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(71) **Applicant** *(for all designated States except US):* **QTER-OS, INC.** [US/US]; 100 Campus Drive, 6th Floor, Marlborough, MA 01752 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only):* **GRAY, Kevin** [US/US]; 114 Avalon Drive, Northborough, MA 01532 (US). **O'MULLAN, Patrick** [US/US]; 20 Lincoln Lane, Grafton, MA 01532 (US). **BOWMAN, Tim** [US/US]; 36 Jenks Drive, Amherst, MA 01002 (US). **WARNER, Ian** [US/US]; 12 Plymouth Street, Cambridge, MA 02141 (US).

(74) **Agents: VAN GOOR, David, W.** et al; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (US).

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(54) Title: NEW BIOCATALYSTS AND PRIMERS FOR PRODUCTION OF CHEMICALS

(57) Abstract: Provided are isolated *Clostridium phytofermentans* biocatalysts, *Clostridium phytofermentans* Q.12 and *Clostridium phytofermentans* Q.13, that can produce high yields of products. Also provided are primers useful to identify and quantify any strain of *Clostridium phytofermentans* . Further provided are methods of using the biocatalysts and primers to degrade organic material and for use in industrial processes.

NEW BIOCATALYSTS AND PRIMERS FOR PRODUCTION OF CHEMICALS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/415,787, filed November 19, 2010, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Increasing cost of petroleum-based transportation fuels, dwindling petroleum reserves and concerns over the environmental impact of petroleum-fuel combustion are driving a strong demand for viable alternatives to replace petroleum-based fuels. In particular, recent years have highlighted the promise of producing biofuels through bio-conversion of a variety of pretreated biomass material, such as lignocellulosic material, starch, or agriculture waste/byproducts, in combination with enzymes and yeast/bacterial systems. A particular challenge is developing technology with the potential to economically convert polysaccharide containing materials such as woody or non-woody plant material, algae, and nonvascular plants as well as waste materials and side products from the processing of plant or algal matter into high value transportation fuels and other energy forms or chemical feedstocks. Various examples of these polysaccharide containing materials include cellulosic, lignocellulosic, and hemicellulosic material; pectin containing material; starch; wood; corn stover; switchgrass; paper; and paper pulp sludge.

[0003] Various options for generating effective and sustainable biofuels and other biochemicals have been studied. Bioenergy sources can include alcohols, diesel, gases, bioelectricity (microbial fuel cells) and specialty chemicals. Ethanol fermentation from biomass including cellulosic, lignocellulosic, pectin, polyglucose and/or polyfructose containing biomass can provide much needed solutions for the world energy problem. Species of yeast, fungi and bacteria have been reported to be able to convert cellulosic biomass of its monomeric sugars to ethanol. However, many of these microorganisms grow slowly and/or produce ethanol only to low concentrations. This can be due to a general lack of tolerance to ethanol by the microorganism, or a feedback inhibition or suppression mechanism present in the microorganism, or to some other mechanism as well as some combination of these mechanisms. Such ethanol production issues can, in addition to affecting the ethanol titer, can also affect the ethanol productivity.

[0004] In the last few years genotypic approaches have proven to be useful for solving taxonomic problems regarding microorganisms; in fact, genotypic differences are considered more stable and more precise than phenotypic characteristics. These differences can be exploited to identify species strains and variants quickly and efficiently.

SUMMARY OF THE INVENTION

[0005] Disclosed herein are primers for amplifying a target polynucleotide sequence, wherein the target nucleotide sequence is from a *Clostridium phytofermentans* species or variant. In one embodiment, the target nucleotide sequence has at least about 80% identity to all or a part of SEQ ID NO:l 1, SEQ ID

NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence has at least about 90% identity to all or a part of SEQ ID NO:l 1, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence has at least about 99% identity to all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence comprises all or a part of SEQ ID NO:l 1, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the primer is between about 10 and about 50 nucleotides in length. In one embodiment, the primer is between about 15 and about 30 nucleotides in length. In one embodiment, the primer is SEQ ID NO:l, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO: 10.

[0006] Also disclosed herein are methods of detecting a *Clostridium phytofermentans* species or variant in a sample comprising: (a) providing a pair of primers, wherein each of the primers hybridize to a target nucleotide sequence from the *Clostridium phytofermentans* species or variant; (b) performing a polymerase chain reaction (PCR), wherein the PCR comprises a derivative of the sample and the pair of primers; and, (c) determining the presence or absence of the *Clostridium phytofermentans* species or variant based upon a level of a product of the PCR. In one embodiment, the target nucleotide sequence has at least about 80% identity to all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence has at least about 90%> identity to all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence has at least about 99%> identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the target nucleotide sequence comprises all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20. In one embodiment, the primer is between about 10 and about 50 nucleotides in length. In one embodiment, the primer is between about 15 and about 30 nucleotides in length. In one embodiment, the primer is SEQ ID NO:l, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO: 10. In one embodiment, the sample is an environmental sample. In one embodiment, the sample is from a fermentation reaction. In one embodiment, the sample is from a culture. In one embodiment, the sample is from a culture comprising two or more microorganisms. In one embodiment, the derivative of the sample comprises polynucleotides isolated from the sample. In one embodiment, the derivative of the sample comprises one or more microorganisms. Some embodiments

further comprise estimating a level of the *Clostridium phytofermentans* species or variant based upon the level of the product of the PCR.

[0007] Disclosed herein are isolated bacteria, wherein the bacteria are *Clostridium phytofermentans* Q.13 or *Clostridium phytofermentans* Q.12. In one embodiment, the bacterium is deposited under NRRL Accession Number NRRL B-·50436 or NRRL Accession Number NRRL B-50437. In one embodiment, the bacterium can hydrolyze polysaccharides. In one embodiment, the bacterium can hydrolyze hexose or pentose sugars. In one embodiment, the bacterium can utilize cellulose or xylose as its sole carbon source. In one embodiment, the bacterium can hydrolyze and ferment hemicellulosic or lignocellulosic material. In one embodiment, the bacterium produces alcohol dehydrogenase, wherein the alcohol dehydrogenase reduces acetaldehyde into ethanol. In one embodiment, the bacterium can produce ethanol at greater than 90% theoretical yield from a biomass. In one embodiment, the bacterium can produce ethanol at greater than 90% theoretical yield from a biomass comprising cellulosic, hemicellulosic, or lignocellulosic material.

Also disclosed herein are high-yielding mutants of *Clostridium phytofermentans* that produce ethanol at a rate of over 45 g/L from biomass.

[0008] Also disclosed herein are methodsof producing one or more fermentation end-products comprising: (a) providing a biomass in a media; (b) contacting the biomass with one or more microorganisms, wherein at least one of the microorganisms is *Clostridium phytofermentans* Q.12 or Q.13; and (c) allowing sufficient time for the microorganisms to hydrolyze and ferment the biomass to produce the fermentation end-products. In one embodiment, the biomass comprises hemicellulosic or lignocellulosic material In one embodiment, the biomass comprises corn, wheat, rice, barley, soybeans, bamboo, cotton, crambe, jute, sorghum, high biomass sorghum, oats, tobacco, grasses, miscanthus, switchgrass, trees, beans, rape/canola, alfalfa, flax, sunflowers, safflowers, millet, rye, sugarcane, sugar beets, cocoa, tea, Brassica sp., cotton, coffee, sweet potatoes, flax, peanuts, clover; lettuce, tomatoes, cucurbits, cassava, potatoes, carrots, radishes, peas, lentils, cabbages, cauliflower, broccoli, Brussels sprouts, grapes, peppers, apples, pears, peaches, apricots, walnuts, almonds, olives, avocadoes, bananas, coconuts, stillage, bagasse, leaves, pomace, distillers grains, Distillers Dried Solubles (DDS), Distillers Dried Grains (DDG), Condensed Distillers Solubles (CDS), Distillers Wet Grains (DWG), Distillers Dried Grains with Solubles (DDGS), peels, pits, fermentation waste, skins, straw, seeds, shells, beancake, sawdust, wood flour, wood pulp, paper pulp, paper pulp waste streams, rice or oat hulls, grass clippings, lumber, food leftovers, or a combination thereof. In one embodiment, the biomass is pretreated by acid, steam explosion, hot water treatment, alkali, catalase, or a detoxifying or chelating agent. In one embodiment, the fermentation end-products comprise one or more alcohols. In one embodiment, the fermentation end-products comprise ethanol. In one embodiment, the *Clostridium phytofermentans* Q.12 or Q.13 is deposited under NRRL Accession Numbers NRRL B-50436 or NRRL B-50437. In one embodiment, the biomass is pretreated to make the polysaccharides more available to the microorganisms.

[0009] Also disclosed herein are methods of hydrolyzing and fermenting a carbonaceous biomass wherein the biomass is contacted by a *C. phytofermentans* Q.12 or *C. phytofermentans* Q.13 bacterium for a period long enough to produce ethanol at greater than 90% theoretical yield from the carbonaceous biomass. In one embodiment, the contacting is at a temperature from about 30°C to about 40° C. In one embodiment, the contacting is at a temperature from about 35°C to about 39°C. In one embodiment, the contacting is at a pH from about 5.5 to about 7.5. In one embodiment, the bacterium uses biomass as a major carbon source. In one embodiment, the bacterium is deposited under NRRL Accession Numbers NRRL B–50436 or NRRL B-50437. In one embodiment, the bacterium is genetically-modified.

[0010] Also disclosed herein are methods for producing one or more fermentation end-product comprising: (a) culturing a medium comprising a non-recombinant or recombinant Q.12 or Q.13 bacterium for a period of time under conditions suitable for production of one or more fermentation end-products by the Q.12 or Q.13 bacterium; and (b) harvesting the fermentation end-products from the medium. In one embodiment, the Q.12 or Q.13 bacterium is a mesophile. In one embodiment, the fermentation end-products comprise an alcohol. In one embodiment, the fermentation end-products comprise ethanol. In one embodiment, the medium comprises a cellulosic and/or lignocellulosic material. In one embodiment, the cellulosic or lignocellulosic material is not enzymatically treated with a sufficient quantity of enzymes to convert more than 15% of the cellulosic or lignocellulosic material to simple sugars within 24 hours. In one embodiment, the cellulosic or lignocellulosic material is pretreated with acid, hot water, steam explosion, alkali, a chelating agent and/or a detoxifying agent. In one embodiment, a second microorganism is added to the medium.

[0011] Also disclosed herein are fuel plants comprising a fermenter configured to house a medium and a strain of Q.12 or Q.13 bacteria, wherein the fermenter comprises a cellulosic or lignocellulosic material. In one embodiment, the cellulosic or lignocellulosic material is pretreated.

Also disclosed herein are sets of primer pairs for amplifying a polynucleotide of a *C. phytofermentans* bacteria, wherein the primer pairs are SEQ ID NO:l/SEQ ID NO:2, SEQ ID NO:3/SEQ ID NO:4, SEQ ID NO:5/SEQ ID NO:6, SEQ ID NO:7/SEQ ID NO:8, or SEQ ID NO:9/SEQ ID NO: 10. In one embodiment, the bacteria are mutants, variants, or recombinant.

[0012] Also disclosed herein are methods for detecting a *Clostridium phytofermentans* bacteria in a sample comprising: performing a PCR reaction comprising the sample in the presence of forward primers selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, and SEQ ID NO: 9 and mixtures thereof and reverse primers selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 and mixtures thereof to amplify a PCR product, wherein the presence or absence of the PCR product indicates the presence or absence of the *Clostridium phytofermentans* bacteria in the sample. In one embodiment, the sample is an environmental sample. In one embodiment, the sample is taken from a fermentation production operation. In one embodiment, the sample is taken from a culture operation.

[0013] Also disclosed herein are assays to detect a *C. phytofermentans* bacteria in a culture of microorganisms comprising: (a) extracting a fixed volume from the culture of microorganisms; (b) performing a PCR reaction comprising the sample in the presence of any of the primer pairs selected from the group consisting of SEQ ID NO:l / SEQ ID NO:2 , SEQ ID NO: 3/ SEQ ID NO:4, SEQ ID NO: 5/ SEQ ID NO:6, SEQ ID NO:7 / SEQ ID NO:8, and SEQ ID NO:9 / SEQ ID NO: 10 to amplify a PCR product; and (c) determining the presence of the *C. phytofermentans* in the culture based on the level of the PCR product.

[0014] Also disclosed herein are assays to identify *C. phytofermentans* in a culture of microorganisms comprising: (a) extracting a fixed volume from the culture of microorganisms; (b) amplifying a polynucleotide in the fixed volume with any of the primer pairs selected from the group consisting of SEQ ID NO:l / SEQ ID NO:2 , SEQ ID NO: 3/ SEQ ID NO:4, SEQ ID NO: 5/ SEQ ID NO:6, SEQ ID NO:7 / SEQ ID NO:8, and SEQ ID NO:9 / SEQ ID NO: 10; and (c) calculating the correlation between the amount of the amplified polynucleotide and the cell density to determine the *C phytofermentans* and non-C. *phytofermentans* cell densities in the culture. In one embodiment the method comprises PCR amplification.

[0015] Also disclosed herein are methods for quantification of a *Clostridium* bacteria comprising hybridizing an oligonucleotide of the *Clostridium* to any of SEQ ID NO: 1 through 10. In one embodiment, the method comprises array hybridization.

INCORPORATION BY REFERENCE

[0016] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0018] **Figure 1** shows Q.13 ethanol production on cellobiose over time compared to Q.8.

[0019] **Figure 2** shows Q.12 ethanol production on cellobiose over time compared to Q.8.

[0020] **Figure 3** illustrates primer pairs of C. *phytofermentans.*

[0021] **Figure 4** depicts a method for producing fermentation end products from biomass by first treating biomass with an acid at elevated temperature and pressure in a hydrolysis unit.

[0022] **Figure 5** depicts a method for producing fermentation end products from biomass by charging biomass to a fermentation vessel.

[0023] **Figure 6** discloses pretreatments that produce hexose or pentose saccharides or oligomers that are then unprocessed or processed further and either fermented separately or together.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present disclosure can be understood more readily by reference to the following detailed description, the Examples included therein and to the Figures and their previous and following description.

[0025] Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this disclosure is not limited to specific synthetic methods, specific purified proteins, or to particular nucleic acids, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0026] In one embodiment a wild type or a genetically improved microorganism can be used for alcohol production by fermentation. For example *Clostridium phytofermentans* , *Thermoanaerobacter ethanolicus, Clostridium thermocellum, Clostridium beijerinickii, Clostridium acetobutylicum, Clostridium tyrobutyricum, Clostridium thermobutyricum, Thermoanaerobacterium saccharolyticum, Thermoanaerobacter thermohydrosulfuricus* , and *Saccharomyces cerevisiae, Clostridium acetobutylicum, Moorella ssp. , Carboxydocella ssp. , Zymomonas mobilis,* recombinant *E. Coli, Klebsiella oxytoca* and *Clostridium beijerickii* as well as other microorganisms.

[0027] In one embodiment a microorganism that hydrolyzes and ferments polysaccharides can be used as a biocatalyst in the biofuel or biochemical industry. In one embodiment the microorganism that both hydrolyzes and ferments biomass efficiently is *Clostridium phytofermentans* ($ISDg^T$, American Type Culture Collection 700394^T). See U.S. Patent No. 7,682,81 1 B2. In another embodiment a microorganism is genetically modified to express one or more proteins that modulate the activity of a metabolic pathway to produce energy-rich products from the conversion of carbohydrates. See, *e.g.,* Lynd, *et al.* Curr. Opinion Biotechnol. 16:577-583 (2005). In another embodiment strain development provides processes by which new microorganisms can be derived and screened that possess the attributes for enhanced yields on industrial scales.

[0028] In another embodiment, processes that identify such microorganisms can be useful in screening of other microorganisms that show the same basic characteristics. Routine procedures for microbial species identification rely on examination of the colony (pigmentation of the surface and reverse sides, topography, texture, and rate of growth) and microscopic morphology (size and shape of cells and spores) and staining (gram-positive v. gram-negative). Further identification characteristics include nutritional requirements (vitamins and amino acids) and temperature tolerance, as well as product production, *etc.* Morphological and physiological characteristics can frequently vary; in fact, the phenotypic features can be easily influenced by outside factors such as temperature variation, medium, and chemotherapy and therefore strain identification is often difficult.

[0029] Definitions

[0030] Unless characterized otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0031] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a purified polypeptide" includes mixtures of two or more purified polypeptides.

[0032] The term "about" as used herein refers to a range that is 15% plus or minus from a stated numerical value within the context of the particular usage. For example, about 10 would indicate a range from 8.5 to 11.5. All numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that can vary depending upon the desired properties sought to be obtained

[0033] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

[0034] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase "the medium can optionally contain glucose" means that the medium may or may not contain glucose as an ingredient and that the description includes both media containing glucose and media not containing glucose.

[0035] In one embodiment, provided herein are isolated Gram-positive *Clostridium phytofermentans* bacterial strains, wherein the bacteria are obligate anaerobic, mesophilic, cellulolytic microorganisms that can use polysaccharides as a sole carbon source and can oxidize glucose into ethanol or one or more organic acids as its fermentation product. In another embodiment, provided herein are bacteria designated *Clostridium phytofermentans* Q.12 or *Clostridium phytofermentans* Q.13, having the NRRL patent deposit designations NRRL B-50436 or NRRL B-50437, respectively. As used herein, "obligate" means required or compulsory. As used herein, a "mesophilic" is a bacterium that preferentially ferments a carbon source at about 30-40° C. Q.12 or Q.13 consist of motile rods that form terminal spores.

[0036] In one embodiment, the bacteria Q.12 or Q.13 are obligate anaerobic mesophiles that can ferment biomass or carbonaceous material into ethanol, organic acids and other fermentation end products. For example, these bacteria can degrade cellulose and/or xylose into ethanol and acetic or lactic acid.

[0037] It is understood that as discussed herein, the terms "similar" or "similarity" mean the same thing as "homology" and "identity." Thus, for example, if the use of the word homology is used to refer to two non-natural sequences, it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid or amino acid sequences. Many of the methods for determining similarity between two evolutionarily

related molecules are routinely applied to any two or more nucleic acids or polypeptides for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related.

[0038] The term "increased" or "increasing" as used herein, refers to the ability of one or more recombinant microorganisms to produce a greater amount of a given product or molecule *(e.g.,* commodity chemical, biofuel, or intermediate product thereof) as compared to a control microorganism, such as an unmodified microorganism or a differently-modified microorganism. An "increased" amount is typically a "statistically significant" amount, and can include an increase that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (including all integers and decimal points in between, *e.g.,* 1.5, 1.6, 1.7. 1.8, *etc.)* the amount produced by an unmodified microorganism or a differently modified microorganism.

[0039] In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed nucleic acids and polypeptides herein, is through defining the variants and derivatives in terms of similarity, or homology, to specific known sequences. In general, variants of nucleic acids and polypeptides herein disclosed can typically have at least, about 50%, 51%, 52%>, 53%>, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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%, 99%, or 100% percent similarity, or homology, to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the similarity of two polypeptides or nucleic acids. For example, the similarity can be calculated after aligning the two sequences so that the similarity is at its highest level.

[0040] Another way of calculating similarity, or homology, can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.; the BLAST algorithm of Tatusova and Madden FEMS Microbiol. Lett. 174: 247-250 (1999) available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html)), or by inspection.

[0041] The same types of similarity, or homology, can be obtained for nucleic acids by, for example, the algorithms disclosed in Zuker, M. Science 244:48-52, 1989, Jaeger et al. Proc. Natl. Acad. Sci. USA 86:77067710, 1989, Jaeger et al. Methods Enzymol. 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods can differ, but the skilled artisan understands if similarity is found with at least one of these methods, the sequences would be understood to have the stated similarity.

[0042] For example, as used herein, a sequence recited as having a particular percent similarity to another sequence refers to sequences that have the recited similarity as calculated by any one or more of

the calculation methods described above. For example, a first sequence has 80 percent similarity, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent similarity to the second sequence using the Zuker calculation method even if the first sequence does not have 80 percent similarity to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent similarity, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent similarity to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent similarity to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent similarity, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent similarity to the second sequence using each of the calculation methods (although, in practice, the different calculation methods will often result in different calculated similarity percentages).

