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61/251,610 14 October 2009 (14.10.2009) US(71) Applicant (for all designated States except US): **XYLECO, INC.** [US/US]; 271 Salem St., Unit L, Woburn, MA 01801 (US).

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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

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(54) Title: PRODUCING EDIBLE RESIDUES FROM ETHANOL PRODUCTION

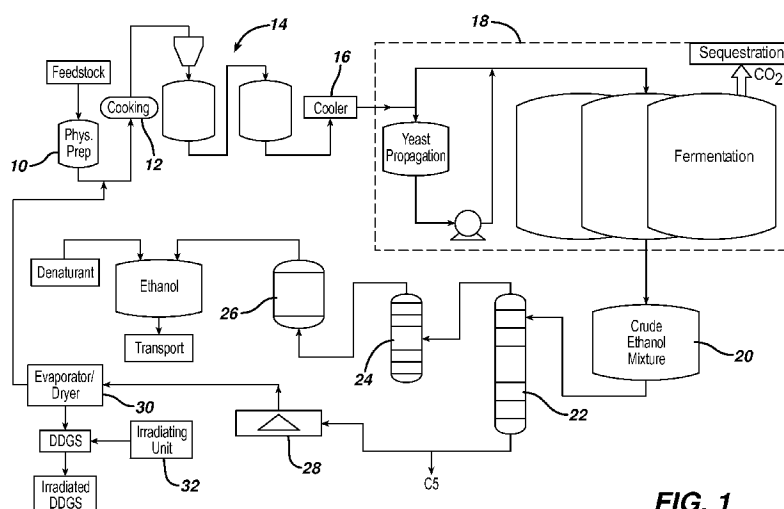


FIG. 1

(57) Abstract: Edible residues of ethanol production, e.g., distillers grains and solubles, are produced that are low in, or substantially free from, antibiotic residues. Antibiotics or bacteria present in edible residues resulting from ethanol production are inactivated by irradiating the edible residues.

PRODUCING EDIBLE RESIDUES FROM **ETHANOL PRODUCTION**

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 61/251,610, filed October 14, 2009.

TECHNICAL FIELD

This invention relates to producing edible residues from ethanol production, e.g., distillers grains and residues from cellulosic ethanol production.

BACKGROUND

Manufacturing plants exist for the production of ethanol from grains, e.g., corn, and from sugars. Ethanol manufacturing is discussed in many sources, e.g., in The Alcohol Textbook, 4th Ed., ed. K.A. Jacques, et al., Nottingham University Press, 2003. Distillers grains (also referred to as distillers grains and solubles (DGS) or dry distillers grains (DDGS)) are a by-product of ethanol production. Distillers grains are a valuable by-product, as they are a major source of low-cost livestock feed. However, recently concerns have arisen due to the presence of antibiotics in distillers grains. The antibiotics in the distillers grains are generally present as a result of the use of antibiotics in the ethanol manufacturing process. Antibiotics such as penicillin and virginiamycin are used to control bacteria that compete with yeast during fermentation, converting sugar into lactic acid rather than ethanol. If restrictions on the sale or use of distillers grains are imposed due to concerns over antibiotic content, this will further damage the profit margins of ethanol producers, as well as depriving livestock farmers of a good source of livestock feed.

U.S. Patent Application No. 20060127999, "Process for producing ethanol from corn dry milling," and U.S. Patent Application No. 20030077771, "Process for producing ethanol," are each incorporated by reference herein in their entireties. In addition, U.S. Patent No. 7,351,559 "Process for producing ethanol," U.S. Patent No. 7,074,603, "Process for producing ethanol from corn dry milling" and U.S. Patent No. 6,509,180,

“Process for producing ethanol” are each incorporated by reference herein in their entireties.

SUMMARY

Generally, this invention relates to edible residues from ethanol production, and to methods of producing edible residues that are low in antibiotic content, or, in preferred embodiments, substantially free of antibiotics. By “low in antibiotic content,” or “substantially free of antibiotics,” we mean that the edible residue contains little or no active antibiotic, e.g., less than 100 ppm; the edible residue may contain inactivated antibiotics, as will be discussed herein.

The edible residue may be, for example, distillers dry grains (DDG), in the case of corn ethanol production, or a mixture of lignin, unfermented sugars (e.g., xylose, arabinose), minerals (e.g., clay, silica, silicates), and in some cases undigested cellulose.

