of which is incorporated herein by reference. Steps 114, 118 and 120 (saccharification, fermentation, and distillation), which pertain to the production of alcohol by bioprocessing, will now be discussed further.

Saccharification

In order to convert the feedstock to fermentable sugars, the cellulose in the feedstock is hydrolyzed by a saccharifying agent, e.g., an enzyme, a process referred to as saccharification. The materials that include the cellulose are treated with the enzyme, e.g., by combining the material and the enzyme in a solvent, e.g., in an aqueous solution.

Enzymes and biomass-destroying organisms that break down biomass, such as the cellulose and/or the lignin portions of the biomass, contain or manufacture various cellulolytic enzymes (cellulases), ligninases or various small molecule biomass-destroying metabolites. These enzymes may be a complex of enzymes that act synergistically to degrade crystalline cellulose or the lignin portions of biomass. Examples of cellulolytic enzymes include: endoglucanases, cellobiohydrolases, and cellobiases (β-glucosidases). Referring to FIG. 3, a cellulosic substrate is 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.

Suitable saccharifying agents are described, for example, in the Materials section below.

As noted above, the food-based nutrient source or nutrient package is preferably added prior to or during saccharification, and an enzyme is added that is selected to release nutrients from the food-based nutrient source. Suitable enzymes are described, for example, in the Materials section below.

The saccharification process can be partially or completely performed in a tank (e.g., a tank having a volume of at least 4000, 40,000, 400,000, or 1 ,000,000 L) in a manufacturing plant, and/or can be partially or completely performed in transit, e.g., in a

rail car, tanker truck, or in a supertanker or the hold of a ship. The time required for complete saccharification will depend on the process conditions and the feedstock and enzyme used. If saccharification is performed in a manufacturing plant under controlled conditions, the cellulose may be substantially entirely converted to glucose in about 12-96 hours. If saccharification is performed partially or completely in transit, saccharification may take longer.

It is generally preferred that the tank contents be mixed during saccharification, e.g., using jet mixing as described in U.S. Provisional Application No. 61/218,832~~, 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 resulting glucose solution be relatively high, e.g., greater than 40%, or greater than 50, 60, 70, 80, 90 or even greater than 95% by weight. This reduces the volume to be shipped, if saccharification and fermentation are performed at different locations, and also inhibits microbial growth in the solution. However, lower concentrations may be used, in which case it may be desirable to add an antimicrobial additive, e.g., a broad spectrum antibiotic, in a low concentration, e.g., 50 to 150 ppm. Other suitable antibiotics include amphotericin B, ampicillin, chloramphenicol, ciprofloxacin, gentamicin, hygromycin B, kanamycin, neomycin, penicillin, puromycin, streptomycin. Antibiotics will inhibit growth of microorganisms during transport and storage, and can be used at appropriate concentrations, e.g., between 15 and 1000 ppm by weight, e.g., between 25 and 500 ppm, or between 50 and 150 ppm. If desired, an antibiotic can be included even if the sugar concentration is relatively high.

A relatively high concentration solution can be obtained by limiting the amount of water added to the feedstock 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 feedstock 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 some embodiments, the feedstock is processed to convert it to a convenient and concentrated solid material, e.g., in a powdered, granulate or particulate form. The concentrated material can be in a purified, or a raw or crude form. The concentrated form can have, for example, a total sugar concentration of between about 90 percent by weight and about 100 percent by weight, e.g., 92, 94, 96 or 98 percent by weight sugar. Such a form can be particularly cost effective to ship, e.g., to a bioprocessing facility, such as a biofuel manufacturing plant. Such a form can also be advantageous to store and handle, easier to manufacture and becomes both an intermediate and a product, providing an option to the biorefmery as to which products to manufacture.

In some instances, the powdered, granulate or particulate material can also include one or more of the materials, e.g., additives or chemicals, described herein, such as the food-based nutrient or nutrient package, a nitrogen source, e.g., urea, a surfactant, an enzyme, or any microorganism described herein. In some instances, all materials needed for a bio-process are combined in the powdered, granulate or particulate material. Such a form can be a particularly convenient form for transporting to a remote bioprocessing facility, such as a remote biofuels manufacturing facility. Such a form can also be advantageous to store and handle.

In some instances, the powdered, granulate or particulate material (with or without added materials, such as additives and chemicals) can be treated by any of the physical treatments described in U.S. Serial No. 12/429,045~~, incorporated by reference above~~. For example, irradiating the powdered, granulate or particulate material can increase its solubility and can sterilize the material so that a bioprocessing facility can integrate the material into their process directly as may be required for a contemplated intermediate or product.

In certain instances, the powdered, granulate or particulate material (with or without added materials, such as additives and chemicals) can be carried in a structure or a carrier for ease of transport, storage or handling. For example, the structure or carrier can include or incorporate a bag or liner, such as a degradable bag or liner. Such a form can be particularly useful for adding directly to a bioprocess system.

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.