[0043] As used herein, the term "nucleic acid" refers to single or multiple stranded molecules which can be DNA or RNA, or any combination thereof, including modifications to those nucleic acids. The nucleic acid can represent a coding strand or its complement, or any combination thereof. Nucleic acids can be identical in sequence to the sequences which are naturally occurring for any of the moieties discussed herein or can include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence. These nucleic acids can also be modified from their typical structure. Such modifications include, but are not limited to, methylated nucleic acids, the substitution of a non-bridging oxygen on the phosphate residue with either a sulfur (yielding phosphorothioate deoxynucleotides), selenium (yielding phosphorselenoate deoxynucleotides), or methyl groups (yielding methylphosphonate deoxynucleotides), a reduction in the AT content of AT rich regions, or replacement of non-preferred codon usage of the expression system to preferred codon usage of the expression system. The nucleic acid can be directly cloned into an appropriate vector, or if desired, can be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers which contain restriction sites to the termini of the nucleic acid. General methods are set forth in Sambrook et al. (2001) Molecular Cloning—A Laboratory Manual (3rd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook).

[0044] The nucleic acid can be detected with a probe capable of hybridizing to the nucleic acid of a cell or a sample. This probe can be a nucleic acid comprising the nucleotide sequence of a coding strand or its complementary strand or the nucleotide sequence of a sense strand or antisense strand, or a fragment thereof The nucleic acid can comprise the nucleic acid of the bacterial genome, or fragments thereof In one embodiment, the probe can be either DNA or RNA and can bind either DNA or RNA, or both, in the biological sample.

[0045] In one embodiment, the nucleic acid of SEQ ID NO:l, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10 or any

of the other disclosed nucleic acid, and fragments thereof, is utilized as probes or primers to detect nucleic acids of the disclosed bacterium *(e.g.,* sequences of the Q.12 or Q.13 16S rRNA). In one embodiment, a polynucleotide probe or primer comprising at least 15 contiguous nucleotides can be utilized to detect a nucleic acid of the disclosed bacterial strains. In one embodiment, the polynucleotide probe or primer can be at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or at least 200 nucleotides in length.

[0046] As used herein, the term "nucleic acid probe" refers to a nucleic acid fragment that selectively hybridizes under stringent conditions with a nucleic acid comprising a nucleic acid set forth in a sequence listed herein. This hybridization can be specific. The degree of complementarity between the hybridizing nucleic acid and the sequence to which it hybridizes should be at least enough to exclude hybridization with a nucleic acid encoding an unrelated protein.

[0047] As used herein, the term "primer" refers to a single-stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a nucleic acid molecule.

[0048] The terms "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, rRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0049] As will be understood by those skilled in the art, a polynucleotide sequence can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or can be adapted to express, proteins, polypeptides, peptides and the like. Such segments can be naturally isolated, or modified synthetically by the hand of man.

[0050] The terms "polynucleotide variant" and "variant" and the like refer to polynucleotides that display substantial sequence identity with any of the reference polynucleotide sequences or genes described herein, and to polynucleotides that hybridize with any polynucleotide reference sequence described herein, or any polynucleotide coding sequence of any gene or protein referred to herein, under low stringency, medium stringency, high stringency, or very high stringency conditions that are defined hereinafter and known in the art. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide, or has increased activity in relation to the reference polynucleotide *(e.g.,* optimized). Polynucleotide variants include, for example, polynucleotides

having at least 50% (and at least 51% to at least 99% and all integer percentages in between) sequence identity with a reference polynucleotide described herein.

[0051] The terms "polynucleotide variant" and "variant" also include naturally-occurring allelic variants that encode these enzymes. Examples of naturally-occurring variants include allelic variants (same locus), homologs (different locus), and orthologs (different microorganism). Naturally occurring variants such as these can be identified and isolated using well-known molecular biology techniques including, for example, various polymerase chain reaction (PCR) and hybridization-based techniques as known in the art. Naturally-occurring variants can be isolated from any microorganism that encodes one or more genes having a suitable enzymatic activity described herein *(e.g.,* C-C ligase, diol dehydrogenase, pectate lyase, alginate lyase, diol dehydratase, transporter, *etc.).*

[0052] Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or microorganisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. In certain aspects, non-naturally occurring variants can have been optimized for use in a given microorganism *(e.g.,* E. coli), such as by engineering and screening the enzymes for increased activity, stability, or any other desirable feature. The variations can produce both conservative and non-conservative amino acid substitutions (as compared to the originally encoded product). For polynucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of a reference polypeptide. Variant polynucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a biologically active polypeptide. Variants of a reference polynucleotide sequence can have at least about 30%, 40% 50%, 55%, 60%, 65%, 70%, 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% or more sequence identity with the reference polynucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters. In one embodiment a variant polynucleotide sequence encodes a protein with substantially similar activity compared to a protein encoded by the respective reference polynucleotide sequence. Substantially similar activity means variant protein activity that is within +/- 15% of the activity of a protein encoded by the respective reference polynucleotide sequence. In another embodiment a variant polynucleotide sequence encodes a protein with greater activity compared to a protein encoded by the respective reference polynucleotide sequence.

[0053] In one embodiment a method is disclosed which uses variants of full-length polypeptides having any of the enzymatic activities described herein, truncated fragments of these full-length polypeptides, variants of truncated fragments, as well as their related biologically active fragments. Typically, biologically active fragments of a polypeptide can participate in an interaction, for example, an intra-molecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction *(e.g.,* the interaction can be transient and a covalent bond is

formed or broken). Biologically active fragments of a polypeptide/enzyme an enzymatic activity described herein include peptides comprising amino acid sequences sufficiently similar to, or derived from, the amino acid sequences of a (putative) full-length reference polypeptide sequence. Typically, biologically active fragments comprise a domain or motif with at least one enzymatic activity, and can include one or more (and in some cases all) of the various active domains. A biologically active fragment of an enzyme can be a polypeptide fragment which is, for example, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 450, 500, 600 or more contiguous amino acids, including all integers in between, of a reference polypeptide sequence. In certain embodiments, a biologically active fragment comprises a conserved enzymatic sequence, domain, or motif, as described elsewhere herein and known in the art. Suitably, the biologically-active fragment has no less than about 1%, 10%, 25%, or 50% of an activity of the wild-type polypeptide from which it is derived. Additional methods for genetic modification can be found in U.S. Patent Publication US20100086981A1, which is herein incorporated by reference in its entirety.

[0054] The term "exogenous" as used herein, refers to a polynucleotide sequence or polypeptide that does not naturally occur in a given wild-type cell or microorganism, but is typically introduced into the cell by a molecular biological technique, *e.g.,* engineering to produce a recombinant microorganism. Examples of "exogenous" polynucleotides include vectors, plasmids, and/or man-made nucleic acid constructs encoding a desired protein or enzyme.

[0055] The term "endogenous" as used herein, refers to naturally- occurring polynucleotide sequences or polypeptides that can be found in a given wild-type cell or microorganism. For example, certain naturally- occurring bacterial or yeast species do not typically contain a benzaldehyde lyase gene, and, therefore, do not comprise an "endogenous" polynucleotide sequence that encodes a benzaldehyde lyase. In this regard, it is also noted that even though a microorganism can comprise an endogenous copy of a given polynucleotide sequence or gene, the introduction of a plasmid or vector encoding that sequence, such as to over-express or otherwise regulate the expression of the encoded protein, represents an "exogenous" copy of that gene or polynucleotide sequence. Any of the pathways, genes, or enzymes described herein can utilize or rely on an "endogenous" sequence, or can be provided as one or more "exogenous" polynucleotide sequences, and/or can be used according to the endogenous sequences already contained within a given microorganism.

[0056] The term "heterologous" as used herein, refers to an exogenous polynucleotide sequence, an additional copy of an endogenous polynucleotide sequence, or a polypeptide encoded by either.

[0057] The term "sequence identity" for example, comprising a "sequence 50%> identical to," as used herein, refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" can be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base *(e.g.,* A, T, C, G, I) or the identical amino

acid residue *(e.g.,* Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gin, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison *(e.g.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0058] The term "transformation" as used herein, refers to the permanent, heritable alteration in a cell resulting from the uptake and incorporation of foreign DNA into the host-cell genome. This includes the transfer of an exogenous gene from one microorganism into the genome of another microorganism as well as the addition of additional copies of an endogenous gene into a microorganism.

[0059] The term "vector" as used herein, refers to a polynucleotide molecule, such as a DNA molecule. It can be derived, from a plasmid, bacteriophage, yeast or virus, into which a polynucleotide can be inserted or cloned. A vector can contain one or more restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, *e.g.,* a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, *e.g.,* a linear or closed circular plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Such a vector can comprise specific sequences that allow recombination into a particular, desired site of the host chromosome. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. A vector can be one which is operably functional in a bacterial cell, such as a cyanobacterial cell. The vector can include a reporter gene, such as a green fluorescent protein (GFP), which can be either fused in frame to one or more of the encoded polypeptides, or expressed separately. The vector can also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants.

[0060] The terms "wild-type" and "naturally-occurring" as used herein are used interchangeably to refer to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild type gene or gene product *(e.g.,* a polypeptide) is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

[0061] "Stringent conditions" refers to the washing conditions used in a hybridization protocol. In general, the washing conditions should be a combination of temperature and salt concentration chosen so that the denaturation temperature is approximately 5° C. to 20° C. below the calculated Tm of the nucleic acid hybrid under study. In one embodiment, the denaturation temperature is approximately 5° C , 6° C ,

7° C , 8° C., 9° C., 10° C , 11° C., 12° C , 13° C., 14° C., 15° C., 16° C , 17° C., 18° C , 19° C., or 20° C. below the calculated Tm of the nucleic acid hybrid under study. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to the probe or polypeptide-coding nucleic acid of interest and then washed under conditions of different stringencies. The Tm of such an oligonucleotide can be estimated by allowing 20° C for each A or T nucleotide, and 4 °C for each G or C. For example, an 18 nucleotide probe of 50% G+C would, therefore, have an approximate Tm of 54° C. Stringent conditions are known to one of skill in the art. See, for example, Sambrook et al. (2001). The following is an exemplary set of hybridization conditions and is not limiting:

[0062] Very High Stringency

[0063] Hybridization: 5XSSC at 65° C. for 16 hours. Wash twice: 2XSSC at room temperature (RT) for 15 minutes each. Wash twice: 0.5XSSC at 65° C. for 20 minutes each.

[0064] High Stringency

[0065] Hybridization: 5X-6XSSC at 65°C-70° C. for 16-20 hours. Wash twice: 2XSSC at RT for 5-20 minutes each. Wash twice: 1XSSC at 55° C.-70° C. for 30 minutes each.

[0066] Low Stringency

[0067] Hybridization: 6XSSC at RT to 55° C. for 16-20 hours. Wash at least twice: 2X-3XSSC at RT to 55° C. for 20-30 minutes each.

[0068] In one embodiment, provided is a method of fermenting a carbonaceous material, comprising contacting the carbonaceous material with an effective, fermenting amount of an isolated Gram-positive bacterium, wherein the bacterium is an anaerobic, obligate mesophile, wherein the bacterium can use polysaccharides as a sole carbon source and can reduce acetaldehyde into ethanol, whereby contacting the carbonaceous material with the bacterium ferments the carbonaceous material. As used herein, an "effective amount" is within the knowledge of one skilled in the art. Various methods are known by which a person of skill can determine the amount of bacteria useful to effectively ferment a carbonaceous material, *e.g.,* biomass, of interest. The carbonaceous materials can be any one or more of the materials disclosed herein. In one aspect, the bacterial strain is Q.12. In another aspect, the bacterial strain is Q.13.

[0069] In one embodiment, the contacting step of the disclosed method occurs at a pH of from about 5.0 to about 7.5. In one embodiment, the contacting step occurs at a pH of from about 6.0 to about 6.5. The contacting step can occur at a pH of about 5, 6, 7, or 8. In one embodiment, the method disclosed is be carried out at a temperature from about 30° C to about 40° C In one embodiment, the disclosed method is be carried out at a temperature from about 35° C to about 37° C Additional temperatures at which the disclosed method can be carried out are 31° C, 32° C, 33° C, 34° C, 35° C, 36° C, 37° C, 38° C, and 39° C.

[0070] In one embodiment, provided herein is a method of growing an isolated Gram-positive bacterium, designated Q.12 or Q.13 and deposited under NRRL Accession Nos. B-50436 andNRRL B-50437, respectively. In another embodiment, the bacterial strains, designated Q.12 or Q.13, and deposited

under NRRL Accession No. B-50436 or NRRL Accession No. B-50437 are provided. In one embodiment, the bacterium is an anaerobic, obligate mesophile, wherein the bacterium can use cellulose as a sole carbon source and can oxidize acetaldehyde into ethanol, comprising culturing the bacterium at a temperature and on a medium effective to promote growth of the bacterium. The bacterium can grow at a temperature from about 30° C to about 40° C In one aspect, the bacterium can grow at a temperature from about 35° C to about 37° C Further, the bacterium can grow on medium wherein the pH is from about 5.0 to about 7.5. In one aspect, the pH of the medium can be from about 6.0 to about 6.5. Media are currently known that are effective in promoting growth of the disclosed bacterium. Therefore, a person of skill would know which media would be effective in promoting the growth of the bacterium

[0071] "Fermentation end-product" is used herein to include biofuels, chemicals, and compounds suitable as liquid fuels, gaseous fuels, reagents, chemical feedstocks, chemical additives, processing aids, food additives, and other products. Examples of fermentation end-products include but are not limited to 1,4 diacids (succinic, fumaric and malic), 2,5 furan dicarboxylic acid, 3 hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyro lactone, glycerol, sorbitol, xylitol/arabinitol, butanediol, butanol, methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-l -butene, 4-phenyl-2-butene, l-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, l-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, l-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl)ethanol, 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-l -butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)- 1-butene, l-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, l-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, l-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, l-(4-hydroxyphenyl)-2,3-butanonedione, indolylethane, indolylethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl- 1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl- 1-pentene, l-phenyl-2-pentene, 1-phenyl-3-pentene, l-phenyl-2-pentanol, l-phenyl-3-pentanol, l-phenyl-2-pentanone, l-phenyl-3-pentanone, l-phenyl-2,3-pentanediol, l-phenyl-2-hydroxy-3-pentanone, l-phenyl-3-hydroxy-2-pentanone, l-phenyl-2,3-pentanedione, 4-methyl- 1-phenylpentane, 4-methyl-l -phenyl-l -pentene, 4-methyl- 1-phenyl-2-pentene, 4-methyl- 1-phenyl-3-pentene, 4-methyl- 1-phenyl-3-pentanol, 4-methyl- 1-

phenyl-2-pentanol, 4-methyl-l-phenyl-3-pentanone, 4-methyl- l-phenyl-2-pentanone, 4-methyl- 1-phenyl-2,3-pentanediol, 4-methyl-l-phenyl-2,3-pentanedione, 4-methyl-l-phenyl-3-hydroxy-2-pentanone, 4-methyl- 1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)- 1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, l-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, l-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl- 1-(4-hydroxyphenyl) pentane, 4-methyl- 1-(4-hydroxyphenyl)-2-pentene, 4-methyl- 1-(4-hydroxyphenyl)-3-pentene, 4-methyl- 1-(4-hydroxyphenyl)- 1-pentene, 4-methyl- 1-(4-hydroxyphenyl)-3-pentanol, 4-methyl- 1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-l-(4-hydroxyphenyl)-3-pentanone, 4-methyl- l-(4-hydroxyphenyl)-2-pentanone, 4-methyl-l-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-l-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-l-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-l-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, l-indole-3-pentane, l-(indole-3)-l -pentene, l-(indole-3)-2-pentene, l-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, l-(indole-3)-3-pentanol, l-(indole-3)-2-pentanone, l-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, l-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-l-(indole-3-)pentane, 4-methyl- l-(indole-3)-2-pentene, 4-methyl-l-(indole-3)-3-pentene, 4-methyl- l-(indole-3)-l -pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-l-(indole-3)-2-pentanol, 4-methyl-l-(indole-3)-3-pentanone, 4-methyl- l-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl- l-(indole-3)-2,3-pentanedione, 4-methyl-l-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-l-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl- 1-hexene, 5-methyl-2-hexene, 4-methyl- 1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl- 1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2.3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3.4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl- 1-phenylhexane, 4-methyl-l-phenylhexane, 5-methyl- 1-phenyl- 1-hexene, 5-methyl- 1-phenyl-2-hexene, 5-methyl- 1-phenyl-3-hexene, 4-methyl- 1-phenyl- 1-hexene, 4-methyl- 1-phenyl-2-hexene, 4-methyl- 1-phenyl-3-hexene, 5-methyl- 1-phenyl-2-hexanol, 5-methyl-l-phenyl-3-hexanol, 4-methyl- 1-phenyl-2-hexanol, 4-methyl- 1-phenyl-3-hexanol, 5-methyl- 1-phenyl-2-hexanone, 5-methyl- 1-phenyl-3-hexanone, 4-methyl- 1-phenyl-2-hexanone, 4-methyl- 1-phenyl-3-hexanone, 5-methyl- 1-phenyl-2,3-hexanediol, 4-methyl- 1-phenyl-2,3-hexanediol, 5-

methyl- 1-phenyl-3 -hydroxy-2-hexanone, 5-methyl- 1-phenyl-2-hydroxy-3 -hexanone, 4-methyl- 1-phenyl-3-hydroxy-2-hexanone, 4-methyl-l-phenyl-2-hydroxy-3-hexanone, 5-methyl-l-phenyl-2,3-hexanedione, 4-methyl-l-phenyl-2,3-hexanedione, 4-methyl- l-(4-hydroxyphenyl)hexane, 5-methyl-l -(4-hydroxyphenyl)- 1-hexene, 5-methyl- 1-(4-hydroxyphenyl)-2-hexene, 5-methyl- 1-(4-hydroxyphenyl)-3-hexene, 4-methyl- 1-(4-hydroxyphenyl)- 1-hexene, 4-methyl- 1-(4-hydroxyphenyl)-2-hexene, 4-methyl- 1-(4-hydroxyphenyl)-3 -hexene, 5-methyl- 1-(4-hydroxyphenyl)-2-hexanol, 5-methyl- 1-(4-hydroxyphenyl)-3-hexanol, 4-methyl- l-(4-hydroxyphenyl)-2-hexanol, 4-methyl- l-(4-hydroxyphenyl)-3-hexanol, 5-methyl- 1-(4-hydroxyphenyl)-2-hexanone, 5-methyl- 1-(4-hydroxyphenyl)-3 -hexanone, 4-methyl- 1-(4-hydroxyphenyl)-2-hexanone, 4-methyl- 1-(4-hydroxyphenyl)-3 -hexanone, 5-methyl- 1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-l-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-l -(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-l -(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl- 1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl- l-(4-hydroxyphenyl)-2-hydroxy-3 -hexanone, 5-methyl-l-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-l-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-l-(indole-3-)hexane, 5-methyl- l-(indole-3)-l -hexene, 5-methyl- l-(indole-3)-2-hexene, 5-methyl-l-(indole-3)-3-hexene, 4-methyl-l-(indole-3)-l -hexene, 4-methyl- l-(indole-3)-2-hexene, 4-methyl-l-(indole-3)-3 -hexene, 5-methyl- l-(indole-3)-2-hexanol, 5-methyl- l-(indole-3)-3-hexanol, 4-methyl- 1-(indole-3)-2-hexanol, 4-methyl-l-(indole-3)-3-hexanol, 5-methyl-l -(indole-3)-2-hexanone, 5-methyl-l -(indole-3)-3 -hexanone, 4-methyl- l-(indole-3)-2-hexanone, 4-methyl-l-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-l-(indole-3)-2,3-hexanediol, 5-methyl- l-(indole-3)-3 -hydroxy-2-hexanone, 5-methyl-l -(indole-3)-2-hydroxy-3-hexanone, 4-methyl-l-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-l-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-l -(indole-3)-2,3-hexanedione, 4-methyl-l-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2.5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2.6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-

heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedionen-undecane, 1-undecene, 1-undecanol, undecanal, undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decadecene, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1,3-propanediol, 4-hydroxybutanal, 1,4-butanediol, 3-hydroxy-2-butanone, 2,3-butandiol, 1,5-pentane diol, homocitrate, homoisocitorate, b-hydroxy adipate, glutarate, glutarsemialdehyde, glutaraldehyde, 2-

hydroxy- 1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2.3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, l,10-diamino-5-decene, l,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetoaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-l -butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, l,4-diphenyl-2,3-butanediol, l,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydeoxyphenyl)-4-phenylbutane, 1-(4-hydeoxyphenyl)-4-phenyl- 1-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanol, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanone, 1-(4-hydeoxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydeoxyphenyl)-4-phenyl-3 -hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl- 1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetoaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)- 1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1.4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3-)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-l -butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetoaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)- 1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, l,4-di(indole-3)-2,3-butanediol, l,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-l ,8-dicarboxylic acid, 3-hydroxy-hexane-l ,8-dicarboxylic acid, 3-hexanone-l ,8-dicarboxylic acid, 3,4-hexanediol-l ,8-dicarboxylic acid, 4-hydroxy-3-hexanone-l ,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, phosphate, lactic acid, acetic acid, formic acid, isoprenoids, and polyisoprenes, including rubber. Further, such products can include succinic acid, pyruvic acid, enzymes such as cellulases, polysaccharases, lipases, proteases, ligninases, and hemicellulases and can be present as a pure compound, a mixture, or an impure or diluted form.