In some implementations, the edible residue contains less than 50 ppm by weight active antibiotic, e.g., less than 25 ppm, less than 10 ppm, or even less than 1 ppm.

In one aspect, the invention features a method comprising irradiating edible residues that have been produced as a by-product of an ethanol manufacturing process.

Some implementations include one or more of the following features. The edible residues comprise distillers grains and solubles, e.g., from a corn ethanol process. Alternatively, the edible residues may comprise lignin, xylose and minerals, and in some cases undigested cellulose, for example when the ethanol manufacturing process utilizes a cellulosic feedstock and/or a lignocellulosic feedstock.

In some cases, the edible residues contain an antibiotic, and irradiating is performed under conditions that are selected to inactivate or destroy the antibiotic, e.g., by changing the molecular structure of the antibiotic. In such cases, after irradiation the edible residues can contain less than 100 ppm, such as less than 50, 25, 10, or 1 ppm, by weight of active antibiotic, or can be substantially free of active antibiotic. In some implementations, prior to irradiation the edible residues contain from about 500 ppm to about 10,000 ppm by weight of active antibiotic.

In other cases, the ethanol manufacturing process may be conducted without addition of antibiotics. In such cases, prior to irradiation the edible residues may contain bacteria, and irradiation is performed under conditions that destroy bacteria.

In some implementations, irradiation is delivered at a dose of greater than about 0.5 Mrad, and/or less than about 5Mrad, e.g., at a dose of from about 1 to about 3 Mrad.

If the edible residues are distillers grains and solubles, the distillers grains and solubles may be dried, producing dried distillers grains and solubles (DDGS). Drying may be performed prior to, during or after irradiation.

All publications, patent applications, patents, and other references mentioned herein or attached hereto are incorporated by reference in their entirety for all that they contain.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram illustrating a process for making ethanol and distillers grains.

DETAILED DESCRIPTION

Referring to FIG. 1, a plant for manufacturing ethanol can include, for example, one or more operating units (10) for receiving and physically treating a feedstock, which in a typical grain-based (e.g., corn or grain) ethanol plant generally includes grain receiving equipment and a hammermill. If the feedstock to be used is a non-grain cellulosic or lignocellulosic material the operating units 10 may be configured to reduce the size of the feedstock in a manner that exposes internal fibers of the feedstock, e.g., as disclosed in U.S. Patent No. 7,470,463, the full disclosure of which is incorporated herein by reference.

In some cases, for instance if the feedstock includes a material that is difficult to treat by fermentation, e.g., crop residues or other lignocellulosic feedstocks, the plant may include an optional operating unit configured to treat the feedstock to reduce its recalcitrance. In some implementations, recalcitrance is reduced by at least 5%, or at least

10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95%. In some cases recalcitrance is substantially completely eliminated. Treatment processes utilized by the recalcitrance reducing operating unit can include one or more of irradiation, sonication, oxidation, pyrolysis, and steam explosion. Treatment methods can be used in combinations of two, three, four, or even all of these technologies (in any order). Operating units that pretreat feedstocks to reduce recalcitrance are described in WO 2008/03186, the full disclosure of which is incorporated herein by reference.

The feedstock can then be processed in a series of cooking devices (12), as is well known, subjected to liquefaction (14), and cooled (16) to a suitable temperature for contact with microorganisms such as yeasts. The cooled stream then flows to a bio-processing system (18) where it is bio-processed, e.g., fermented, to produce a crude ethanol mixture which flows into a holding tank (20).

The bio-processing system may in some cases utilize antibiotics to prevent excessive generation of lactic acid by bacteria in the mixture. For example, antibiotic may be added in a concentration of from about 500 ppm to about 10,000 ppm by weight of the feedstock.

Alternatively, the use of antibiotics may be avoided, for example by cleaning the feedstock and processing equipment, running the process at low pH levels, and maintaining high throughput during steeping, mashing and fermentation. Non-antibiotic additives may also be used, for example the hop extract sold by BetaTec Hop Products under the tradename IsoStab™. If these alternatives are used, it is desirable to sterilize the edible residues of the process, to ensure their safety.