INTERMEDIATES AND PRODUCTS

The processes and nutrients discussed herein can be used to convert carbohydrate-containing materials, e.g., cellulosic or lignocellulosic materials, to one or more products, such as energy, fuels, foods and materials. Specific examples of products include, but are not limited to, hydrogen, alcohols (e.g., monohydric alcohols or dihydric alcohols, such as ethanol, n-propanol or n-butanol), hydrated or hydrous alcohols, e.g., containing greater than 10%, 20%, 30% or even greater than 40% water, sugars, biodiesel, organic acids (e.g., acetic acid and/or lactic acid), hydrocarbons, 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, such as acetic acid or butyric acid, 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, aldehydes, alpha, beta unsaturated acids, such as acrylic acid and olefins, such as ethylene. Other alcohols and alcohol derivatives include propanol, propylene glycol, 1,4-butanediol, 1,3 -propanediol, methyl or ethyl esters of any of these alcohols. Other products include methyl acrylate, methylmethacrylate, lactic acid, propionic acid, butyric acid, succinic acid, 3-hydroxypropionic acid, a salt of any of the acids and a mixture of any of the acids and respective salts.

Other intermediates and products, including food and pharmaceutical products, are described in U.S. Serial No. 12/417,900~~, the full disclosure of which is hereby incorporated by reference herein~~.

WHAT IS CLAIMED IS:

1. A method comprising

combining a feedstock, comprising a cellulosic or lignocellulosic material and/or a saccharified cellulosic or lignocellulosic material, with a microorganism and a food-based nutrient source to form a mixture, the microorganism utilizing the feedstock to

produce a product or intermediate;

wherein the food-based nutrient source is used primarily or solely as a nutrient source for the microorganism; and

wherein the mixture further comprises an enzyme system comprising one or more enzymes selected to release nutrients from the food-based nutrient source.

2. The method of claim 1 wherein the food-based nutrient source is selected from the group consisting of grains, vegetables, residues of grains, residues of vegetables, residues of meat products, and mixtures thereof.

3. The method of claim 1 or 2 wherein the product comprises a fuel selected from the group consisting of hydrogen, alcohols, organic acids, hydrocarbons, and mixtures thereof.

4. The method of claim 1, 2 or 3 wherein utilizing comprises fermentation.

5. The method of claim 4 wherein the microorganism comprises a yeast.

6. The method of claim 4 wherein fermentation is conducted at a pH of ~~about~~ 4.8 to 5.2.

7. The method of any one of the above claims wherein utilizing comprises saccharification.

8. The method of claim 7 wherein the microorganism comprises an enzyme.

9. The method of claim 8 wherein the enzyme comprises a hemicellulase or a cellulase.

10. The method of claim 7 wherein saccharification is conducted at a pH of ~~about~~ 3.8 to 4.2.

11. The method of any one of the above claims wherein the mixture further comprises a nitrogen source.

12. The method of claim 11 wherein the nitrogen source is selected from the group consisting of urea, ammonia, ammonium sulfate, and mixtures thereof.

13. The method of claim 2 wherein the food-based nutrient source is selected from the group consisting of wheat, oats, barley, soybeans, peas, legumes, potatoes, corn, rice bran, corn meal, wheat bran, and mixtures thereof.

~~14. The method of any one of the above claims wherein the mixture further comprises an enzyme system selected to release nutrients from the food-based nutrient source.~~

~~15~~14. The method of claim ~~14~~ 1 wherein the enzyme system comprises one or more enzymes selected from the group consisting of amylases, proteases, and mixtures thereof.

~~16~~15. The method of claim ~~14~~ 1 wherein the enzyme system comprises a protease and an amylase.

~~17~~16. The method of claim ~~16~~ 15 wherein the enzyme system further comprises a hemicellulase or a cellulase.

~~18~~17. The method of any one of the above claims wherein the mixture further comprises a medium.

~~19~~18. The method of claim ~~18~~ 17 wherein the concentration of the food-based nutrient source in the medium is from ~~about~~ 0.1 to 10 g/L.

~~20~~19. The method of claim 3, wherein the alcohol is selected from the group consisting of methanol, ethanol, propanol, isopropanol, n-butanol, ethylene glycol, propylene glycol, 1 ,4-butane diol, glycerin, and mixtures thereof.

~~21~~20. The method of claim 3, wherein the organic acid is selected from the group consisting of formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic, palmitic acid, stearic acid, oxalic acid, malonic acid, succinic acid, glutaric acid, oleic acid, linoleic acid, glycolic acid, lactic acid, γ-hydroxybutyric acid and mixtures thereof.

~~22~~21. The method of claim 3, wherein the hydrocarbon is selected from the group consisting of methane, ethane, propane, isobutene, pentane, n-hexane, and mixtures thereof.

~~23~~22. The method of any one of the above claims wherein the cellulosic or lignocellulosic material has been treated to reduce its recalcitrance.

~~24~~23. The method of claim ~~23~~ 22 wherein the cellulosic or lignocellulosic material has been treated with radiation.

~~25~~24. The method of claim ~~24~~ 23 wherein the radiation comprises an electron beam.