[0072] The term "fatty acid comprising material" as used herein has its ordinary meaning as known to those skilled in the art and can comprise one or more chemical compounds that include one or more fatty acid moieties as well as derivatives of these compounds and materials that comprise one or more of these compounds. Common examples of compounds that include one or more fatty acid moieties include triacylglycerides, diacylglycerides, monoacylglycerides, phospholipids, lysophospholipids, free fatty acids, fatty acid salts, soaps, fatty acid comprising amides, esters of fatty acids and monohydric alcohols, esters of fatty acids and polyhydric alcohols including glycols *(e.g.,* ethylene glycol, propylene glycol, *etc.),* esters of fatty acids and polyethylene glycol, esters of fatty acids and polyethers, esters of fatty acids and polyglycol, esters of fatty acids and saccharides, esters of fatty acids with other hydroxyl-containing compounds, *etc.* A fatty acid comprising material can be one or more of these compounds in an isolated

or purified form. It can be a material that includes one or more of these compounds that is combined or blended with other similar or different materials. It can be a material where the fatty acid comprising material occurs with or is provided with other similar or different materials, such as vegetable and animal oils; mixtures of vegetable and animal oils; vegetable and animal oil byproducts; mixtures of vegetable and animal oil byproducts; vegetable and animal wax esters; mixtures, derivatives and byproducts of vegetable and animal wax esters; seeds; processed seeds; seed byproducts; nuts; processed nuts; nut byproducts; animal matter; processed animal matter; byproducts of animal matter; corn; processed corn; corn byproducts; distiller's grains; beans; processed beans; bean byproducts; soy products; lipid containing plant, fish or animal matter; processed lipid containing plant or animal matter; byproducts of lipid containing plant, fish or animal matter; lipid containing microbial material; processed lipid containing microbial material; and byproducts of lipid containing microbial matter. Such materials can be utilized in liquid or solid forms. Solid forms include whole forms, such as cells, beans, and seeds; ground, chopped, slurried, extracted, flaked, milled, *etc.* The fatty acid portion of the fatty acid comprising compound can be a simple fatty acid, such as one that includes a carboxyl group attached to a substituted or un-substituted alkyl group. The substituted or unsubstituted alkyl group can be straight or branched, saturated or unsaturated. Substitutions on the alkyl group can include hydroxyls, phosphates, halogens, alkoxy, or aryl groups. The substituted or unsubstituted alkyl group can have 7 to 29 carbons *(e.g.*, 8 to 30 carbons counting the carboxyl group). The substituted or unsubstituted alkyl group can have **11** to 23 carbons *(e.g.*, *\2* to 24 carbons counting the carboxyl group). The carbons can be arranged in a linear chain with or without side chains and/or substitutions. Addition of the fatty acid comprising compound can be by way of adding a material comprising the fatty acid comprising compound.

[0073] The term "pH modifier" as used herein has its ordinary meaning as known to those skilled in the art and can include any material that will tend to increase, decrease or hold steady the pH of the broth or medium. A pH modifier can be an acid, a base, a buffer, or a material that reacts with other materials present to serve to raise, lower, or hold steady the pH. In one embodiment, more than one pH modifier can be used, such as more than one acid, more than one base, one or more acid with one or more bases, one or more acids with one or more buffers, one or more bases with one or more buffers, or one or more acids with one or more bases with one or more buffers. In one embodiment, a buffer can be produced in the broth or medium or separately and used as an ingredient by at least partially reacting in acid or base with a base or an acid, respectively. When more than one pH modifiers are utilized, they can be added at the same time or at different times. In one embodiment, one or more acids and one or more bases are combined, resulting in a buffer. In one embodiment, media components, such as a carbon source or a nitrogen source serve as a pH modifier; suitable media components include those with high or low pH or those with buffering capacity. Exemplary media components include acid- or base-hydrolyzed plant polysaccharides having residual acid or base, ammonia fiber explosion (AFEX) treated plant material with residual ammonia, lactic acid, corn steep solids or liquor.

[0074] The term "fermentation" as used herein has its ordinary meaning as known to those skilled in the art and can include culturing of a microorganism or group of microorganisms in or on a suitable medium for the microorganisms. The microorganisms can be aerobes, anaerobes, facultative anaerobes, heterotrophs, autotrophs, photoautotrophs, photoheterotrophs, chemoautotrophs, and/or chemoheterotrophs. The microorganisms can be growing aerobically or anaerobically. They can be in any phase of growth, including lag (or conduction), exponential, transition, stationary, death, dormant, vegetative, sporulating, *etc.*

[0075] "Growth phase" is used herein to describe the type of cellular growth that occurs after the "Initiation phase" and before the "Stationary phase" and the "Death phase." The growth phase is sometimes referred to as the exponential phase or log phase or logarithmic phase.

[0076] The term "plant polysaccharide" as used herein has its ordinary meaning as known to those skilled in the art and can comprise one or more polymers of sugars and sugar derivatives as well as derivatives of sugar polymers and/or other polymeric materials that occur in plant matter. Exemplary plant polysaccharides include lignin, cellulose, starch, pectin, and hemicellulose. Others are chitin, sulfonated polysaccharides such as alginic acid, agarose, carrageenan, porphyran, furcelleran and funoran. Generally, the polysaccharide can have two or more sugar units or derivatives of sugar units. The sugar units and/or derivatives of sugar units can repeat in a regular pattern, or otherwise. The sugar units can be hexose units or pentose units, or combinations of these. The derivatives of sugar units can be sugar alcohols, sugar acids, amino sugars, *etc.* The polysaccharides can be linear, branched, cross-linked, or a mixture thereof. One type or class of polysaccharide can be cross-linked to another type or class of polysaccharide.

[0077] The term "fermentable sugars" as used herein has its ordinary meaning as known to those skilled in the art and can include one or more sugars and/or sugar derivatives that can be utilized as a carbon source by the microorganism, including monomers, dimers, and polymers of these compounds including two or more of these compounds. In some cases, the microorganism can break down these polymers, such as by hydrolysis, prior to incorporating the broken down material. Exemplary fermentable sugars include, but are not limited to glucose, xylose, arabinose, galactose, mannose, rhamnose, cellobiose, lactose, sucrose, maltose, and fructose.

[0078] The term "saccharification" as used herein has its ordinary meaning as known to those skilled in the art and can include conversion of plant polysaccharides to lower molecular weight species that can be utilized by the microorganism at hand. For some microorganisms, this would include conversion to monosaccharides, disaccharides, trisaccharides, and oligosaccharides of up to about seven monomer units, as well as similar sized chains of sugar derivatives and combinations of sugars and sugar derivatives. For some microorganisms, the allowable chain-length can be longer and for some microorganisms the allowable chain-length can be shorter.

[0079] The term "biomass" as used herein refers to organic material derived from living microorganisms, including any member from the kingdoms: Monera, Protista, Fungi, Plantae, or

Animalia. Organic material that comprises oligosaccharides *(e.g.,* pentose saccharides, hexose saccharides, or longer saccharides) is of particular use in the processes disclosed herein. Organic material includes microorganisms or material derived therefrom. Organic material includes cellulosic, hemicellulosic, and/or lignocellulosic material. In one embodiment biomass comprises genetically-modified microorganisms or parts of microorganisms, such as genetically-modified plant matter, algal matter, animal matter. In another embodiment biomass comprises non-genetically modified microorganisms or parts of microorganisms, such as non-genetically modified plant matter, algal matter, animal matter The term "feedstock" is also used to refer to biomass being used in a process, such as those described herein.

[0080] Plant matter comprises members of the kingdom Plantae, such as terrestrial plants and aquatic or marine plants. In one embodiment terrestrial plants comprise crop plants (such as fruit, vegetable or grain plants). In one embodiment aquatic or marine plants include, but are not limited to, sea grass, salt marsh grasses (such as Spartina sp. or Phragmites sp.) or the like. In one embodiment a crop plant comprises a plant that is cultivated or harvested for oral consumption, or for utilization in an industrial, pharmaceutical, or commercial process. In one embodiment, crop plants include but are not limited to corn, wheat, rice, barley, soybeans, bamboo, cotton, crambe, jute, sorghum, high biomass sorghum, oats, tobacco, grasses, *(e.g.,* Miscanthus grass or switchgrass), trees (softwoods and hardwoods) or tree leaves, beans, rape/canola, alfalfa, flax, sunflowers, safflowers, millet, rye, sugarcane, sugar beets, cocoa, tea, Brassica sp., cotton, coffee, sweet potatoes, flax, peanuts, clover; lettuce, tomatoes, cucurbits, cassava, potatoes, carrots, radishes, peas, lentils, cabbages, cauliflower, broccoli, Brussels sprouts, grapes, peppers, or pineapples; tree fruits or nuts such as citrus, apples, pears, peaches, apricots, walnuts, almonds, olives, avocadoes, bananas, or coconuts; flowers such as orchids, carnations and roses; nonvascular plants such as ferns; oil producing plants (such as castor beans, jatropha, or olives); or gymnosperms such as palms. Plant matter also comprises material derived from a member of the kingdom Plantae, such as woody plant matter, non-woody plant matter, cellulosic material, lignocellulosic material, or hemicellulosic material. Plant matter includes carbohydrates (such as pectin, starch, inulin, fructans, glucans, lignin, cellulose, or xylan). Plant matter also includes sugar alcohols, such as glycerol. In one embodiment plant matter comprises a corn product, *(e.g.,* corn stover, corn cobs, corn grain, corn steep liquor, corn steep solids, or corn grind), stillage, bagasse, leaves, pomace, or material derived therefrom. In another embodiment plant matter comprises distillers grains, Distillers Dried Solubles (DDS), Distillers Dried Grains (DDG), Condensed Distillers Solubles (CDS), Distillers Wet Grains (DWG), Distillers Dried Grains with Solubles (DDGS), peels, pits, fermentation waste, skins, straw, seeds, shells, beancake, sawdust, wood flour, wood pulp, paper pulp, paper pulp waste streams, rice or oat hulls, bagasse, grass clippings, lumber, or food leftovers. These materials can come from farms, forestry, industrial sources, households, *etc.* In another embodiment plant matter comprises an agricultural waste byproduct or side stream. In another embodiment plant matter comprises a source of pectin such as citrus fruit *(e.g.,* orange, grapefruit, lemon, or limes), potato, tomato, grape, mango, gooseberry, carrot, sugar-

beet, and apple, among others. In another embodiment plant matter comprises plant peel *(e.g.,* citrus peels) and/or pomace *(e.g.,* grape pomace). In one embodiment plant matter is characterized by the chemical species present, such as proteins, polysaccharides or oils. In one embodiment plant matter is from a genetically modified plant. In one embodiment a genetically-modified plant produces hydrolytic enzymes (such as a cellulase, hemicellulase, or pectinase *etc.)* at or near the end of its life cycles. In another embodiment a genetically-modified plant encompasses a mutated species or a species that can initiate the breakdown of cell wall components. In another embodiment plant matter is from a non-genetically modified plant.

[0081] Animal matter comprises material derived from a member of the kingdom Animaliae *(e.g.,* bone meal, hair, heads, tails, beaks, eyes, feathers, entrails, skin, shells, scales, meat trimmings, hooves or feet) or animal excrement *(e.g.,* manure). In one embodiment animal matter comprises animal carcasses, milk, meat, fat, animal processing waste, or animal waste (manure from cattle, poultry, and hogs).

[0082] Algal matter comprises material derived from a member of the kingdoms Monera *(e.g.,* Cyanobacteria) or Protista *(e.g.,* algae (such as green algae, red algae, glaucophytes, cyanobacteria,) or fungus-like members of Protista (such as slime molds, water molds, etc). Algal matter includes seaweed (such as kelp or red macroalgae), or marine microflora, including plankton.

[0083] Organic material comprises waste from farms, forestry, industrial sources, households or municipalities. In one embodiment organic material comprises sewage, garbage, food waste *(e.g.,* restaurant waste), waste paper, toilet paper, yard clippings, or cardboard.

[0084] The term "carbonaceous biomass" as used herein has its ordinary meaning as known to those skilled in the art and can include one or more biological materials that can be converted into a biofuel, chemical or other product. Carbonaceous biomass can comprise municipal waste (waste paper, recycled toilet papers, yard clippings, *etc.),* wood, plant material, plant matter, plant extract, bacterial matter *(e.g.,* bacterial cellulose), distillers' grains, a natural or synthetic polymer, or a combination thereof.

[0085] In one embodiment, biomass does not include fossilized sources of carbon, such as hydrocarbons that are typically found within the top layer of the Earth's crust *(e.g.,* natural gas, nonvolatile materials composed of almost pure carbon, like anthracite coal, *etc.).*

[0086] "Broth" is used herein to refer to inoculated medium at any stage of growth, including the point immediately after inoculation and the period after any or all cellular activity has ceased and can include the material after post-fermentation processing. It includes the entire contents of the combination of soluble and insoluble matter, suspended matter, cells and medium, as appropriate.

[0087] The term "productivity" as used herein has its ordinary meaning as known to those skilled in the art and can include the mass of a material of interest produced in a given time in a given volume. Units can be, for example, grams per liter-hour, or some other combination of mass, volume, and time. In fermentation, productivity is frequently used to characterize how fast a product can be made within a given fermentation volume. The volume can be referenced to the total volume of the fermentation vessel, the working volume of the fermentation vessel, or the actual volume of broth being fermented. The

context of the phrase will indicate the meaning intended to one of skill in the art. Productivity is different from "titer" in that productivity includes a time term, and titer is analogous to concentration. Titer and Productivity can generally be measured at any time during the fermentation, such as at the beginning, the end, or at some intermediate time, with titer relating the amount of a particular material present or produced at the point in time of interest and the productivity relating the amount of a particular material produced per liter in a given amount of time. The amount of time used in the productivity determination can be from the beginning of the fermentation or from some other time, and go to the end of the fermentation, such as when no additional material is produced or when harvest occurs, or some other time as indicated by the context of the use of the term. "Overall productivity" refers to the productivity determined by utilizing the final titer and the overall fermentation time. "Productivity to maximum titer" refers to the productivity determined utilizing the maximum titer and the time to achieve the maximum titer. "Instantaneous productivity" refers to the productivity at a moment in time and can be determined from the slope of the titer v. time curve for the compound of interest, or by other appropriate means as determined by the circumstances of the operation and the context of the language. "Incremental productivity" refers to productivity over a portion of the fermentation time, such as several minutes, an hour, or several hours. Frequently, an incremental productivity is used to imply or approximate instantaneous productivity. Other types of productivity can be used as well, with the context indicating how the value should be determined.

[0088] "Titer" refers to the amount of a particular material present in a fermentation broth. It is similar to concentration and can refer to the amount of material made by the microorganism in the broth from all fermentation cycles, or the amount of material made in the current fermentation cycle or over a given period of time, or the amount of material present from whatever source, such as produced by the microorganism or added to the broth. Frequently, the titer of soluble species will be referenced to the liquid portion of the broth, with insolubles removed, and the titer of insoluble species will be referenced to the total amount of broth with insoluble species being present, however, the titer of soluble species can be referenced to the total broth volume and the titer of insoluble species can be referenced to the liquid portion, with the context indicating the which system is used with both reference systems intended in some cases. Frequently, the value determined referenced to one system will be the same or a sufficient approximation of the value referenced to the other. "Concentration" when referring to material in the broth generally refers to the amount of a material present from all sources, whether made by the microorganism or added to the broth. Concentration can refer to soluble species or insoluble species, and is referenced to either the liquid portion of the broth or the total volume of the broth, as for "titer."

[0089] The term "biocatalyst" as used herein has its ordinary meaning as known to those skilled in the art and can include one or more enzymes and microorganisms, including solutions, suspensions, and mixtures of enzymes and microorganisms. In some contexts this word will refer to the possible use of either enzymes or microorganisms to serve a particular function, in other contexts the word will refer to

the combined use of the two, and in other contexts the word will refer to only one of the two. The context of the phrase will indicate the meaning intended to one of skill in the art.

[0090] The terms "conversion efficiency" or "yield" as used herein have their ordinary meaning as known to those skilled in the art and can include the mass of product made from a mass of substrate. The term can be expressed as a percentage yield of the product from a starting mass of substrate. For the production of ethanol from glucose, the net reaction is generally accepted as:

$$C_6H_{12}0_6 \rightarrow 2\ C_2H_5OH + 2\ C0_2$$

and the theoretical maximum conversion efficiency or yield is 51% (wt). Frequently, the conversion efficiency will be referenced to the theoretical maximum, for example, "80% of the theoretical maximum." In the case of conversion of glucose to ethanol, this statement would indicate a conversion efficiency of 41%(wt.). The context of the phrase will indicate the substrate and product intended to one of skill in the art. For substrates comprising a mixture of different carbon sources such as found in biomass (xylan, xylose, glucose, cellobiose, arabinose cellulose, hemicellulose *etc.)*, the theoretical maximum conversion efficiency of the biomass to ethanol is an average of the maximum conversion efficiencies of the individual carbon source constituents weighted by the relative concentration of each carbon source. In some cases, the theoretical maximum conversion efficiency is calculated based on an assumed saccharification yield. In one embodiment, given carbon source comprising lOg of cellulose, the theoretical maximum conversion efficiency can be calculated by assuming saccharification of the cellulose to the assimilable carbon source glucose of about 75% by weight. In this embodiment, lOg of cellulose can provide 7.5 g of glucose which can provide a maximum theoretical conversion efficiency of about 7.5 g»51% or 3.8 g of ethanol. In other cases, the efficiency of the saccharification step can be calculated or determined, *e.g.*, saccharification yield.

[0091] Saccharification yields can include between about 10-100%, about 20-90%, about 30-80%, about 40-70% or about 50-60%, such as about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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%, 99% or about 100% for any carbohydrate carbon sources larger than a single monosaccharide subunit.

[0092] The saccharification yield takes into account the amount of ethanol, and acidic products produced plus the amount of residual monomeric sugars detected in the media. The ethanol figures resulting from media components are not adjusted in this experiment. These can account for up to 3 g/L ethanol production or equivalent of up to 6 g/L sugar as much as +/- 10%-15% saccharification yield (or saccharification efficiency). For this reason the saccharification yield % can be greater than 100%, for some plots. The saccharification yield takes into account the amount of ethanol, and acidic products produced plus the amount of residual monomeric sugars detected in the media. The ethanol figures

resulting from media components are not adjusted in this experiment. These can account for up to 3 g/l ethanol production or equivalent of up to 6g/l sugar as much as +/- 10%-15% saccharification yield (or saccharification efficiency). For this reason the saccharification yield %> can be greater than 100%, for some plots.