Water or other solvent, and other non-ethanol components, are stripped from the crude ethanol mixture using a stripping column (22), and the ethanol is then distilled using a distillation unit (24), e.g., a rectifier. Finally, the ethanol can be dried using a molecular sieve (26), denatured if necessary, and output to a desired shipping method.

Another stream comes off of the bottom of stripping column (22) and is passed through a centrifuge (28). A liquid fraction, or “thin stillage (backset)” is then returned to the process, generally prior to the cooking devices (12). The solids (“wet cake”) are subjected to further processing, including drying, in an evaporator/dryer operating unit

(30), resulting in the production of an edible residue, e.g., dried Distillers Grains and Solubles (DDGS) if the feedstock was corn.

The edible residue is then irradiated, using an irradiating unit (32). Irradiation serves both to inactivate any antibiotic present in the edible residue from the fermentation process, e.g., by changing the molecular structure of the antibiotic, and to sterilize the edible residue, killing any undesirable bacteria or other microorganisms present in the edible residue.

Irradiation can be performed using any suitable device. If the edible residue is in the form of a thin section, e.g., small pellets, electron beam irradiation may be preferred to provide high throughput. If deeper penetration is required, e.g., if the edible residue is in the form of a thick cake, gamma radiation can be used.

The radiation may be delivered in any dose that is sufficient to inactivate the antibiotic and/or destroy bacteria and undesirable microorganisms, without deleteriously affecting the nutrient availability of the edible residue. For example, the dose may be from about 0.5 MRad to about 5 MRad, e.g., about 1 MRad to about 3 MRad.

Drying of the edible residue may be performed before (as shown), during, or after irradiation, or may be omitted if desired.

Generally, all of the processing equipment used in the process described above is typically utilized in existing ethanol manufacturing plants, with the exception of optional recalcitrance reducing operating unit and the device used to irradiate the edible residue.

In some cases, the feedstock can be a cellulosic or lignocellulosic material that has been physically treated and optionally pre-treated at a remote location and then shipped to the plant, e.g., by rail, truck, ship (e.g., barge or supertanker), or air. In such cases, the material may be shipped in a densified state for volume efficiency. For example, the feedstock can be physically treated, e.g., using the size reduction techniques described below, to a bulk density of less than about 0.35 g/cc, and then densified to have a bulk density of at least about 0.5 g/cc. In some implementations, the densified material can have a bulk density of at least 0.6, 0.7, 0.8, or 0.85 g/cc. Fibrous materials can be densified using any suitable process, e.g., as disclosed in WO 2008/073186.

The feedstock can in some cases be fibrous in nature. Fiber sources include cellulosic fiber sources, including paper and paper products (e.g., polycoated paper and

Kraft paper), and lignocellulosic fiber sources, including wood, and wood-related materials, e.g., particle board. Other suitable fiber sources include natural fiber sources, e.g., grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, rice hulls, coconut hair; fiber sources high in α -cellulose content, e.g., cotton. Fiber sources can be obtained from virgin scrap textile materials, e.g., remnants, post consumer waste, e.g., rags. When paper products are used as fiber sources, they can be virgin materials, e.g., scrap virgin materials, or they can be post-consumer waste. Aside from virgin raw materials, post-consumer, industrial (e.g., offal), and processing waste (e.g., effluent from paper processing) can also be used as fiber sources. Also, the fiber source can be obtained or derived from human (e.g., sewage), animal or plant wastes. Additional fiber sources have been described in U.S. Patent Nos. 6,448,307, 6,258,876, 6,207,729, 5,973,035 and 5,952,105.

The sugars liberated during bioprocessing can be converted into a variety of products, such as alcohols or organic acids. The product obtained depends upon the microorganism utilized and the conditions under which the bio-processing occurs. These steps can be performed utilizing the existing equipment of the grain-based ethanol manufacturing facility, with little or no modification. A xylose (C5) stream may be produced during bio-processing, if hemi-cellulose is present in the feedstock, and thus in some cases provision is made for removing this stream after the stripping column.