[0093] The terms "pretreatment" or "pretreated" as used herein refer to any mechanical, chemical, thermal, biochemical process or combination of these processes whether in a combined step or performed sequentially, that achieves disruption or expansion of a biomass so as to render the biomass more susceptible to attack by enzymes and/or microorganisms. In some embodiments, pretreatment can include removal or disruption of lignin so is to make the cellulose and hemicellulose polymers in the plant biomass more available to cellulolytic enzymes and/or microorganisms, for example, by treatment with acid or base. In some embodiments, pretreatment can include the use of a microorganism of one type to render plant polysaccharides more accessible to microorganisms of another type. In some embodiments, pretreatment can also include disruption or expansion of cellulosic and/or hemicellulosic material. Steam explosion, and ammonia fiber expansion (or explosion) (AFEX) are well known thermal/chemical techniques. Hydrolysis, including methods that utilize acids and/or enzymes can be used. Other thermal, chemical, biochemical, enzymatic techniques can also be used. For example, other chemicals can be added to neutralize or detoxify the biomass or saccharide streams resulting from earlier pretreatment.

[0094] The terms "fed-batch" or "fed-batch fermentation" as used herein has its ordinary meaning as known to those skilled in the art and can include a method of culturing microorganisms where nutrients, other medium components, or biocatalysts (including, for example, enzymes, fresh microorganisms, extracellular broth, *etc.)* are supplied to the fermentor during cultivation, but culture broth is not harvested from the fermentor until the end of the fermentation, although it can also include "self seeding" or "partial harvest" techniques where a portion of the fermentor volume is harvested and then fresh medium is added to the remaining broth in the fermentor, with at least a portion of the inoculum being the broth that was left in the fermentor. During a fed-batch fermentation, the broth volume can increase, at least for a period, by adding medium or nutrients to the broth while fermentation microorganisms are present. In some fed-batch fermentations, the broth volume can be insensitive to the addition of nutrients and in some cases not change from the addition of nutrients. Suitable nutrients which can be utilized include those that are soluble, insoluble, and partially soluble, including gasses, liquids and solids. In one embodiment In some embodiments, a fed-batch process might be referred to with a phrase such as, "fed-batch with cell augmentation." This phrase can include an operation where nutrients and microbial cells are added or one where microbial cells with no substantial amount of nutrients are added. The more general phrase "fed-batch" encompasses these operations as well. The context where any of these phrases is used will indicate to one of skill in the art the techniques being considered.

[0095] The term "SSF" as used herein, refers to simultaneous saccharification fermentation. The term "SHF" means sequential hydrolysis followed by subsequent fermentation.

[0096] A term "phytate" as used herein has its ordinary meaning as known to those skilled in the art can be include phytic acid, its salts, and its combined forms as well as combinations of these.

[0097] The term "recombinant" as used herein, refers to a microorganism is genetically modified to comprise one or more heterologous or endogenous nucleic acid molecules. Such nucleic acid molecules can be comprised extrachromosomally or integrated into the chromosome of a microorganism. The term "non-recombinant" means a microorganism is not genetically modified. For example, a recombinant microorganism can be modified to overexpress an endogenous gene encoding an enzyme through modification of promoter elements *(e.g.,* replacing an endogenous promoter element with a constitutive or highly active promoter). Alternatively, a recombinant microorganism can be modified by introducing a heterologous or another copy of an endogenous nucleic acid molecule encoding a protein that is not otherwise expressed in the host microorganism.

[0098] The term "sugar compounds" as used herein has its ordinary meaning as known to those skilled in the art and can include monosaccharide sugars, including but not limited to hexoses and pentoses; sugar alcohols; sugar acids; sugar amines; compounds containing two or more of these linked together directly or indirectly through covalent or ionic bonds; and mixtures thereof. Included within this description are disaccharides; trisaccharides; oligosaccharides; polysaccharides; and sugar chains, branched and/or linear, of any length.

[0099] "Dry cell weight" is used herein to refer to a method of determining the cell content of a broth or inoculum, and the value so determined. Generally, the method can include rinsing or washing a volume of broth followed by drying and weighing the residue. In some cases, a sample of broth is simply centrifuged with the layer containing cells collected, dried, and weighed. Frequently, the broth is centrifuged, then resuspended in water or a mixture of water and other ingredients, such as a buffer, ingredients to create an isotonic condition, ingredients to control any change in osmotic pressure, *etc.* The centrifuge-resuspend steps can be repeated, if desired, and different resuspending solutions can be used prior to the final centrifuging and drying. When an insoluble medium component is present, the presence of the insoluble component can be ignored, with the value determined as above. Methods when insoluble medium components are present include those where the insoluble component is reacted to a soluble form, dissolved or extracted into a different solvent that can include water, or separated by an appropriate method, such as by centrifugation, gradient centrifugation, flotation, filtration, or other suitable technique or combination of techniques.

[00100] Description

[00101] The following description and examples illustrate some exemplary embodiments of the disclosure in detail. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure that are encompassed by its scope. Accordingly, the description of a certain exemplary embodiment should not be deemed to limit the scope of the present disclosure.

[00102] Biocatalysts *Clostridium phytofermentans* Q.12 (Q.12) *and Clostridium phytofermentans* Q.13 (Q.13) are fast-growing, high yielding strains of *Clostridium phytofermentans,* and can in some

embodiments be defined based on the phenotypic and genotypic characteristics of the cultured strain as described *infra*. Aspects described herein generally include systems, methods, and compositions for producing fuels, such as ethanol, and/or other useful organic products involving, for example, **Q**.12 and **Q**.13 and/or any other strain of the species, including those which can be derived from *Clostridium phytofermentans*, including genetically modified strains, or strains separately isolated. Some exemplary species can be defined using standard taxonomic considerations (Stackebrandt and Goebel, International Journal of Systematic Bacteriology, 44:846-9, 1994): Strains with 16S rRNA sequence homology values of 98% and higher as compared to the type **Q**.12 or **Q**.13, and strains with DNA re-association values of at least about 70% can be considered **Q**.12 or **Q**.13. For example, strains with 16S rRNA sequence homology values of at least 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.9, 98.0, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99.0, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9% can be considered **Q**.12 or **Q**.13. In one embodiment, strains with DNA re-association values of at least about 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, 99% can be considered **Q**.12 or **Q**.13. Considerable evidence exists to indicate that many microorganisms which have 70%, or greater DNA re-association values also have at least 98%> DNA sequence identity and share phenotypic traits defining a species. Analyses of the genome sequence of **Q**.12 or **Q**.13 indicate the presence of large numbers of genes and genetic loci that are likely to be involved in mechanisms and pathways for plant polysaccharide fermentation, giving rise to the unusual fermentation properties of these biocatalysts which can be found in all or nearly all strains of the species **Q**.12 or **Q**.13 and can be natural isolates, or genetically modified strains.

[00103] Attributes of Q.12 or Q.13

[00104] In one embodiment, the microorganisms, **Q**.12 or **Q**.13, provide useful advantages for the conversion of biomass to ethanol and other products. One advantage of this microorganism is its ability to produce enzymes capable of hydrolyzing polysaccharides and higher molecular weight saccharides to lower molecular weight saccharides, such as oligosaccharides, disaccharides, and monosaccharides. In one embodiment, **Q**.12 or **Q**.13 produce a hydrolytic enzyme which facilitates fermenting of a biomass material. Examples of biomass material that can be fermented include, but are not limited to, cellulosic, hemicellulosic, lignocellulosic materials; pectins; starches; wood; paper; agricultural products; forest waste; tree waste; tree bark; leaves; grasses; sawgrass; woody plant matter; non-woody plant matter; carbohydrates; pectin; starch; inulin; fructans; glucans; corn; sugar cane; grasses; bamboo, algae, and material derived from these materials. The microorganisms can usually produce these enzymes as needed, frequently without excessive production of unnecessary hydrolytic enzymes, or in one embodiment, one or more enzymes is added to further improve the microorganism's production capability. This ability to produce a very wide range of hydrolytic enzymes gives **Q**.12 or **Q**.13 and the associated technology distinct advantages in biomass fermentation, especially those fermentations not utilizing simple sugars as the feedstock. Various fermentation conditions can enhance the activities of the microorganism, resulting in higher yields, higher productivity, greater product selectivity, and/or greater conversion efficiency. In

one embodiment, fermentation conditions include fed batch operation and fed batch operation with cell augmentation; addition of complex nitrogen sources such as corn steep powder or yeast extract; addition of specific amino acids including proline, glycine, isoleucine, and/or histidine; addition of a complex material containing one or more of these amino acids; addition of other nutrients or other compounds such as phytate, proteases enzymes, or polysaccharase enzymes. In one embodiment, fermentation conditions can include supplementation of a medium with an organic nitrogen source. In another embodiment, fermentation conditions can include supplementation of a medium with an inorganic nitrogen source. In one embodiment, the addition of one material provides supplements that fit into more than one category, such as providing amino acids and phytate.

[00105] In one embodiment, the Q.12 or Q.13 microorganism is used to hydrolyze various higher saccharides (higher molecular weight) present in biomass to lower saccharides (lower molecular weight), such as in preparation for fermentation to produce ethanol, hydrogen, or other chemicals such as organic acids including formic acid, acetic acid, and lactic acid. Another advantage of Q.12 or Q.13 is its ability to hydrolyze polysaccharides and higher saccharides that contain hexose sugar units or that contain pentose sugar units, and that contain both, into lower saccharides and in some cases monosaccharides. These enzymes and/or the hydrolysate can be used in fermentations to produce various products including fuels, and other chemicals. Another advantage of Q.12 or Q.13 is its ability to produce ethanol, hydrogen, and other fuels or compounds such as organic acids including acetic acid, formic acid, and lactic acid from lower sugars (lower molecular weight) such as monosaccharides. Another advantage of Q.12 or Q.13 is its ability to perform the combined steps of hydrolyzing a higher molecular weight biomass containing sugars and/or higher saccharides or polysaccharides to lower sugars and fermenting these lower sugars into desirable products including ethanol, hydrogen, and other compounds such as organic acids including formic acid, acetic acid, and lactic acid.

[00106] Another advantage of Q.12 or Q.13 is its ability to grow under conditions that include elevated ethanol concentration, high sugar concentration, low sugar concentration, utilize insoluble carbon sources, and/or operate under anaerobic conditions. These characteristics, in various combinations, can be used to achieve operation with long fermentation cycles and can be used in combination with batch fermentations, fed batch fermentations, self-seeding/partial harvest fermentations, and recycle of cells from the final fermentation as inoculum.

[00107] In one embodiment, Q.12 is a high ethanol-producing strain of *Clostridium phytofermentans* derived from the naturally-occurring species. Q.13 is derived from Q.12 and is more ethanol tolerant than Q.12.

[00108] In one example, the process for converting biomass material into ethanol includes pretreating the biomass material *(e.g.,* "feedstock"), hydrolyzing the pretreated biomass to convert polysaccharides to oligosaccharides, further hydrolyzing the oligosaccharides to monosaccharides, and converting the monosaccharides to ethanol. In one example, the biomass can be hydrolyzed directly to monosaccharides or other saccharides that are utilized by the fermentation microorganism to produce ethanol or other

products. If a different final product is desired, such as hydrocarbons, hydrogen, methane, hydroxy compounds such as alcohols *(e.g.*, butanol, propanol, methanol, *etc.)*, carbonyl compounds such as aldehydes and ketones *(e.g.*, acetone, formaldehyde, 1-propanal, *etc.)*, organic acids, derivatives of organic acids such as esters *(e.g.*, wax esters, glycerides, *etc.)* and other functional compounds including, but not limited to, 1, 2-propanediol, 1, 3- propanediol, lactic acid, formic acid, acetic acid, succinic acid, pyruvic acid, enzymes such as cellulases, polysaccharases, lipases, proteases, ligninases, and hemicellulases, the monosaccharides can be used in the biosynthesis of that particular compound. Biomass material that can be utilized includes woody plant matter, non-woody plant matter, cellulosic material, lignocellulosic material, hemicellulosic material, carbohydrates, pectin, starch, inulin, fructans, glucans, corn, algae, sugar cane, grasses, switchgrass, bamboo, citrus peels, sorghum, high biomass sorghum, oat hulls, and material derived from these. The final product can then be separated and/or purified, as indicated by the properties for the desired final product. In some instances, compounds related to sugars such as sugar alcohols or sugar acids can be utilized as well.

[00109] In one embodiment,, more than one of these steps can occur at any given time. For example, hydrolysis of the pretreated feedstock and hydrolysis of the oligosaccharides can occur simultaneously, and one or more of these can occur simultaneously to the conversion of monosaccharides to ethanol.

[00110] In another embodiment, an enzyme can directly convert the polysaccharide to monosaccharides. In some instances, an enzyme can hydrolyze the polysaccharide to oligosaccharides and the enzyme or another enzyme can hydrolyze the oligosaccharides to monosaccharides.

[00111] In another embodiment, the enzymes present in the fermentation can be produced separately and then added to the fermentation or they can be produced by microorganisms present in the fermentation. In one embodiment, the microorganisms present in the fermentation produces some enzymes. In another embodiment, enzymes are produced separately and added to the fermentation.

[00112] For the overall conversion of pretreated biomass to final product to occur at high rates, each of the enzymes for each conversion step can be present with sufficiently high activity. If one of these enzymes is missing or is present in insufficient quantities, the production rate of ethanol, or other desired product can be reduced. The production rate can also be reduced if the microorganisms responsible for the conversion of monosaccharides to product only slowly take up monosaccharides and/or have only limited capability for translocation of the monosaccharides and intermediates produced during the conversion to ethanol.

[00113] In another embodiment, the enzymes of the method are produced by Q.12 or Q.13, including a range of hydrolytic enzymes suitable for the biomass materials used in the fermentation methods. In one embodiment, Q.12 or Q.13 is grown under conditions appropriate to induce and/or promote production of the enzymes needed for the saccharification of the polysaccharide present. The production of these enzymes can occur in a separate vessel, such as a seed fermentation vessel or other fermentation vessel, or in the production fermentation vessel where ethanol production occurs. When the enzymes are produced in a separate vessel, they can, for example, be transferred to the production fermentation vessel along with

the cells, or as a relatively cell free solution liquid containing the intercellular medium with the enzymes. When the enzymes are produced in a separate vessel, they can also be dried and/or purified prior to adding them to the production fermentation vessel. The conditions appropriate for production of the enzymes are frequently managed by growing the cells in a medium that includes the biomass that the cells will be expected to hydrolyze in subsequent fermentation steps. Additional medium components, such as salt supplements, growth factors, and cofactors including, but not limited to phytate, amino acids, and peptides can also assist in the production of the enzymes utilized by the microorganism in the production of the desired products.

[00114] Feedstock and Pretreatment of Feedstock

[00115] In one embodiment, the feedstock contains cellulosic, hemicellulosic, and/or lignocellulosic material. The feedstock can be derived from agricultural crops, crop residues, trees, woodchips, sawdust, paper, cardboard, grasses, algae and other sources.

[00116] Cellulose is a linear polymer of glucose where the glucose units are connected via PO —'4) linkages. Hemicellulose is a branched polymer of a number of sugar monomers including glucose, xylose, mannose, galactose, rhamnose and arabinose, and can have sugar acids such as mannuronic acid and galacturonic acid present as well. Lignin is a cross-linked, racemic macromolecule of mostly p-coumaryl alcohol, conferyl alcohol and sinapyl alcohol. These three polymers occur together in lignocellusic materials in plant biomass. The different characteristics of the three polymers can make hydrolysis of the combination difficult as each polymer tends to shield the others from enzymatic attack.

[00117] In one embodiment, methods are provided for the pretreatment of feedstock used in the fermentation and production of the biofuels and ethanol. The pretreatment steps can include mechanical, thermal, pressure, chemical, thermochemical, and/or biochemical tests pretreatment prior to being used in a bioprocess for the production of fuels and chemicals, but untreated biomass material can be used in the process as well. Mechanical processes can reduce the particle size of the biomass material so that it can be more conveniently handled in the bioprocess and can increase the surface area of the feedstock to facilitate contact with chemicals/biochemicals/biocatalysts. Mechanical processes can also separate one type of biomass material from another. The biomass material can also be subjected to thermal and/or chemical pretreatments to render plant polymers more accessible. Multiple steps of treatment can also be used.

[00118] Mechanical processes include, are not limited to, washing, soaking, milling, size reduction, screening, shearing, size classification and density classification processes. Chemical processes include, but are not limited to, bleaching, oxidation, reduction, acid treatment, base treatment, sulfite treatment, acid sulfite treatment, basic sulfite treatment, ammonia treatment, and hydrolysis. Thermal processes include, but are not limited to, sterilization, ammonia fiber expansion or explosion ("AFEX"), steam explosion, holding at elevated temperatures, pressurized or unpressurized, in the presence or absence of water, and freezing. Biochemical processes include, but are not limited to, treatment with enzymes, including enzymes produced by genetically-modified plants, and treatment with microorganisms. Various

enzymes that can be utilized include cellulase, amylase, β-glucosidase, xylanase, gluconase, and other polysaccharases; lysozyme; laccase, and other lignin-modifying enzymes; lipoxygenase, peroxidase, and other oxidative enzymes; proteases; and lipases. One or more of the mechanical, chemical, thermal, thermochemical, and biochemical processes can be combined or used separately. Such combined processes can also include those used in the production of paper, cellulose products, microcrystalline cellulose, and cellulosics and can include pulping, kraft pulping, acidic sulfite processing. The feedstock can be a side stream or waste stream from a facility that utilizes one or more of these processes on a biomass material, such as cellulosic, hemicellulosic or lignocellulosic material. Examples include paper plants, cellulosics plants cotton processing plants, and microcrystalline cellulose plants. The feedstock can also include cellulose-containing or cellulosic containing waste materials. The feedstock can also be biomass materials, such as wood, grasses, corn, starch, or sugar, produced or harvested as an intended feedstock for production of ethanol or other products such as by Q.12 or Q.13 biocatalysts.

[00119] In another embodiment, a method can utilize a pretreatment process disclosed in U.S. Patents and Patent Applications US20040152881, US20040171 136, US20040168960, US20080121359, US20060069244, US200601 88980, US20080176301, 5693296, 6262313, US20060024801, 5969189, 6043392, US20020038058, US5865898, US5865898, US6478965, 5986133, or US20080280338, each of which is incorporated by reference herein in its entirety.

[00120] In another embodiment, the AFEX process is be used for pretreatment of biomass. In one embodiment, the AFEX process is used in the preparation of cellulosic, hemicellulosic or lignocellulosic materials for fermentation to ethanol or other products. The process generally includes combining the feedstock with ammonia, heating under pressure, and suddenly releasing the pressure. Water can be present in various amounts. The AFEX process has been the subject of numerous patents and publications.

[00121] In another embodiment, the pretreatment of biomass comprises the addition of calcium hydroxide to a biomass to render the biomass susceptible to degradation. Pretreatment comprises the addition of calcium hydroxide and water to the biomass to form a mixture, and maintaining the mixture at a relatively high temperature. Alternatively, an oxidizing agent, selected from the group consisting of oxygen and oxygen-containing gasses, can be added under pressure to the mixture. Examples of carbon hydroxide treatments are disclosed in U.S. Patent No. 5865898 to Holtzapple and S. Kim and M. T. Holzapple, Bioresource Technology, 96, (2005) 1994, incorporated by reference herein in its entirety.

[00122] In one embodiment, pretreatment of biomass comprises dilute acid hydrolysis. Example of dilute acid hydrolysis treatment are disclosed in T. A. Lloyd and C. E Wyman, Bioresource Technology, (2005) 96, 1967), incorporated by reference herein in its entirety. Dilute acid for hydrolysis can be derived from inorganic acids, such as sulfuric acid or hydrochloric acid, or from organic acids, such as acetic acid, malic acid, lactic acid, carboxylic acid, or citric acid.

[00123] In another embodiment, pretreatment of biomass comprises pH controlled liquid hot water treatment. Examples of pH controlled liquid hot water treatments are disclosed in N. Mosier et al., Bioresource Technology, (2005) 96, 1986, incorporated by reference herein in its entirety.

[00124] In one embodiment, pretreatment of biomass comprises aqueous ammonia recycle process (ARP). Examples of aqueous ammonia recycle process are described in T. H. Kim and Y. Y. Lee, Bioresource Technology, (2005)96, 2007, incorporated by reference herein in its entirety.

[00125] In one embodiment, the above mentioned methods have two steps: a pretreatment step that leads to a wash stream, and an enzymatic hydrolysis step of pretreated-biomass that produces a hydrolysate stream. In the above methods, the pH at which the pretreatment step is carried out includes acid hydrolysis, hot water pretreatment, steam explosion or alkaline reagent based methods (AFEX, ARP, and lime pretreatments). Dilute acid and hot water treatment methods solubilize mostly hemicellulose, whereas methods employing alkaline reagents remove most lignin during the pretreatment step. As a result, the wash stream from the pretreatment step in the former methods contains mostly hemicellulose-based sugars, whereas this stream has mostly lignin for the high-pH methods. The subsequent enzymatic hydrolysis of the residual biomass leads to mixed sugars (C5 and C6) in the alkali based pretreatment methods, while glucose is the major product in the hydrolyzate from the low and neutral pH methods. In one embodiment, the treated material is additionally treated with catalase or another similar chemical, chelating agents, surfactants, and other compounds to remove impurities or toxic chemicals or further release polysaccharides.

[00126] In one embodiment, pretreatment of biomass comprises ionic liquid pretreatment. Biomass can be pretreated by incubation with an ionic liquid, followed by ionic liquid extraction with a wash solvent such as alcohol or water. The treated biomass can then be separated from the ionic liquid/wash-solvent solution by centrifugation or filtration, and sent to the saccharification reactor or vessel. Examples of ionic liquid pretreatment are disclosed in US publication No. 2008/0227162, incorporated herein by reference in its entirety.

[00127] In another embodiment, a method can utilize a pretreatment process disclosed in U.S. Patent No. 4600590 to Dale, U.S. Patent No. 4644060 to Chou, U.S. Patent No. 5037663 to Dale. U.S. Patent No. 5171592 to Holtzapple, et al., U.S. Patent No. 5939544 to Karstens, et al., U.S. Patent No. 5473061 to Bredereck, et al., U.S. Patent No. 6416621 to Karstens., U.S. Patent No. 6106888 to Dale, et al., U.S. Patent No. 6176176 to Dale, et al., PCT publication W02008/020901 to Dale, et al., Felix, A., et al., Anim. Prod. 51, 47-61 (1990)., Wais, A.C., Jr., et al., Journal of Animal Science, 35, No. 1,109-112 (1972), which are incorporated herein by reference in their entireties.

[00128] Alteration of the pH of a pretreated feedstock can be accomplished by washing the feedstock *(e.g.,* with water) one or more times to remove an alkaline or acidic substance, or other substance used or produced during pretreatment. Washing can comprise exposing the pretreated feedstock to an equal volume of water 2, 3, 4, 5, 6, 7 , 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more times. In another embodiment, a pH modifier can be added. For example, an acid, a buffer, or a material

that reacts with other materials present can be added to modulate the pH of the feedstock. In one embodiment, more than one pH modifier can be used, such as one or more bases, one or more bases with one or more buffers, one or more acids, one or more acids with one or more buffers, or one or more buffers. When more than one pH modifiers are utilized, they can be added at the same time or at different times. Other non-limiting exemplary methods for neutralizing feedstocks treated with alkaline substances have been described, for example in U.S. Patent Nos. 4,048,341; 4,182,780; and 5,693,296.

[00129] In one embodiment, one or more acids can be combined, resulting in a buffer. Suitable acids and buffers that can be used as pH modifiers include any liquid or gaseous acid that is compatible with the microorganism. Non-limiting examples include peroxyacetic acid, sulfuric acid, lactic acid, citric acid, phosphoric acid, and hydrochloric acid. In some instances, the pH can be lowered to neutral pH or acidic pH, for example a pH of 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, or lower. In some embodiments, the pH is lowered and/or maintained within a range of about pH 4.5 to about 7.1, or about 4.5 to about 6.9, or about pH 5.0 to about 6.3, or about pH 5.5 to about 6.3, or about pH 6.0 to about 6.5, or about pH 5.5 to about 6.9 or about pH 6.2 to about 6.7.

[00130] In another embodiment, biomass can be pre-treated at an elevated temperature and/or pressure. In one embodiment biomass is pre treated at a temperature range of 20°C to 400°C. In another embodiment biomass is pretreated at a temperature of about 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 80°C, 90°C, 100°C, 120°C, 150°C, 200°C, 250°C, 300°C ,350°C, 400°C or higher. In another embodiment, elevated temperatures are provided by the use of steam, hot water, or hot gases. In one embodiment steam can be injected into a biomass containing vessel. In another embodiment the steam, hot water, or hot gas can be injected into a vessel jacket such that it heats, but does not directly contact the biomass.

[00131] In another embodiment, a biomass can be treated at an elevated pressure. In one embodiment biomass is pre treated at a pressure range of about 1 psi to about 30 psi. In another embodiment biomass is pre treated at a pressure or about 1 psi, 2 psi, 3 psi, 4 psi, 5 psi, 6 psi, 7 psi, 8 psi, 9 psi, 10 psi, 12 psi, 15 psi, 18 psi, 20 psi, 22 psi, 24 psi, 26 psi, 28 psi, 30 psi or more. In some embodiments, biomass can be treated with elevated pressures by the injection of steam into a biomass containing vessel. In one embodiment, the biomass can be treated to vacuum conditions prior or subsequent to alkaline or acid treatment or any other treatment methods provided herein.

[00132] In one embodiment alkaline or acid pretreated biomass is washed *(e.g.,* with water (hot or cold) or other solvent such as alcohol *(e.g.,* ethanol)), pH neutralized with an acid, base, or buffering agent *(e.g.,* phosphate, citrate, borate, or carbonate salt) or dried prior to fermentation. In one embodiment, the drying step can be performed under vacuum to increase the rate of evaporation of water or other solvents. Alternatively, or additionally, the drying step can be performed at elevated temperatures such as about 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 80°C, 90°C, 100°C, 120°C, 150°C, 200°C, 250°C, 300°C or more.

[00133] In one embodiment, the pretreatment step includes a step of solids recovery. The solids recovery step can be during or after pretreatment *(e.g.,* acid or alkali pretreatment), or before the drying step. In one embodiment, the solids recovery step includes the use of a sieve, filter, screen, or a membrane for separating the liquid and solids fractions. In one embodiment a suitable sieve pore diameter size ranges from about 0.001 microns to 8mm, such as about 0.005microns to 3mm or about 0.01 microns to 1mm. In one embodiment a sieve pore size has a pore diameter of about O.Olmicrons, 0.02 microns, 0.05 microns, 0.1 microns, 0.5 microns, 1 micron, 2 microns, 4 microns, 5 microns, 10 microns, 20 microns, 25 microns, 50 microns, 75 microns, 100 microns, 125 microns, 150 microns, 200 microns, 250 microns, 300 microns, 400 microns, 500 microns, 750 microns, 1mm or more.

[00134] In one embodiment, biomass *(eg.,* corn stover) is processed or pretreated prior to fermentation hi one embodiment a method of pre-treatment includes but is not limited to, biomass particle size reduction, such as for example shredding, milling, chipping, crushing, grinding, or pulverizing. In one embodiment, biomass particle size reduction can include size separation methods such as sieving, or other suitable methods known in the art to separate materials based on size. In one embodiment size separation can provide for enhanced yields. In one embodiment, separation of finely shredded biomass *(e.g.,* particles smaller than about 8 mm in diameter, such as, 8, 7.9, 7.7, 7.5, 7.3, 7, 6.9, 6.7, 6.5, 6.3, 6, 5.9, 5.7, 5.5, 5.3, 5, 4.9, 4.7, 4.5, 4.3, 4, 3.9, 3.7, 3.5, 3.3, 3, 2.9, 2.7, 2.5, 2.3, 2, 1.9, 1.7, 1.5, 1.3, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or 0.1 mm) from larger particles allows the recycling of the larger particles back into the size reduction process, thereby increasing the final yield of processed biomass. In one embodiment, a fermentative mixture is provided which comprises a pretreated lignocellulosic feedstock comprising less than about 50% of a lignin component present in the feedstock prior to pretreatment and comprising more than about 60% of a hemicellulose component present in the feedstock prior to pretreatment; and a microorganism capable of fermenting a five-carbon sugar, such as xylose, arabinose or a combination thereof, and a six-carbon sugar, such as glucose, galactose, mannose or a combination thereof. In some instances, pretreatment of the lignocellulosic feedstock comprises adding an alkaline substance which raises the pH to an alkaline level, for example NaOH. In one embodiment, NaOH is added at a concentration of about 0.5%> to about 2%> by weight of the feedstock. In one embodiment, pretreatment also comprises addition of a chelating agent. In one embodiment, the microorganism is a bacterium, such as a member of the genus *Clostridium,* for example *Clostridium phytofermentans,* Q.12 or Q.13.

[00135] The present disclosure also provides a fermentative mixture comprising: a cellulosic feedstock pretreated with an alkaline substance which maintains an alkaline pH, and at a temperature of from about 80°C to about 120°C; and a microorganism capable of fermenting a five-carbon sugar and a six-carbon sugar. In one embodiment, the five-carbon sugar is xylose, arabinose, or a combination thereof. In one embodiment, the six-carbon sugar is glucose, galactose, mannose, or a combination thereof. In one embodiment, the alkaline substance is NaOH. In some embodiments, NaOH is added at a concentration of about 0.5%, to about *2%* by weight of the feedstock. In one embodiment, the microorganism is a bacterium, such as a member of the genus *Clostridium,* for example *Clostridium phytofermentans* , Q.12

or Q.13. In still another embodiment, the microorganism is genetically modified to enhance activity of one or more hydrolytic enzymes.

[00136] Further provided herein is a fermentative mixture comprising a cellulosic feedstock pre-treated with an alkaline substance which increases the pH to an alkaline level, at a temperature of from about 80°C to about 120°C; and a microorganism capable of uptake and fermentation of an oligosaccharide. In one embodiment the alkaline substance is NaOH. In some embodiments, NaOH is added at a concentration of about 0.5% to about 2% by weight of the feedstock. In one embodiment, the microorganism is a bacterium, such as a member of the genus *Clostridium,* for example *Clostridium phytofermentans,* Q.12 or Q.13. In one embodiment, the microorganism is genetically modified to express or increase expression of an enzyme capable of hydrolyzing said oligosaccharide, a transporter capable of transporting the oligosaccharide, or a combination thereof.

[00137] In one embodiment, pretreatment of biomass comprises enzyme hydrolysis. In one embodiment a biomass is pretreated with an enzyme or a mixture of enzymes, *e.g.*, endonucleases, exonucleases, cellobiohydrolases, cellulase, beta-glucosidases, glycoside hydrolases, glycosyltransferases, lyases, esterases and proteins containing carbohydrate-binding modules. In one embodiment, the enzyme or mixture of enzymes is one or more individual enzymes with distinct activities. In another embodiment, the enzyme or mixture of enzymes can be enzyme domains with a particular catalytic activity. For example, an enzyme with multiple activities can have multiple enzyme domains, including for example glycoside hydrolases, glycosyltransferases, lyases and/or esterases catalytic domains.

[00138] In one embodiment, pretreatment of biomass comprises enzyme hydrolysis with one or more enzymes from a Q.12 or Q.13 biocatalyst. In one embodiment, pretreatment of biomass comprises enzyme hydrolysis with one or more enzymes from Q.12 or Q.13, wherein the one or more enzyme is selected from the group consisting of endonucleases, exonucleases, cellobiohydrolases, beta-glucosidases, glycoside hydrolases, glycosyltransferases, lyases, esterases and proteins containing carbohydrate-binding modules. In one embodiment, biomass can be pretreated with a hydrolase identified in C. *phytofermentans.*

[00139] In one embodiment, pretreatment of biomass comprises enzyme hydrolysis with one or more of enzymes listed in Table 1. Table 1 show examples of known activities of some of the glycoside hydrolases, lyases, esterases, and proteins containing carbohydrate-binding modules family members predicted to be present in *Clostridia,* for example, *C. phytofermentans.* Known activities are listed by activity and corresponding PC number as determined by the International Union of Biochemistry and Molecular Biology.

[00140] Table 1: Known activities of glycoside hydrolase family members

Glycoside Hydrolase Family	Known activities	Number of domains predicted in *C. phytofermentans*
1	beta-glucosidase (EC 3.2.1.21); beta-galactosidase (EC 3.2.1.23); beta-mannosidase (EC 3.2.1.25); beta-glucuronidase (EC 3.2.1.31); beta-D-fucosidase (EC 3.2.1.38); phlorizin hydrolase (EC 3.2.1.62); 6-phospho--galactosidase (EC 3.2.1.85); 6-phospho- beta-glucosidase (EC 3.2.1.86); strictosidinebeta-glucosidase (EC 3.2.1.105); lactase (EC 3.2.1.108); amygdalinbeta-glucosidase (EC 1 3.2.1.117); prunasin beta-glucosidase (EC 3.2.1.118); raucaifricine beta-glucosidase (EC 3.2.1.125); thioglucosidase (EC 3.2.1.147); beta-primeverosidase (EC 3.2.1.149); isoflavonod 7-0-beta- apiosyl--glucosidase (EC 3.2.1.161); hydroxyisourate hydrolase (EC_3.-.-.-); _beta-glycosidase_(EC_3.2.1.-)	1
2	beta-galactosidase (EC 3.2.1.23) ;beta-mannosidase (EC 3.2.1.25); beta-glucuronidase (EC 3.2.1.31); mannosylglycoprotein 5 endo-beta-mannosidase (EC 3.2.1.152); exo-beta glucosaminidase_(E 3.2.1.-)	5
3	beta-glucosidase (EC 3.2.1.21); xylan 1,4-beta-xylosidase (EC 3.2.1.37); beta -N-acetylhexosaminidase (EC 3.2.1.52); glucan 1,3-beta-glucosiclase (EC 3.2.1.58); glucan 1,4-beta-glucosidase (EC 3.2.1 .74); exo-1 ,3-1,4-glucanase (EC 3.2.1.-); alpha-L arabinofuranosidase (EC 3.2.1.55).	8
4	maltose-6-phosphate glucosidase (EC 3.2.1.122); alpha glucosidase (EC 3.2.1.20); alpha-galactosidase (EC 3.2.1.22); 6-phospho-beta-glucosidase (EC 3.2.1.86); alpha -glucuronidase (EC 3.2.1.139).	3
5	chitosanase (EC 3.2.1.132); beta-mannosidase (EC 3.2.1.25); Cellulase (EC 3.2.1.4); glucan 1,3-beta-glucosidase (EC 3.2.1.58); licheninase (EC 3.2.1.73); glucan endo-1,6-beta-glucosidase (EC 3.2.1.75); mannan endo-1,4-beta-mannosidase (EC 3.2.1.78); 3 Endo-1,4-beta-xylanase (EC 3.2.1.8); cellulose 1,4-beta-cellobiosidase (EC 3.2.1.91); endo-1,6-beta-galactanase (EC 3.2.1 .-); beta -1,3-mannanase (EC 3.2.1.-); xyloglucan-specific endo-beta-1,4-glucanase (EC 3.2.1.151)	3
8	chitosanase (EC 3.2.1.132); cellulase (EC 3.2.1.4); licheninase (EC 3.2.1.73); endo-1,4-beta-xylanase (EC 3.2.1.8); reducing-end-xylose releasing exo-oligoxylanase (EC 3.2.1.156)	1
9	endoglucanase (EC 3.2.1.4); cellobiohydrolase (EC 3.2.1.91); beta-glucosidase (EC 3.2.1.21)	1
10	xylanase (EC 3.2.1.8); endo-1,3-beta-xylanase (EC 3.2.1.32)	6
11	xylanase (EC 3.2.1.8).	1
12	endoglucanase (EC 3.2.1.4); xyloglucan hydrolase (EC 3.2.1.151); beta-1,3-1 ,4-glucanase (EC 3.2.1.73); xyloglucan endotransglycosylase (EC 2.4.1.207)	1
13	apha-amylase (EC 3.2.1.1); pullulanase (EC 3.2.1 .41); cyclomaltodextrin glucanotransferase (EC 2.4.1.19); cyclornaltodextrinase (EC 3.2.1.54); trehalose-6-phosphate hydrolase (EC 3.2.1.93); oligo-alpha-glucosiclase (EC 3.2.1.10); maltogenic amylase (EC 3.2.1.133); neopullulanase (EC 3.2.1.135); alpha-glucosidase (EC 3.2.1.20); maltotetraose-forming 3 alpha-amylase (EC 3.2.1.60); isoamylase (EC 3.2.1.68); glucodextranase (EC 12.170); maltohexaose-forming alphaamylase (EC 3.2.1.98); branching enzyme (EC 2.4.1.18); trehalose synthase (EC	7

Glycoside Hydrolase Family	Known activities	Number of domains predicted in *C. phytofermentans*
	5.4.99.16); 4--glucanotransferase (EC 2.4.1.25); maltopentaose-forming -amylase (EC 3.2.1.-); amylosucrase (EC 2.4.1.4): sucrose phosphorylase (EC 2.4.1.7); malto-oligosyltrehalose trehalohydrolase (EC 3.2.1.141); isomaltulose synthase (EC 5.4.99.11).	
16	xyloglucan:xyloglucosyltransferase (EC 2.4.1.207); keratan-sulfate endo-1,4-beta-galactosidase (EC 3.2.1.103); Glucan endo-1,3-beta-D-glucosidase (EC 3.2.1.39); endo-1,3(4)-beta-glucanase (EC 3.21.6); Licheninase (EC 3.2.1.73): agarase (EC 3.2.1.81);betacarrageenase (EC 3.2.1.83); xyioglucanase (EC 3.2.1.151)	1
18	chitinase (EC 3.2.1.14); endo-beta-N-acetylglucosaminidase (EC 3.2.1.96); non-catalytic proteins: xylanase inhibitors; concanavalin B; narbonin	6
19	chitinase(EC 3.2.1.14).	2
20	beta-hexosaminidase (EC 3.2.1.52); lacto-N-biosidase (EC 3.2.1.140); -1,6-N-acetylglucosaminidase) (EC 3.2.1.-)	3
25	lysozyme (EC 3.2.1.17)	1
26	beta-mannanase (EC 3.2.1.78);beta-1,3-xylanase (EC 3.2.1.32)	3
28	polygalacturonase (EC 3.2.1.15); exo-polygalacturonase (EC 3.2.1.67); exo-polygalacturonosidase (EC 3.2.1.82); rhamnogalacturonase (EC 3.2.1.-); endo-xylogalacturonan hydrolase (EC 3.2.1.-); rhamnogalacturonan alpha-L-rhamnopyranohydrolase (EC 3.2.1.40)	5
29	alpha-L-fucosidase (EC 3.2.1.51)	3
30	glucosylceramidase (EC 3.2.1.45); beta-1,6-glucanase (EC 3.2.1.75); beta-xylosidase (EC 3.2.1.37)	2
31	alpha-glucosidase (EC 3.2.1.20): alpha-1,3-glucosidase (EC 3.2.1.84); sucrase-isomaltase (EC 3.2.1.48) (EC 3.2.1.10); alpha-xylosidase (EC 3.2.1.-); alpha-glucan lyase (EC 4.2.2.13); isomaltosyltransferase_(EC 2.4.1.-).	3
36	alpha-galactosidase (EC 3.2.1.22); alpha-N-acetylgalactosaminidase (EC 3.2.1.49); stachyose synthase (EC 2.4.1.67); raffinose synthase (EC 2.4.1.82)	2
38	alpha-mannosidase (EC 3.2.1.24); alpha-mannosidase (EC 3.2.1.114)	1
43	beta-xylosidase (EC 3.2.1.37); beta-1,3-xylosidase (EC 3.2.1.-);alpha-L-arabinofuranosidase (EC 3.2.1.55); arabinanase (EC 3.2.1.99); xylanase (EC 3.2.1.8); galactan 1,3-beta-galactosidase (EC 3.2.1.145)	8
48	endoglucanase (EC 3.2.1.4); chitinase (EC 3.2.1.14); cellobiohydrolases some cellobiohydrolases of this family have been reported to act from the reducing ends of cellulose (EC 3.2.1.-), while others have been reported to operate from the non- reducing ends to liberate cellobiose or cellotriose or cellotetraose (EC 3.2.1.-). This family also contains endo-processive celtulases (EC 3.2.1.-), whose activity is hard to distinguish from that of cellobiohydrolases.	1
51	alpha-L-arabinofuranosidase (EC 3.2.1.55); endoglucanase (EC 3.2.1.4)	1
65	trehalase (EC 3.2.1.28); maltose phosphorylase (EC 2.4.1.8); trehalose phosphorylase (EC 2.4.1.64); kojibiose phosphorylase	4