The microorganism utilized in bioprocessing can be a natural microorganism or an engineered microorganism. For example, the microorganism can be a bacterium, e.g., a cellulolytic bacterium, a fungus, e.g., a yeast, a plant or a protist, e.g., an algae, a protozoa or a fungus-like protist, e.g., a slime mold. When the organisms are compatible, mixtures of organisms can be utilized. The microorganism can be an aerobe or an anaerobe. The microorganism can be a homofermentative microorganism (produces a single or a substantially single end product). The microorganism can be a homoacetogenic microorganism, a homolactic microorganism, a propionic acid bacterium, a butyric acid bacterium, a succinic acid bacterium or a 3-hydroxypropionic acid bacterium. The microorganism can be of a genus selected from the group *Clostridium*, *Lactobacillus*, *Moorella*, *Thermoanaerobacter*, *Propionibacterium*, *Propionispora*, *Anaerobiospirillum*, and *Bacteriodes*. In specific instances, the

microorganism can be *Clostridium formicoaceticum*, *Clostridium butyricum*, *Moorella thermoacetica*, *Thermoanaerobacter kivui*, *Lactobacillus delbrukii*, *Propionibacterium acidipropionici*, *Propionispora arboris*, *Anaerobiospirillum succinicproduces*, *Bacteriodes amylophilus* or *Bacteriodes ruminicola*. For example, the microorganism can be a recombinant microorganism engineered to produce a desired product, such as a recombinant *Escherichia coli* transformed with one or more genes capable of encoding proteins that direct the production of the desired product is used (see, e.g., U.S. Pat. No. 6,852,517, issued Feb. 8, 2005).

Bacteria that can ferment biomass to ethanol and other products include, e.g., *Zymomonas mobilis* and *Clostridium thermocellum* (Philippidis, 1996, *supra*). Leschine et al. (*International Journal of Systematic and Evolutionary Microbiology* 2002, **52**, 1155-1160) isolated an anaerobic, mesophilic, cellulolytic bacterium from forest soil, *Clostridium phytofermentans* sp. nov., which converts cellulose to ethanol.

Bio-processing, e.g., fermentation, of biomass to ethanol and other products may be carried out using certain types of thermophilic or genetically engineered microorganisms, such *Thermoanaerobacter* species, including *T. mathranii*, and yeast species such as *Pichia species*. An example of a strain of *T. mathranii* is A3M4 described in Sonne-Hansen et al. (*Applied Microbiology and Biotechnology* 1993, 38, 537-541) or Ahring et al. (*Arch. Microbiol.* 1997, 168, 114-119).

To aid in the breakdown of the materials that include the cellulose (treated by any method described herein or even untreated), one or more enzymes, e.g., a cellulolytic enzyme can be utilized. In some embodiments, the materials that include the cellulose are first treated with the enzyme, e.g., by combining the material and the enzyme in an aqueous solution. This material can then be combined with any microorganism described herein. In other embodiments, the materials that include the cellulose, the one or more enzymes and the microorganism are combined concurrently, e.g., by combining in an aqueous solution.

The carboxylic acid groups in these products generally lower the pH of the fermentation solution, tending to inhibit fermentation with some microorganisms, such as *Pichia stipitis*. Accordingly, it is in some cases desirable to add base and/or a buffer, before or during fermentation, to bring up the pH of the solution. For

example, sodium hydroxide or lime can be added to the fermentation medium to elevate the pH of the medium to range that is optimum for the microorganism utilized.

Fermentation is generally conducted in an aqueous growth medium, which can contain a nitrogen source or other nutrient source, e.g., urea, along with vitamins and trace minerals and metals. It is generally preferable that the growth medium be sterile, or at least have a low microbial load, e.g., bacterial count. Sterilization of the growth medium may be accomplished in any desired manner. However, in preferred implementations, sterilization is accomplished by irradiating the growth medium or the individual components of the growth medium prior to mixing. The dosage of radiation is generally as low as possible while still obtaining adequate results, in order to minimize energy consumption and resulting cost. For example, in many instances, the growth medium itself or components of the growth medium can be treated with a radiation dose of less than 5 Mrad, such as less than 4, 3, 2 or 1 Mrad. In specific instances, the growth medium is treated with a dose of between about 1 and 3 Mrad.

OTHER EMBODIMENTS

A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the disclosure.

For example, while the production of dried distillers grains and solubles (DDGS) is discussed above, in some cases the end product may instead be wet distillers grains and solubles (WDGS). While WDGS, with its high moisture content, is generally expensive to transport and subject to spoilage, in some cases it may be used, for instance where the livestock feed is to be used close to the ethanol manufacturing facility. Such applications are described, for example, in U.S. Patent No. 6,355,456, the full disclosure of which is incorporated herein by reference.