Glycoside Hydrolase Family	Known activities	Number of domains predicted in *C. phytofermentans*
	(EC 2.4.1.230)	
67	alpha-glucuronidase (EC 3.2.1.139); xylan alpha-I,2-glucuronosidase (EC_3.2.1.131)	1
73	peptidoglycan hydrolases with endo-beta-N-acetylglucosam inidase (EC 3.2.1.-) specificity; there is only one, unconfirmed, report of beta-i,4-N-acetylmuramoylhydrolase (EC 3.2.1.17) activity	1
77	amylomaltase or 4-aipha-glucanotransferase (EC 2.4.1.25)	1
85	endo-beta-N-acetylglucosaminidase (EC 3.2.1.96)	1
87	mycodextranase (EC 3.2.1.61); alpha-1,3-glucanase (EC 3.2.1.59)	3
88	d-4,5 unsaturated beta-glucuronyl hydrolase (EC 3.2.1.-)	4
94	cellobiose phosphorylase (EC 2.4.1.20); cellodextrin phosphorylase (EC 2.4.1.49); chitobiose phosphorylase (EC 2.4.1.-); cyclic beta-1,2-glucan synthase (EC 2.4.1.-)	5
95	alpha-1,2-L-fucosidase (EC 3.2.1.63); alpha-L-fucosidase (EC 3.2.1.51)	2
105	unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.-)	3
106	alpha-L-rhamnosidase (EC 3.2.1.40)	1
112	lacto-N-biose phosphorylase or galacto-N-biose phosphorylase (EC 2.4.1.211)	3

[00141] In one embodiment, enzymes that degrade polysaccharides are used for the pretreatment of biomass and can include enzymes that degrade cellulose, namely, cellulases. Examples of some cellulases include endocellulases (EC 3.2.1.4) and exo-cellulases (EC 3.2.1.91), that hydrolyze beta-l,4-glucosidic bonds. Members of the GH5, GH9 and GH48 families can have both exo- and endo-cellulase activity.

[00142] In one embodiment, enzymes that degrade polysaccharides are used for the pretreatment of biomass and can include enzymes that have the ability to degrade hemicellulose, namely, hemicellulases. Hemicellulose can be a major component of plant biomass and can contain a mixture of pentoses and hexoses, for example, **D**-xylopyranose, L-arabinofuranose, **D**-mannopyranose, **D**-glucopyranose, **D**-galactopyranose, **D**-glucopyranosyluronic acid and other sugars. In one embodiment, predicted hemicellulases identified in ***C. phytofermentans*** that can be used in the pretreatment of biomass include enzymes active on the linear backbone of hemicellulose, for example, endo-beta- 1,4-**D**-xylanase (EC 3.2.1.8), such as GH5, GH10, GHll, and GH43 family members; 1,4-beta-**D**-xyloside xylohydrolase (EC 3.2.1.37), such as GH30, GH43, and GH3 family members; and beta-mannanase (EC 3.2.1.78), such as GH26 family members.

[00143] In one embodiment, enzymes that degrade polysaccharides are used for the pretreatment of biomass and can include enzymes that have the ability to degrade pectin, namely, pectinases. In plant cell walls, the cross-linked cellulose network can be embedded in a matrix of pectins that can be covalently cross-linked to xyloglucans and certain structural proteins. Pectin can comprise homogalacturonan (HG) or rhamnogalacturonan (RH).

[00144] In one embodiment, pretreatment of biomass includes enzymes that can hydrolyze starch. C. *phytofermentans* can degrade starch and chitin (Warnick, T. A., Methe, **B.** A. & Leschine, S. **B.**

Clostridium phytofermentans sp. nov., a cellulolytic mesophile from forest soil. *Int. J. Syst. Evol. Microbiol.* 52, 1155-1160 (2002); Leschine, S. B. in *Handbook on Clostridia* (ed Diirre, P.) (CRC Press, Boca Raton, 2005); Reguera, G. & Leschine, S. B. Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments. *FEMS Micro biol. Lett.* 204, 367-374 (2001)). Enzymes that hydrolyze starch include alpha-amylase, glucoamylase, beta-amylase, exo-alpha-l,4-glucanase, and pullulanase.

[00145] In one embodiment, pretreatment of biomass comprises hydrolases that can include enzymes that hydrolyze chitin. Examples of enzymes that can hydrolyze chitin include GH18 and GH19 family members. In another embodiment, hydrolases can include enzymes that hydrolyze lichen, namely, lichenase, for example, GH16 family members.

[00146] In one embodiment, after pretreatment by any of the above methods the feedstock contains cellulose, hemicellulose, soluble oligomers, simple sugars, lignin, volatiles and ash. The parameters of the pretreatment can be changed to vary the concentration of the components of the pretreated feedstock. For example, in one embodiment a pretreatment is chosen so that the concentration of soluble oligomers is high and the concentration of lignin is low after pretreatment. Examples of parameters of the pretreatment include temperature, pressure, time, and pH.

[00147] In one embodiment, the parameters of the pretreatment are changed to vary the concentration of the components of the pretreated feedstock such that concentration of the components in the pretreated stock is optimal for fermentation with a microorganism such as a Q.12 or Q.13 microorganism.

[00148] In one embodiment, the parameters of the pretreatment are changed to encourage the release of the components of a genetically modified feedstock such as enzymes stored within a vacuole to increase or complement the enzymes synthesized by Q.12 or Q.13 to produce optimal release of the fermentable components during hydrolysis and fermentation.

[00149] In one embodiment, the parameters of the pretreatment are changed such that concentration of accessible cellulose in the pretreated feedstock is 1%, 5%, 10%, 12%, 13%, 14%, 15%, 16%, 17%, 19%, 20%, 30%, 40% or 50%>. In one embodiment, the parameters of the pretreatment are changed such that concentration of accessible cellulose in the pretreated feedstock is 5% to 30%. In one embodiment, the parameters of the pretreatment are changed such that concentration of accessible cellulose in the pretreated feedstock is 10% to 20%.

[00150] In one embodiment, the parameters of the pretreatment are changed such that concentration of hemicellulose in the pretreated feedstock is 1%, 5%, 10%, 12%, 13%, 14%, 15%, 16%, 17%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 40% or 50%. In one embodiment, the parameters of the pretreatment are changed such that concentration of hemicellulose in the pretreated feedstock is 5% to 40%. In one embodiment, the parameters of the pretreatment are changed such that concentration of hemicellulose in the pretreated feedstock is 10% to 30%.

[00151] In one embodiment, the parameters of the pretreatment are changed such that concentration of soluble oligomers in the pretreated feedstock is 1%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%,

55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. Examples of soluble oligomers include, but are not limited to, cellobiose and xylobiose. In one embodiment, the parameters of the pretreatment are changed such that concentration of soluble oligomers in the pretreated feedstock is 30%> to 90%>. In one embodiment, the parameters of the pretreatment are changed such that concentration of soluble oligomers in the pretreated feedstock is 45% to 80%. In one embodiment, the parameters of the pretreatment are changed such that concentration of soluble oligomers in the pretreated feedstock is 45% to 80% and the soluble oligomers are primarily cellobiose and xylobiose.

[00152] In one embodiment, the parameters of the pretreatment are changed such that concentration of simple sugars in the pretreated feedstock is 1%, 5%, 10%, 12%, 13%, 14%, 15%, 16%, 17%, 19%, 20%, 30%, 40%, or 50%. In one embodiment, the parameters of the pretreatment are changed such that concentration of simple sugars in the pretreated feedstock is 0% to 20%. In one embodiment, the parameters of the pretreatment are changed such that concentration of simple sugars in the pretreated feedstock is 0% to 5%. Examples of simple sugars include, but are not limited to, C5 and C6 monomers and dimers.

[00153] In one embodiment, the parameters of the pretreatment are changed such that concentration of lignin in the pretreated feedstock is 1%, 5%, 10%, 12%, 13%, 14%, 15%, 16%, 17%, 19%, 20%, 30%, 40%, or 50%. In one embodiment, the parameters of the pretreatment are changed such that concentration of lignin in the pretreated feedstock is 0% to 20%. In one embodiment, the parameters of the pretreatment are changed such that concentration of lignin in the pretreated feedstock is 0% to 5%. In one embodiment, the parameters of the pretreatment are changed such that concentration of lignin in the pretreated feedstock is less than 1% to 2%. In one embodiment, the parameters of the pretreatment are changed such that the concentration of phenolics is minimized.

[00154] In one embodiment, the parameters of the pretreatment are changed such that concentration of furfural and low molecular weight lignin in the pretreated feedstock is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. In one embodiment, the parameters of the pretreatment are changed such that concentration of furfural and low molecular weight lignin in the pretreated feedstock is less than 1% to 2%.

[00155] In one embodiment, the parameters of the pretreatment are changed such that concentration of accessible cellulose is 10% to 20 %, the concentration of hemicellulose is 10% to 30%, the concentration of soluble oligomers is 45% to 80%, the concentration of simple sugars is 0% to 5%, and the concentration of lignin is 0% to 5% and the concentration of furfural and low molecular weight lignin in the pretreated feedstock is less than 1% to 2%.

[00156] In one embodiment, the parameters of the pretreatment are changed to obtain a high concentration of hemicellulose and a low concentration of lignin. In one embodiment, the parameters of the pretreatment are changed to obtain a high concentration of hemicellulose and a low concentration of lignin such that concentration of the components in the pretreated stock is optimal for fermentation with a microorganism such as Q.12 or Q.13.

Co-culture Fermentations

[00157] In one embodiment, a method of producing one or more fermentation end-products with *Clostridium phytofermentans* Q.12 or Q.13 further comprises a second microorganism. In one embodiment, the second microorganism is a yeast, a bacteria, or a non-yeast fungus, wherein the second microorganism is a different species than Q.12 or Q.13. In one embodiment, the second microorganism is genetically modified. In another embodiment, the second microorganism is not genetically modified. Examples of yeast that can be the second microorganism include, but are not limited to, species found in the genus *Ascoidea, Brettanomyces, Candida, Cephaloascus, Coccidiascus, Dipodascus, Eremothecium, Galactomyces, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces, Sporopachydermia, Torulaspora, Yarrowia, or Zygosaccharomyces;* for example, *Ascoidea rebescens, Brettanomyces anomalus, Brettanomyces bruxellensis, Brettanomyces claussenii, Brettanomyces custersianus, Brettanomyces lambicus, Brettanomyces naardenensis, Brettanomyces nanus, Candida albicans, Candida ascalaphidarum, Candida amphixiae, Candida antarctica, Candida argentea, Candida atlantica, Candida atmosphaerica, Candida blattae, Candida carpophila, Candida cerambycidarum, Candida chauliodes, Candida corydali, Candida dosseyi, Candida dubliniensis, Candida ergatensis, Candida fructus, Candida glabrata, Candida fermentati, Candida guilliermondii, Candida haemulonii, Candida insectamens, Candida insectorum, Candida intermedia, Candida jeffresii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida lyxosophila, Candida maltosa, Candida marina, Candida membranifaciens, Candida milleri, Candida oleophila, Candida oregonensis, Candida parapsilosis, Candida quercitrusa, Candida rugosa, Candida sake, Candida shehatea, Candida temnochilae, Candida tenuis, Candida tropicalis, Candida tsuchiyae, Candida sinolaborantium, Candida sojae, Candida subhashii, Candida viswanathii, Candida utilis, Cephaloascus fragrans, Coccidiascus legeri, Dypodascus albidus, Eremothecium cymbalariae, Galactomyces candidum, Galactomyces geotrichum, Kluyveromyces aestuarii, Kluyveromyces africanus, Kluyveromyces bacillisporus, Kluyveromyces blattae, Kluyveromyces dobzhanskii, Kluyveromyces hubeiensis, Kluyveromyces lactis, Kluyveromyces lodderae, Kluyveromyces marxianus, Kluyveromyces nonfermentans, Kluyveromyces piceae, Kluyveromyces sinensis, Kluyveromyces thermotolerans, Kluyveromyces waltii, Kluyveromyces wickerhamii, Kluyveromyces yarrowii, Pichia anomola, Pichia heedii, Pichia guilliermondii, Pichia kluyveri, Pichia membranifaciens, Pichia norvegensis, Pichia ohmeri, Pichia pastoris, Pichia subpelliculosa, Saccharomyces bayanus, Saccharomyces boulardii, Saccharomyces bulderi, Saccharomyces cariocanus, Saccharomyces cariocus, Saccharomyces cerevisiae, Saccharomyces chevalieri, Saccharomyces dairenensis, Saccharomyces ellipsoideus, Saccharomyces eubayanus, Saccharomyces exiguus, Saccharomyces florentinus, Saccharomyces kluyveri, Saccharomyces martiniae, Saccharomyces monacensis, Saccharomyces norbensis, Saccharomyces paradoxus, Saccharomyces pastorianus, Saccharomyces spencerorum, Saccharomyces turicensis, Saccharomyces unisporus, Saccharomyces uvarum, Saccharomyces zonatus, Schizosaccharomyces cryophilus, Schizosaccharomyces japonicus, Schizosaccharomyces octosporus, Schizosaccharomyces pombe, Sporopachydermia cereana, Sporopachydermia lactativora,*

Sporopachydermia quercuum,Torulaspora delbrueckii, Torulaspora franciscae, Torulaspora globosa, Torulaspora pretoriensis, Yarrowia lipolytica, Zygosaccharomyces bailii, Zygosaccharomyces bisporus, Zygosaccharomyces cidri, Zygosaccharomyces fermentati, Zygosaccharomyces florentinus, Zygosaccharomyces kombuchaensis, Zygosaccharomyces lentus, Zygosaccharomyces mellis, Zygosaccharomyces microellipsoides, Zygosaccharomyces mrakii, Zygosaccharomyces pseudorouxii, or *Zygosaccharomyces rouxii.* Examples of bacteria that can be the second microorganism include, but are not limited to, any bacterium found in the genus of *Butyrivibrio, Ruminococcus, Eubacterium, Bacteroides, Acetivibrio, Caldibacillus, Acidothermus, Cellulomonas, Curtobacterium, Micromonospora, Actinoplanes, Streptomyces, Thermobifida, Thermomonospora, Microbispora, Fibrobacter, Sporocytophaga, Cytophaga, Flavobacterium, Achromobacter, Xanthomonas, Cellvibrio, Pseudomonas, Myxobacter, Escherichia, Klebsiella, Thermoanaerobacterium, Thermoanaerobacter, Geobacillus, Saccharococcus, Paenibacillus, Bacillus, Caldicellulosiruptor, Anaerocellum, Anoxybacillus, Zymomonas, Clostridium;* for example, *Butyrivibrio fibrisolvens, Ruminococcus flavefaciens, Ruminococcus succinogenes, Ruminococcus albus, Eubacterium cellulolyticum, Bacteroides cellulosolvens, Acetivibrio cellulolyticus, Acetivibrio cellulosolvens, Caldibacillus cellulovorans, Bacillus circulans, Acidothermus cellulolyticus, Cellulomonas cartae, Cellulomonas cellasea, Cellulomonas cellulans, Cellulomonas fimi, Cellulomonas flavigena, Cellulomonas gelida, Cellulomonas iranensis, Cellulomonas persica, Cellulomonas uda, Curtobacterium falcumfaciens , Micromonospora melonosporea, Actinoplanes aurantiaca, Streptomyces reticuli, Streptomyces alboguseolus, Streptomyces aureofaciens, Streptomyces cellulolyticus, Streptomyces flavogriseus, Streptomyces lividans, Streptomyces nitrosporeus, Streptomyces olivochromogenes, Streptomyces rochei, Streptomyces thermovulgaris, Streptomyces viridosporus, Thermobifida alba, Thermobifida fiusca, Thermobifida cellulolytica, Thermomonospora curvata, Microbispora bispora, Fibrobacter succinogenes, Sporocytophaga myxococcoides, Cytophaga sp., Flavobacterium johnsoniae, Achromobacter piechaudii, Xanthomonas sp., Cellvibrio vulgaris, Cellvibrio fulvus, Cellvibrio gilvus, Cellvibrio mixtus, Pseudomonas fluorescens, Pseudomonas mendocina, Myxobacter sp. AL-1, Escherichia albertii, Escherichia blattae, Escherichia coli, Escherichia fergusonii, Escherichia hermannii, Escherichia vulneris, Klebsiella granulomatis, Klebsiella oxytoca, Klebsiella pneumonia, Klebsiella terrigena, Thermoanaerobacterium thermosulfurigenes, Thermoanaerobacterium aotearoense, Thermoanaerobacterium polysaccharolyticum, Thermoanaerobacterium zeae, Thermoanaerobacterium xylanolyticum, Thermoanaerobacterium saccharolyticum, Thermoanaerobium brockii, Thermoanaerobacterium thermosaccharolyticum, Thermoanaerobacter thermohydrosulfuricus, Thermoanaerobacter ethanolicus, Thermoanaerobacter brocki, Geobacillus thermoglucosidasius, Geobacillus stearothermophilus, Saccharococcus caldoxylosilyticus, Saccharoccus thermophilus, Paenibacillus campinasensis, Bacillus flavothermus, Anoxybacillus kamchatkensis, Anoxybacillus gonensis, Caldicellulosiruptor acetigenus, Caldicellulosiruptor saccharolyticus, Caldicellulosiruptor kristjanssonii, Caldicellulosiruptor owensensis, Caldicellulosiruptor lactoaceticus, Anaerocellum*

thermophilum, Clostridium thermocellum, Clostridium cellulolyticum, Clostridium straminosolvens, Clostridium acetobutylicum, Clostridium aerotolerans, Clostridium beijerinckii, Clostridium bifermentans, Clostridium botulinum, Clostridium butyricum, Clostridium cadaveris, Clostridium chauvoei, Clostridium clostridioforme, Clostridium colicanis, Clostridium difficile, Clostridium fallax, Clostridium formicaceticum, Clostridium histolyticum, Clostridium innocuum, Clostridium Ijungdahlii, Clostridium laramie, Clostridium lavalense, Clostridium novyi, Clostridium oedematiens, Clostridium paraputrificum, Clostridium perfringens, Clostridium phytofermentans (including NRRL B-50364 or NRRL B-50351), *Clostridium piliforme, Clostridium ramosum, Clostridium scatologenes, Clostridium septicum, Clostridium sordellu, Clostridium sporogenes, Clostridium* sp. Q.D (such as NRRL B-50361, NRRL B-50362, or NRRL B-50363), *Clostridium tertium, Clostridium tetani, Clostridium tyrobutyricum, Clostridium thermobutyricum, Zymomonas mobilis* or variants thereof (e.g. *C. phytofermentans* Q.12 or *C. phytofermentans* Q.13). In one embodiment, the second microorganism is *Saccharomyces cerevisiae, C thermocellum, C acetobutylicum, C cellovorans,* or *Zymomonas mobilis.* In another embodiment, the second microorganism is *Thermoanaerobacter pseudethanolicus, Thermoanaerobacter mathranii, Thermoanaerobacter italicus, Thermoanaerobacter brockii, T. acetoethylicus, Thermoanaerobacter ethanolicus, Thermoanaerobacter kivui, Thermoanaerobacter siderophilus, Thermoanaerobacter sulfuragignens, Thermoanaerobacter sulfurophilus, Thermoanaerobacter thermocopriae, Thermoanaerobacter thermohydrosulfuricus, Thermoanaerobacter uzonensis,* or *Thermoanaerobacter wiegelii.* In another embodiment, the second microorganism is *Eremothecium ashbyii, Ashbya gossypii, Candida flaeri, Candida famata, Candida ammoniagenes, Corynebacterium* sp., *Serratia marcescens, Fusarium oxysporum, Brevibacterium ammoniagenes, Rhodococcus rhodochrous, Brevibacterium* sp., *Arthrobacter* sp., *Candida boidinii, Bacillus* sp., *Gluconobacter* sp., *Arthrobacter* sp., *Saccharomyces sake, Alcaligenes faecalis, Agrobacterium* sp., *Sporoblomyces salmonicolor, Pseudomonas* sp., *Propionibacterium shermanii, Pseudomonas denitrificans, Geotrichum candidum, Flavobacterium* sp., or *Mortierella alpina.*

[00158] Recovery of Ethanol or Other Fermentation End-Products

[00159] In another aspect, methods are provided for the recovery of the fermentation end products, such as an alcohol *(e.g.,* ethanol, propanol, methanol, butanol, *etc.)* another biofuel or chemical product. In one embodiment, broth will be harvested at some point during of the fermentation, and fermentation end product or products will be recovered. The broth with ethanol to be recovered will include both ethanol and impurities. The impurities include materials such as water, cell bodies, cellular debris, excess carbon substrate, excess nitrogen substrate, other remaining nutrients, non-ethanol metabolites, and other medium components or digested medium components. During the course of processing the broth, the broth can be heated and/or reacted with various reagents, resulting in additional impurities in the broth.