Either wet-milling or dry-milling processes may be used in the methods disclosed herein.

Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method comprising:

irradiating edible residues that have been produced as a by-product of an ethanol manufacturing process.

2. The method of claim 1 wherein the edible residues comprise distillers grains and solubles.

3. The method of claim 1 or 2 wherein the ethanol manufacturing process is a corn ethanol process.

4. The method of claim 1 wherein the edible residues comprise lignin, xylose and minerals.

5. The method of claim 4 wherein the edible residue further comprises undigested cellulose.

6. The method of claim 1, 4 or 5 wherein the ethanol manufacturing process utilizes a cellulosic feedstock and/or a lignocellulosic feedstock.

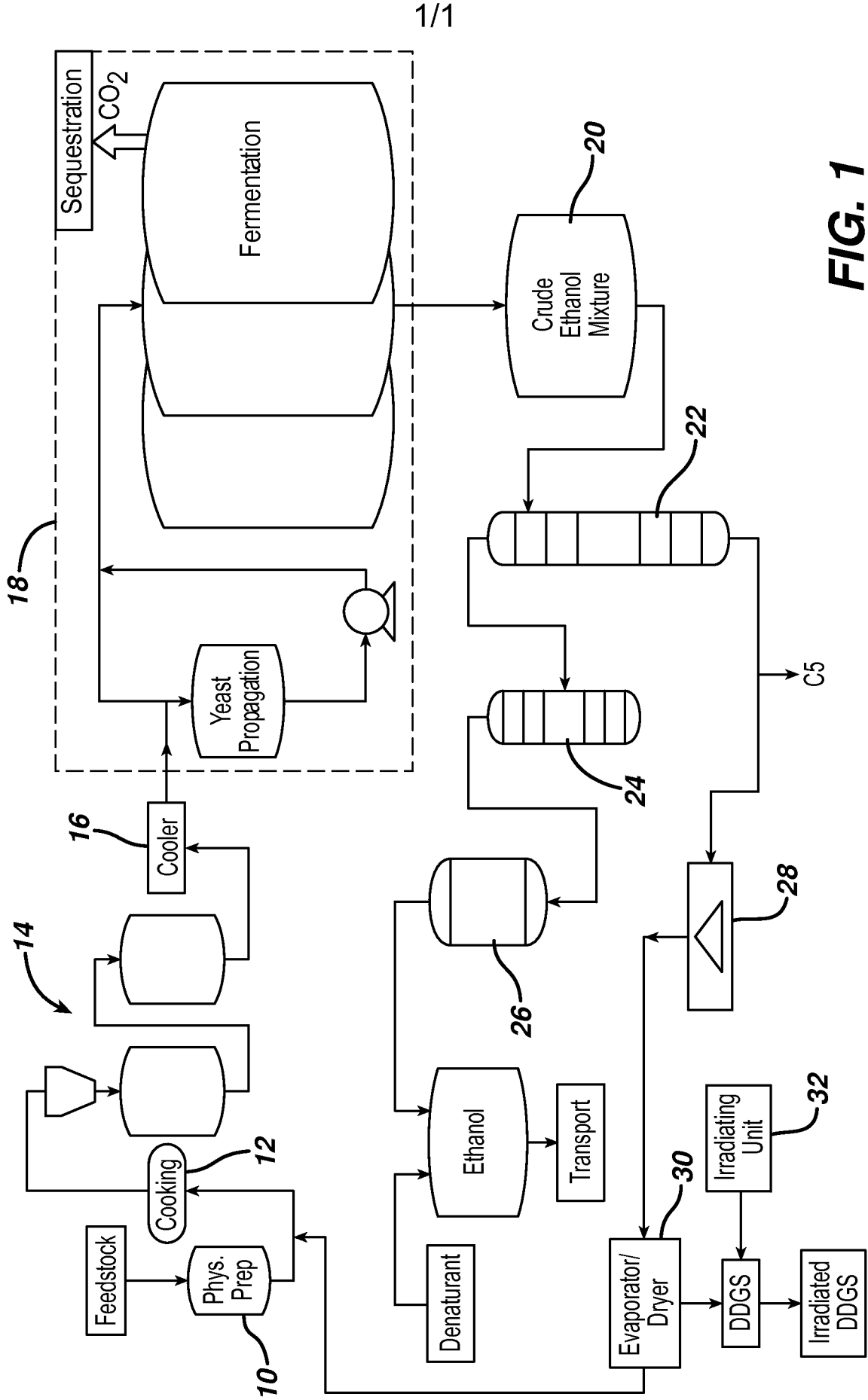
7. The method of claim any of the above claims wherein the edible residues contain an antibiotic, and irradiating is performed under conditions selected to inactivate or destroy the antibiotic.