[00160] In one embodiment, the processing steps to recover ethanol frequently includes several separation steps, including, for example, distillation of a high concentration ethanol material from a less pure ethanol-containing material. In one embodiment, the high concentration ethanol material can be

further concentrated to achieve very high concentration ethanol, such as 98% or 99% or 99.5% (wt.) or even higher. Other separation steps, such as filtration, centrifugation, extraction, adsorption, *etc.* can also be a part of some recovery processes for ethanol as a product or biofuel, or other biofuels or chemical products.

[00161] In one embodiment a process can be scaled to produce commercially useful biofuels. In another embodiment Q.12 or Q.13 is used to produce an alcohol, *e.g.*, ethanol, butanol, propanol, methanol, or a fuel such as hydrocarbons hydrogen, methane, and hydroxy compounds. In another embodiment Q.12 or Q.13 is used to produce a carbonyl compound such as an aldehyde or ketone *(e.g.,* acetone, formaldehyde, 1-propanal, *etc.),* an organic acid, a derivative of an organic acid such as an ester *(e.g.,* wax ester, glyceride, *etc.),* 1, 2- propanediol, 1, 3-propanediol, lactic acid, formic acid, acetic acid, succinic acid, pyruvic acid, or an enzyme such as a cellulase, polysaccharase, lipases, protease, ligninase, and hemicellulase.

[00162] In one embodiment, a fed-batch fermentation for production of fermentation end product is described. In another embodiment, a fed-batch fermentation for production of ethanol is described. Fed-batch culture is a kind of microbial process in which medium components, such as carbon substrate, nitrogen substrate, vitamins, minerals, growth factors, cofactors, *etc.* or biocatalysts (including, for example, fresh microorganisms, enzymes prepared by Q.12 or Q.13 in a separate fermentation, enzymes prepared by other microorganisms, or a combination of these) are supplied to the fermentor during cultivation, but culture broth is not harvested at the same time and volume. To improve bioconversion from soluble and insoluble substrates, such as those that can be used in biofuels production, various feeding strategies can be utilized to improve yields and/or productivity. This technique can be used to achieve a high cell density within a given time. It can also be used to maintain a good supply of nutrients and substrates for the bioconversion process. It can also be used to achieve higher titer and productivity of desirable products that might otherwise be achieved more slowly or not at all.

[00163] In another embodiment, the feeding strategy balances the cell production rate and the rate of hydrolysis of the biomass feedstock with the production of ethanol. Sufficient medium components are added in quantities to achieved sustained cell production and hydrolysis of the biomass feedstock with production of ethanol. In one embodiment, sufficient carbon and nitrogen substrate are added in quantities to achieve sustained production of fresh cells and hydrolytic enzymes for conversion of polysaccharides into lower sugars as well as sustained conversion of the lower sugars into fresh cells and ethanol.

[00164] In another embodiment, the level of a medium component is maintained at a desired level by adding additional medium component as the component is consumed or taken up by the microorganism. Examples of medium components included, but are not limited to, carbon substrate, nitrogen substrate, vitamins, minerals, growth factors, cofactors, and biocatalysts. The medium component can be added continuously or at regular or irregular intervals. In one embodiment, additional medium component is added prior to the complete depletion of the medium component in the medium. In one embodiment, complete depletion can effectively be used, for example to initiate different metabolic pathways, to

simplify downstream operations, or for other reasons as well. In one embodiment, the medium component level is allowed to vary by about 10% around a midpoint, in one embodiment, it is allowed to vary by about 30% around a midpoint, and in one embodiment, it is allowed to vary by 60%> or more around a midpoint. In one embodiment, the medium component level is maintained by allowing the medium component to be depleted to an appropriate level, followed by increasing the medium component level to another appropriate level. In one embodiment, a medium component, such as vitamin, is added at two different time points during fermentation process. For example, one-half of a total amount of vitamin is added at the beginning of fermentation and the other half is added at midpoint of fermentation.

[00165] In another embodiment, the nitrogen level is maintained at a desired level by adding additional nitrogen-containing material as nitrogen is consumed or taken up by the microorganism. The nitrogen-containing material can be added continuously or at regular or irregular intervals. In one embodiment, additional nitrogen-containing material is added prior to the complete depletion of the nitrogen available in the medium. In one embodiment, complete depletion can effectively be used, for example to initiate different metabolic pathways, to simplify downstream operations, or for other reasons as well. In one embodiment, the nitrogen level (as measured by the grams of actual nitrogen in the nitrogen-containing material per liter of broth) is allowed to vary by about 10%> around a midpoint, in some embodiments, it is allowed to vary by about 30% around a midpoint, and in some embodiments, it is allowed to vary by 60% or more around a midpoint. In one embodiment, the nitrogen level is maintained by allowing the nitrogen to be depleted to an appropriate level, followed by increasing the nitrogen level to another appropriate level. Useful nitrogen levels include levels of about 5 to about 10 g/L. In one embodiment levels of about 1 to about 12 g/L can also be usefully employed. In another embodiment levels, such as about 0.5, 0.1 g/L or even lower, and higher levels, such as about 20, 30 g/L or even higher are used. In another embodiment a useful nitrogen level is about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, 24, 25, 26, 27, 28, 29 or 30 g/L. Such nitrogen levels can facilitate the production of fresh cells and of hydrolytic enzymes. Increasing the level of nitrogen can lead to higher levels of enzymes and/or greater production of cells, and result in higher productivity of desired products. Nitrogen can be supplied as a simple nitrogen-containing material, such as an ammonium compounds *(e.g.,* ammonium sulfate, ammonium hydroxide, ammonia, ammonium nitrate, or any other compound or mixture containing an ammonium moiety), nitrate or nitrite compounds *(e.g.,* potassium, sodium, ammonium, calcium, or other compound or mixture containing a nitrate or nitrite moiety), or as a more complex nitrogen-containing material, such as amino acids, proteins, hydrolyzed protein, hydrolyzed yeast, yeast extract, dried brewer's yeast, yeast hydrolysates, distillers' grains, soy protein, hydrolyzed soy protein, fermentation products, and processed or corn steep powder or unprocessed protein-rich vegetable or animal matter, including those derived from bean, seeds, soy, legumes, nuts, milk, pig, cattle, mammal, fish, as well as other parts of plants and other types of animals. Nitrogen-containing materials useful in various embodiments also include materials that contain a nitrogen-containing material, including, but not limited to mixtures of a simple or more complex

nitrogen-containing material mixed with a carbon source, another nitrogen-containing material, or other nutrients or non-nutrients, and AFEX treated plant matter.

[00166] In another embodiment, the carbon level is maintained at a desired level by adding sugar compounds or material containing sugar compounds ("Sugar-Containing Material") as sugar is consumed or taken up by the microorganism. The sugar-containing material can be added continuously or at regular or irregular intervals. In one embodiment, additional sugar-containing material is added prior to the complete depletion of the sugar compounds available in the medium. In one embodiment, complete depletion can effectively be used, for example to initiate different metabolic pathways, to simplify downstream operations, or for other reasons as well. In one embodiment, the carbon level (as measured by the grams of sugar present in the sugar-containing material per liter of broth) is allowed to vary by about 10% around a midpoint, in one embodiment, it is allowed to vary by about 30% around a midpoint, and in one embodiment, it is allowed to vary by 60%> or more around a midpoint. In one embodiment, the carbon level is maintained by allowing the carbon to be depleted to an appropriate level, followed by increasing the carbon level to another appropriate level. In some embodiments, the carbon level can be maintained at a level of about 5 to about 120 g/L. However, levels of about 30 to about 100 g/L can also be usefully employed as well as levels of about 60 to about 80 g/L. In one embodiments, the carbon level is maintained at greater than 25 g/L for a portion of the culturing. In another embodiment, the carbon level is maintained at about 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, 31 g/L, 32 g/L, 33 g/L, 34 g/L, 35 g/L, 36 g/L, 37 g/L, 38 g/L, 39 g/L, 40 g/L, 41 g/L, 42 g/L, 43 g/L, 44 g/L, 45 g/L, 46 g/L, 47 g/L, 48 g/L, 49 g/L, 50 g/L, 51 g/L, 52 g/L, 53 g/L, 54 g/L, 55 g/L, 56 g/L, 57 g/L, 58 g/L, 59 g/L, 60 g/L, 61 g/L, 62 g/L, 63 g/L, 64 g/L, 65 g/L, 66 g/L, 67 g/L, 68 g/L, 69 g/L, 70 g/L, 71 g/L, 72 g/L, 73 g/L, 74 g/L, 75 g/L, 76 g/L, 77 g/L, 78 g/L, 79 g/L, 80 g/L, 81 g/L, 82 g/L, 83 g/L, 84 g/L, 85 g/L, 86 g/L, 87 g/L, 88 g/L, 89 g/L, 90 g/L, 91 g/L, 92 g/L, 93 g/L, 94 g/L, 95 g/L, 96 g/L, 97 g/L, 98 g/L, 99 g/L, 100 g/L, 101 g/L, 102 g/L, 103 g/L, 104 g/L, 105 g/L, 106 g/L, 107 g/L, 108 g/L, 109 g/L, 110 g/L, 111 g/L, 112 g/L, 113 g/L, 114 g/L, 115 g/L, 116 g/L, 117 g/L, 118 g/L, 119 g/L, 120 g/L, 121 g/L, 122 g/L, 123 g/L, 124 g/L, 125 g/L, 126 g/L, 127 g/L, 128 g/L, 129 g/L, 130 g/L, 131 g/L, 132 g/L, 133 g/L, 134 g/L, 135 g/L, 136 g/L, 137 g/L, 138 g/L, 139 g/L, 140 g/L, 141 g/L, 142 g/L, 143 g/L, 144 g/L, 145 g/L, 146 g/L, 147 g/L, 148 g/L, 149 g/L, or 150 g/L.

[00167] The carbon substrate, like the nitrogen substrate, can beutilized for cell production and enzyme production. The carbon substrate can also serve as the raw material for production of fermentation end-products such as ethanol. Frequently, more carbon substrate can lead to greater production of ethanol. In another embodiment, it can be advantageous to operate with the carbon level and nitrogen level related to each other for at least a portion of the fermentation time. In one embodiment, the ratio of carbon to nitrogen is maintained within a range of about 30:1 to about 10:1. In another embodiment, the ratio of carbon nitrogen is maintained from about 20:1 to about 10:1. In another embodiment, the ratio of carbon nitrogen is maintained from about 15:1 to about 10:1. In another embodiment the ratio of carbon nitrogen

is about 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1.

[00168] Maintaining the ratio of carbon and nitrogen ratio within particular ranges can result in benefits to the operation such as the rate of hydrolysis of carbon substrate, which depends on the amount of carbon substrate and the amount and activity of enzymes present, being balanced to the rate of ethanol production. Such balancing can be important, for example, due to the possibility of inhibition of cellular activity due to the presence of a high concentration of low molecular weight saccharides, and the need to maintain enzymatic hydrolytic activity throughout the period where longer chain saccharides are present and available for hydrolysis. Balancing the carbon to nitrogen ratio can, for example, facilitate the sustained production of these enzymes such as to replace those which have lost activity.

[00169] In another embodiment, the amount and/or timing of carbon, nitrogen, or other medium component addition can be related to measurements taken during the fermentation. For example, the amount of monosaccharides present, the amount of insoluble polysaccharide present, the polysaccharase activity, the amount of ethanol present, the amount of cellular material (for example, packed cell volume, dry cell weight, *etc.)* and/or the amount of nitrogen (for example, nitrate, nitrite, ammonia, urea, proteins, amino acids, *etc.)* present can be measured. The concentration of the particular species, the total amount of the species present in the fermentor, the number of hours the fermentation has been running, and the volume of the fermentor can be considered. In various embodiments, these measurements can be compared to each other and/or they can be compared to previous measurements of the same parameter previously taken from the same fermentation or another fermentation. Adjustments to the amount of a medium component can be accomplished such as by changing the flow rate of a stream containing that component or by changing the frequency of the additions for that component. In one embodiment, the amount of polysaccharide can be reduced when the monosaccharides level increases faster than the ethanol level increases. In another embodiment, the amount of polysaccharide can be increased when the amount or level of monosaccharides decreases while the ethanol production approximately remains steady. In another embodiment, the amount of nitrogen can be increased when the monosaccharides level increases faster than the viable cell level. The amount of polysaccharide can also be increased when the cell production increases faster than the ethanol production. In another embodiment the amount of nitrogen can be increased when the enzyme activity level decreases.

[00170] In another embodiment, different levels or complete depletion of a medium component can effectively be used, for example to initiate different metabolic pathways or to change the yield of the different products of the fermentation process. For instance, different levels or complete depletion of a medium component can effectively be used to increase the ethanol yield and productivity, to improve carbon utilization *(e.g.,* g ethanol/g sugar fermented) and reduced acid production *(e.g.,* g acid/g ethanol and g acid/g sugar fermented). In some embodiments, different levels or complete depletion of nitrogen can effectively be used to increase the ethanol yield and productivity, to improve carbon utilization *(e.g.,* g ethanol/g sugar fermented) and reduced acid production *(e.g.,* g acid/g ethanol and g acid/g sugar

fermented). In some embodiments, different levels or complete depletion of carbon can effectively be used to increase the ethanol yield and productivity, to improve carbon utilization *(e.g.,* g ethanol/g sugar fermented) and reduced acid production *(e.g.,* g acid/g ethanol and g acid/g sugar fermented). In some embodiments, the ratio of carbon level to nitrogen level for at least a portion of the fermentation time can effectively be used to increase the ethanol yield and productivity, to improve carbon utilization *(e.g,* g ethanol/g sugar fermented) and reduced acid production *(e.g.,* g acid/g ethanol and g acid/g sugar fermented).

[00171] In another embodiment, a fed batch operation can be employed, wherein medium components and/or fresh cells are added during the fermentation without removal of a portion of the broth for harvest prior to the end of the fermentation. In one embodiment a fed-batch process is based on feeding a growth limiting nutrient medium to a culture of microorganisms. In one embodiment the feed medium is highly concentrated to avoid dilution of the bioreactor. In another embodiment the controlled addition of the nutrient directly affects the growth rate of the culture and avoids overflow metabolism such as the formation of side metabolites. In one embodiment the growth limiting nutrient is a nitrogen source or a saccharide source.

[00172] In another embodiment, a modified fed batch operation can be employed wherein a portion of the broth is harvested at discrete times. Such a modified fed batch operation can be advantageously employed when, for example, very long fermentation cycles are employed. Under very long fermentation conditions, the volume of liquid inside the fermentor increases. In order to operate for very long periods, it can be advantageous to partially empty the fermentor, for example, when the volume is nearly full. A partial harvest of broth followed by supplementation with fresh medium ingredients, such as with a fed batch operation, can improve fermentor utilization and can facilitate higher plant throughputs due to a reduction in the time for tasks such as cleaning and sterilization of equipment. When the "partial harvest" type of operation is employed, the fermentation can be seeded with the broth that remains in the fermentor, or with fresh inoculum, or with a mixture of the two. In addition, broth can be recycled for use as fresh inoculum either alone or in combination with other fresh inoculum.

[00173] In one embodiment, a fed batch operation can be employed, wherein medium components and/or fresh cells are added during the fermentation when the hydrolytic activity of the broth has decreased. In one embodiment, medium components and/or fresh cells are added during the fermentation when the hydrolytic activity of the broth has decreased about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 80%, 85%, 90%, 95%, or 100%.

[00174] While Q.12 or Q.13 can be used in long or short fermentation cycles, it is particularly well-suited for long fermentation cycles and for use in fermentations with partial harvest, self-seeding, and broth recycle operations due to the anaerobic conditions of the fermentation, the presence of alcohol, the very fast growth rate of the strains compared to other Clostridia, and, in one embodiment, the use of a solid carbon substrate, whether or not resulting in low sugar concentrations in the broth.

[00175] In another embodiment, a fermentation to produce ethanol is performed by culturing a strain of Q.12 or Q.13 in a medium having a high concentration of one or more carbon sources, and/or augmenting the culture with addition of fresh cells of Q.12 or Q.13 during the course of the fermentation. The resulting production of ethanol can be up to 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, and in some cases up to 10-fold and higher in volumetric productivity than a batch process and achieve a carbon conversion efficiency approaching the theoretical maximum. The theoretical maximum can vary with the substrate and product. For example, the generally accepted maximum efficiency for conversion of glucose to ethanol is 0.51 g ethanol/g glucose. In one embodiment Q.12 or Q.13 can produce about 40-100% of a theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce up to about 40% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce up to about 50%> of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 70% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 90% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 95% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 95% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 99% of the theoretical maximum yield of ethanol. In another embodiment, Q.12 or Q.13 can produce about 100%, of the theoretical maximum yield of ethanol. In one embodiment Q.12 or Q.13 can produce up to about 1%, *2%,* 3%>, 4%>, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 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%, 99%, 99.99%, 100% of a theoretical maximum yield of ethanol.

[00176] Q.12 or Q.13 cells used for the seed inoculum or for cell augmentation can be prepared or treated in ways that relate to their ability to produce enzymes useful for hydrolyzing the components of the production medium. For example, in one embodiment, the cells can produce useful enzymes after they are transferred to the production medium or production fermentor. In another embodiment, Q.12 or Q.13 cells can have already produced useful enzymes prior to transfer to the production medium or the production fermentor. In another embodiment, Q.12 or Q.13 cells can be ready to produce useful enzymes once transferred to the production medium or the production fermentor, or Q.12 or Q.13 cells can have some combination of these enzyme production characteristics. In one embodiment, the seed can be grown initially in a medium containing a simple sugar source, such as corn syrup or dried brewer's yeast, and then transitioned to the production medium carbon source prior to transfer to the production medium. In another embodiment, the seed is grown on a combination of simple sugars and production medium carbon source prior to transfer to the production medium. In another embodiment, the seed is grown on the production medium carbon source from the start. In another embodiment, the seed is grown on one

production medium carbon source and then transitioned to another production medium carbon source prior to transfer to the production medium. In another embodiment, the seed is grown on a combination of production medium carbon sources prior to transfer to the production medium. In another embodiment, the seed is grown on a carbon source that favors production of hydrolytic enzymes with activity toward the components of the production medium.