8. The method of claim 7 wherein, after irradiation, the edible residues contain less than 100 ppm, such as less than 50, 25, 10, or 1 ppm, by weight of active antibiotic.

9. The method of claim 8 wherein the edible residues are substantially free of active antibiotic.

10. The method of claim 7 wherein prior to irradiation the edible residues contained from about 500 ppm to about 10,000 ppm by weight of active antibiotic.

11. The method of claim any of claims 1-6 wherein, prior to irradiation, the edible residues contain bacteria, and irradiation is performed under conditions that destroy the bacteria.
12. The method of claim 11 wherein the ethanol manufacturing process was conducted without addition of antibiotics.
13. The method of claim 2 wherein the distillers grains and solubles have been dried, producing dried distillers grains and solubles (DDGS).
14. The method of claim 13 wherein drying is performed prior to irradiation.
15. The method of any of the above claims, wherein irradiation is delivered at a dose of greater than about 0.5 MRad.
16. The method of any of the above claims, wherein irradiation is delivered at a dose of less than about 5Mrad.
17. The method of any of the above claims, wherein irradiation is delivered at a dose of from about 1 to about 3 Mrad.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/52382

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23K 1/06; C12P 7/10 (2010.01)

USPC - 435/165, 163, 161

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)

USPC - 435/165, 163, 161

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A23K 1/06; C12P 7/10 (2010.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, USOC, EPAB, JPAB), Google, Google Scholar: ethanol, fermentation, DDGS, corn, stalk, cob, lignin, xylose, animal, livestock, cattle, ruminant, feed, forage, analysis, composition

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	US 2009/0071066 A1 (Meier) 19 March 2009 (19.03.2009). Entire document, particularly para [0028], [0033], [0034], [0057]/Table 2.	1-3, 6/1, 13 and 14 --- 4, 5, 6/4 and 6/5
A	US 2009/0029432 A1 (Abbas et al.) 29 January 2009 (29.01.2009). Entire document, particularly para [0021].	4, 5, 6/4 and 6/5
A	US 2006/0286628 A1 (Everett et al.) 21 December 2006 (21.12.2006). Entire document, particularly para [0032].	4, 5, 6/4 and 6/5
A	US 2007/0037259 A1 (Hennessey et al.) 15 February 2007 (15.02.2007). Entire document, particularly para [0045], [0050], Table 3.	1-5, 6/1, 6/4, 6/5, 13 and 14
A	US 2006/0251764 A1 (Abbas et al.) 9 November 2006 (09.11.2006). Entire document, particularly para [0032].	1-5, 6/1, 6/4, 6/5, 13 and 14
A	Cao et al., ETHANOL PRODUCTION FROM CORN COB PRETREATED BY THE AMMONIA STEEPING PROCESS USING GENETICALLY ENGINEERED YEAST, BIOTECHNOLOGY LETTERS Volume 18 No. 9 (September 1996) pp.1013-1018.	1-5, 6/1, 6/4, 6/5, 13 and 14

☒ 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

19 November 2010 (19.11.2010)

Date of mailing of the international search report

14 DEC 2010

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Facsimile No. 571-273-3201

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/52382

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.: 7-12 and 15-17
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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/52382

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Mosier, Cellulosic Ethanol—Biofuel Beyond Corn, Purdue Agriculture, December 2006.	1-5, 6/1, 6/4, 6/5, 13 and 14
A	Gulati et al., ASSESSMENT OF ETHANOL PRODUCTION OPTIONS FOR CORN PRODUCTS, Bioresource Technology 58 (1996), 253-264.	1-5, 6/1, 6/4, 6/5, 13 and 14