[00177] In another embodiment, a fermentation to produce ethanol is performed by culturing a strain of Q.12 or Q.13 microorganism and adding fresh medium components and fresh Q.12 or Q.13 cells while the cells in the fermentor are growing. Medium components, such as carbon, nitrogen, and combinations of these, can be added as disclosed herein, as well as other nutrients, including vitamins, factors, cofactors, enzymes, minerals, salts, and such, sufficient to maintain an effective level of these nutrients in the medium. The medium and Q.12 or Q.13 can be added simultaneously, or one at a time. In another embodiment, fresh Q.12 or Q.13 cells can be added when hydrolytic enzyme activity decreases, especially when the activity of those hydrolytic enzymes that are more sensitive to the presence of alcohol decreases. After the addition of fresh Q.12 or Q.13 cells, a nitrogen feed or a combination of nitrogen and carbon feed and/or other medium components can be fed, prolonging the enzymatic production or other activity of the cells. In another embodiment, the cells can be added with sufficient carbon and nitrogen to prolong the enzymatic production or other activity of the cells sufficiently until the next addition of fresh cells. In another embodiment, fresh Q.12 or Q.13 cells can be added when both the nitrogen level and carbon level present in the fermentor increase. In another embodiment, Q.12 or Q.13 cells can be added when the viable cell count decreases, especially when the nitrogen level is relatively stable or increasing. In another embodiment, fresh cells can be added when a significant portion of the viable cells are in the process of sporulation, or have sporulated. Appropriate times for adding fresh Q.12 or Q.13 cells can be when the portion of cells in the process of sporulation or have sporulated is about 2% to about 100%, about 10% to about 75%, about 20% to about 50%, or about 25% to about 30% of the cells are in the process of sporulation or have sporulated.

[00178] Medium Compositions

[00179] In various embodiments, particular medium components can have beneficial effects on the performance of the fermentation, such as increasing the titer of desired products, or increasing the rate that the desired products are produced. Specific compounds can be supplied as a specific, pure ingredient, such as a particular amino acid, or it can be supplied as a component of a more complex ingredient, such as using a microbial, plant or animal product as a medium ingredient to provide a particular amino acid, promoter, cofactor, or other beneficial compound. In some cases, the particular compound supplied in the medium ingredient can be combined with other compounds by the microorganism resulting in a fermentation-beneficial compound. One example of this situation would be where a medium ingredient provides a specific amino acid which the microorganism uses to make an enzyme beneficial to the fermentation. Other examples can include medium components that are used to generate growth or product promoters, *etc.* In such cases, it can be possible to obtain a fermentation-beneficial result by

supplementing the enzyme, promoter, growth factor, *etc.* or by adding the precursor. In some situations, the specific mechanism whereby the medium component benefits the fermentation is not known, only that a beneficial result is achieved.

[00180] In one embodiment, beneficial fermentation results can be achieved by adding yeast extract. The addition of the yeast extract can result in increased ethanol titer in batch fermentation, improved productivity and reduced production of side products such as organic acids. In one embodiment beneficial results with yeast extract can be achieved at usage levels of about 0.5 to about 50 g/L, about 5 to about 30 g/L, or about 10 to about 30 g/L. In another embodiment the yeast extract is used at level about 0.5 g/L, 0.6 g/L, 0.7 g/L, 0.8 g/L, 0.9 g/L, 1 g/L, 1.1 g/L, 1.2 g/L, 1.3 g/L, 1.4 g/L, 1.5 g/L, 1.6 g/L, 1.7 g/L, 1.8 g/L, 1.9 g/L, 2 g/L, 2.1 g/L, 2.2 g/L, 2.3 g/L, 2.4 g/L, 2.5 g/L, 2.6 g/L, 2.7 g/L, 2.8 g/L, 2.9 g/L, 3 g/L, 3.1 g/L, 3.2 g/L, 3.3 g/L, 3.4 g/L, 3.5 g/L, 3.6 g/L, 3.7 g/L, 3.8 g/L, 3.9 g/L, 4 g/L, 4.1 g/L, 4.2 g/L, 4.3 g/L, 4.4 g/L, 4.5 g/L, 4.6 g/L, 4.7 g/L, 4.8 g/L, 4.9 g/L, 5 g/L, 5.1 g/L, 5.2 g/L, 5.3 g/L, 5.4 g/L, 5.5 g/L, 5.6 g/L, 5.7 g/L, 5.8 g/L, 5.9 g/L, 6 g/L, 6.1 g/L, 6.2 g/L, 6.3 g/L, 6.4 g/L, 6.5 g/L, 6.6 g/L, 6.7 g/L, 6.8 g/L, 6.9 g/L, 7 g/L, 7.1 g/L, 7.2 g/L, 7.3 g/L, 7.4 g/L, 7.5 g/L, 7.6 g/L, 7.7 g/L, 7.8 g/L, 7.9 g/L, 8 g/L, 8.1 g/L, 8.2 g/L, 8.3 g/L, 8.4 g/L, 8.5 g/L, 8.6 g/L, 8.7 g/L, 8.8 g/L, 8.9 g/L, 9 g/L, 9.1 g/L, 9.2 g/L, 9.3 g/L, 9.4 g/L, 9.5 g/L, 9.6 g/L, 9.7 g/L, 9.8 g/L, 9.9 g/L, 10 g/L, 10.1 g/L, 10.2 g/L, 10.3 g/L, 10.4 g/L, 10.5 g/L, 10.6 g/L, 10.7 g/L, 10.8 g/L, 10.9 g/L, 11 g/L, 11.1 g/L, 11.2 g/L, 11.3 g/L, 11.4 g/L, 11.5 g/L, 11.6 g/L, 11.7 g/L, 11.8 g/L, 11.9 g/L, 12 g/L, 12.1 g/L, 12.2 g/L, 12.3 g/L, 12.4 g/L, 12.5 g/L, 12.6 g/L, 12.7 g/L, 12.8 g/L, 12.9 g/L, 13 g/L, 13.1 g/L, 13.2 g/L, 13.3 g/L, 13.4 g/L, 13.5 g/L, 13.6 g/L, 13.7 g/L, 13.8 g/L, 13.9 g/L, 14 g/L, 14.1 g/L, 14.2 g/L, 14.3 g/L, 14.4 g/L, 14.5 g/L, 14.6 g/L, 14.7 g/L, 14.8 g/L, 14.9 g/L, 15 g/L, 15.1 g/L, 15.2 g/L, 15.3 g/L, 15.4 g/L, 15.5 g/L, 15.6 g/L, 15.7 g/L, 15.8 g/L, 15.9 g/L, 16 g/L, 16.1 g/L, 16.2 g/L, 16.3 g/L, 16.4 g/L, 16.5 g/L, 16.6 g/L, 16.7 g/L, 16.8 g/L, 16.9 g/L, 17 g/L, 17.1 g/L, 17.2 g/L, 17.3 g/L, 17.4 g/L, 17.5 g/L, 17.6 g/L, 17.7 g/L, 17.8 g/L, 17.9 g/L, 18 g/L, 18.1 g/L, 18.2 g/L, 18.3 g/L, 18.4 g/L, 18.5 g/L, 18.6 g/L, 18.7 g/L, 18.8 g/L, 18.9 g/L, 19 g/L, 19.1 g/L, 19.2 g/L, 19.3 g/L, 19.4 g/L, 19.5 g/L, 19.6 g/L, 19.7 g/L, 19.8 g/L, 19.9 g/L, 20 g/L, 20.1 g/L, 20.2 g/L, 20.3 g/L, 20.4 g/L, 20.5 g/L, 20.6 g/L, 20.7 g/L, 20.8 g/L, 20.9 g/L, 21 g/L, 21.1 g/L, 21.2 g/L, 21.3 g/L, 21.4 g/L, 21.5 g/L, 21.6 g/L, 21.7 g/L, 21.8 g/L, 21.9 g/L, 22 g/L, 22.1 g/L, 22.2 g/L, 22.3 g/L, 22.4 g/L, 22.5 g/L, 22.6 g/L, 22.7 g/L, 22.8 g/L, 22.9 g/L, 23 g/L, 23.1 g/L, 23.2 g/L, 23.3 g/L, 23.4 g/L, 23.5 g/L, 23.6 g/L, 23.7 g/L, 23.8 g/L, 23.9 g/L, 24 g/L, 24.1 g/L, 24.2 g/L, 24.3 g/L, 24.4 g/L, 24.5 g/L, 24.6 g/L, 24.7 g/L, 24.8 g/L, 24.9 g/L, 25 g/L, 25.1 g/L, 25.2 g/L, 25.3 g/L, 25.4 g/L, 25.5 g/L, 25.6 g/L, 25.7 g/L, 25.8 g/L, 25.9 g/L, 26 g/L, 26.1 g/L, 26.2 g/L, 26.3 g/L, 26.4 g/L, 26.5 g/L, 26.6 g/L, 26.7 g/L, 26.8 g/L, 26.9 g/L, 27 g/L, 27.1 g/L, 27.2 g/L, 27.3 g/L, 27.4 g/L, 27.5 g/L, 27.6 g/L, 27.7 g/L, 27.8 g/L, 27.9 g/L, 28 g/L, 28.1 g/L, 28.2 g/L, 28.3 g/L, 28.4 g/L, 28.5 g/L, 28.6 g/L, 28.7 g/L, 28.8 g/L, 28.9 g/L, 29 g/L, 29.1 g/L, 29.2 g/L, 29.3 g/L, 29.4 g/L, 29.5 g/L, 29.6 g/L, 29.7 g/L, 29.8 g/L, 29.9 g/L, 30 g/L, 30.1 g/L, 30.2 g/L, 30.3 g/L, 30.4 g/L, 30.5 g/L, 30.6 g/L, 30.7 g/L, 30.8 g/L, 30.9 g/L, 31 g/L, 31.1 g/L, 31.2 g/L, 31.3 g/L, 31.4 g/L, 31.5 g/L, 31.6 g/L, 31.7 g/L, 31.8 g/L, 31.9 g/L, 32 g/L, 32.1 g/L, 32.2 g/L, 32.3 g/L, 32.4 g/L, 32.5 g/L, 32.6 g/L, 32.7 g/L, 32.8 g/L, 32.9 g/L, 33 g/L, 33.1 g/L, 33.2 g/L, 33.3 g/L, 33.4 g/L, 33.5 g/L, 33.6 g/L,

33.7 g/L, 33.8 g/L, 33.9 g/L, 34 g/L, 34.1 g/L, 34.2 g/L, 34.3 g/L, 34.4 g/L, 34.5 g/L, 34.6 g/L, 34.7 g/L, 34.8 g/L, 34.9 g/L, 35 g/L, 35.1 g/L, 35.2 g/L, 35.3 g/L, 35.4 g/L, 35.5 g/L, 35.6 g/L, 35.7 g/L, 35.8 g/L, 35.9 g/L, 36 g/L, 36.1 g/L, 36.2 g/L, 36.3 g/L, 36.4 g/L, 36.5 g/L, 36.6 g/L, 36.7 g/L, 36.8 g/L, 36.9 g/L, 37 g/L, 37.1 g/L, 37.2 g/L, 37.3 g/L, 37.4 g/L, 37.5 g/L, 37.6 g/L, 37.7 g/L, 37.8 g/L, 37.9 g/L, 38 g/L, 38.1 g/L, 38.2 g/L, 38.3 g/L, 38.4 g/L, 38.5 g/L, 38.6 g/L, 38.7 g/L, 38.8 g/L, 38.9 g/L, 39 g/L, 39.1 g/L, 39.2 g/L, 39.3 g/L, 39.4 g/L, 39.5 g/L, 39.6 g/L, 39.7 g/L, 39.8 g/L, 39.9 g/L, 40 g/L, 40.1 g/L, 40.2 g/L, 40.3 g/L, 40.4 g/L, 40.5 g/L, 40.6 g/L, 40.7 g/L, 40.8 g/L, 40.9 g/L, 41 g/L, 41.1 g/L, 41.2 g/L, 41.3 g/L, 41.4 g/L, 41.5 g/L, 41.6 g/L, 41.7 g/L, 41.8 g/L, 41.9 g/L, 42 g/L, 42.1 g/L, 42.2 g/L, 42.3 g/L, 42.4 g/L, 42.5 g/L, 42.6 g/L, 42.7 g/L, 42.8 g/L, 42.9 g/L, 43 g/L, 43.1 g/L, 43.2 g/L, 43.3 g/L, 43.4 g/L, 43.5 g/L, 43.6 g/L, 43.7 g/L, 43.8 g/L, 43.9 g/L, 44 g/L, 44.1 g/L, 44.2 g/L, 44.3 g/L, 44.4 g/L, 44.5 g/L, 44.6 g/L, 44.7 g/L, 44.8 g/L, 44.9 g/L, 45 g/L, 45.1 g/L, 45.2 g/L, 45.3 g/L, 45.4 g/L, 45.5 g/L, 45.6 g/L, 45.7 g/L, 45.8 g/L, 45.9 g/L, 46 g/L, 46.1 g/L, 46.2 g/L, 46.3 g/L, 46.4 g/L, 46.5 g/L, 46.6 g/L, 46.7 g/L, 46.8 g/L, 46.9 g/L, 47 g/L, 47.1 g/L, 47.2 g/L, 47.3 g/L, 47.4 g/L, 47.5 g/L, 47.6 g/L, 47.7 g/L, 47.8 g/L, 47.9 g/L, 48 g/L, 48.1 g/L, 48.2 g/L, 48.3 g/L, 48.4 g/L, 48.5 g/L, 48.6 g/L, 48.7 g/L, 48.8 g/L, 48.9 g/L, 49 g/L, 49.1 g/L, 49.2 g/L, 49.3 g/L, 49.4 g/L, 49.5 g/L, 49.6 g/L, 49.7 g/L, 49.8 g/L, 49.9 g/L or 50 g/L.

[00181] The yeast extract can also be fed throughout the course of the entire fermentation or a portion of the fermentation, continuously or delivered at intervals. In one embodiment usage levels include maintaining a nitrogen concentration of about 0.05 g/L to about 3g/L (as nitrogen), where at least a portion of the nitrogen is supplied from corn steep powder; or about 0.3g/L to 1.3g/L; or 0.4 g/L to about 0.9 g/L. In another embodiment the nitrogen concentration is about 0.05 g/L, 0.06 g/L, 0.07 g/L, 0.08 g/L, 0.09 g/L, 0.1 g/L, 0.11 g/L, 0.12 g/L, 0.13 g/L, 0.14 g/L, 0.15 g/L, 0.16 g/L, 0.17 g/L, 0.18 g/L, 0.19 g/L, 0.2 g/L, 0.21 g/L, 0.22 g/L, 0.23 g/L, 0.24 g/L, 0.25 g/L, 0.26 g/L, 0.27 g/L, 0.28 g/L, 0.29 g/L, 0.3 g/L, 0.31 g/L, 0.32 g/L, 0.33 g/L, 0.34 g/L, 0.35 g/L, 0.36 g/L, 0.37 g/L, 0.38 g/L, 0.39 g/L, 0.4 g/L, 0.41 g/L, 0.42 g/L, 0.43 g/L, 0.44 g/L, 0.45 g/L, 0.46 g/L, 0.47 g/L, 0.48 g/L, 0.49 g/L, 0.5 g/L, 0.51 g/L, 0.52 g/L, 0.53 g/L, 0.54 g/L, 0.55 g/L, 0.56 g/L, 0.57 g/L, 0.58 g/L, 0.59 g/L, 0.6 g/L, 0.61 g/L, 0.62 g/L, 0.63 g/L, 0.64 g/L, 0.65 g/L, 0.66 g/L, 0.67 g/L, 0.68 g/L, 0.69 g/L, 0.7 g/L, 0.71 g/L, 0.72 g/L, 0.73 g/L, 0.74 g/L, 0.75 g/L, 0.76 g/L, 0.77 g/L, 0.78 g/L, 0.79 g/L, 0.8 g/L, 0.81 g/L, 0.82 g/L, 0.83 g/L, 0.84 g/L, 0.85 g/L, 0.86 g/L, 0.87 g/L, 0.88 g/L, 0.89 g/L, 0.9 g/L, 0.91 g/L, 0.92 g/L, 0.93 g/L, 0.94 g/L, 0.95 g/L, 0.96 g/L, 0.97 g/L, 0.98 g/L, 0.99 g/L, 1 g/L, 1.01 g/L, 1.02 g/L, 1.03 g/L, 1.04 g/L, 1.05 g/L, 1.06 g/L, 1.07 g/L, 1.08 g/L, 1.09 g/L, 1.1 g/L, 1.11 g/L, 1.12 g/L, 1.13 g/L, 1.14 g/L, 1.15 g/L, 1.16 g/L, 1.17 g/L, 1.18 g/L, 1.19 g/L, 1.2 g/L, 1.21 g/L, 1.22 g/L, 1.23 g/L, 1.24 g/L, 1.25 g/L, 1.26 g/L, 1.27 g/L, 1.28 g/L, 1.29 g/L, 1.3 g/L, 1.31 g/L, 1.32 g/L, 1.33 g/L, 1.34 g/L, 1.35 g/L, 1.36 g/L, 1.37 g/L, 1.38 g/L, 1.39 g/L, 1.4 g/L, 1.41 g/L, 1.42 g/L, 1.43 g/L, 1.44 g/L, 1.45 g/L, 1.46 g/L, 1.47 g/L, 1.48 g/L, 1.49 g/L, 1.5 g/L, 1.51 g/L, 1.52 g/L, 1.53 g/L, 1.54 g/L, 1.55 g/L, 1.56 g/L, 1.57 g/L, 1.58 g/L, 1.59 g/L, 1.6 g/L, 1.61 g/L, 1.62 g/L, 1.63 g/L, 1.64 g/L, 1.65 g/L, 1.66 g/L, 1.67 g/L, 1.68 g/L, 1.69 g/L, 1.7 g/L, 1.71 g/L, 1.72 g/L, 1.73 g/L, 1.74 g/L, 1.75 g/L, 1.76 g/L, 1.77 g/L, 1.78 g/L, 1.79 g/L, 1.8 g/L, 1.81 g/L, 1.82 g/L, 1.83 g/L, 1.84 g/L, 1.85 g/L, 1.86 g/L, 1.87 g/L, 1.88 g/L, 1.89 g/L, 1.9 g/L, 1.91 g/L, 1.92 g/L, 1.93 g/L, 1.94 g/L, 1.95 g/L,

1.96 g/L, 1.97 g/L, 1.98 g/L, 1.99 g/L, 2 g/L, 2.01 g/L, 2.02 g/L, 2.03 g/L, 2.04 g/L, 2.05 g/L, 2.06 g/L, 2.07 g/L, 2.08 g/L, 2.09 g/L, 2.1 g/L, 2.11 g/L, 2.12 g/L, 2.13 g/L, 2.14 g/L, 2.15 g/L, 2.16 g/L, 2.17 g/L, 2.18 g/L, 2.19 g/L, 2.2 g/L, 2.21 g/L, 2.22 g/L, 2.23 g/L, 2.24 g/L, 2.25 g/L, 2.26 g/L, 2.27 g/L, 2.28 g/L, 2.29 g/L, 2.3 g/L, 2.31 g/L, 2.32 g/L, 2.33 g/L, 2.34 g/L, 2.35 g/L, 2.36 g/L, 2.37 g/L, 2.38 g/L, 2.39 g/L, 2.4 g/L, 2.41 g/L, 2.42 g/L, 2.43 g/L, 2.44 g/L, 2.45 g/L, 2.46 g/L, 2.47 g/L, 2.48 g/L, 2.49 g/L, 2.5 g/L, 2.51 g/L, 2.52 g/L, 2.53 g/L, 2.54 g/L, 2.55 g/L, 2.56 g/L, 2.57 g/L, 2.58 g/L, 2.59 g/L, 2.6 g/L, 2.61 g/L, 2.62 g/L, 2.63 g/L, 2.64 g/L, 2.65 g/L, 2.66 g/L, 2.67 g/L, 2.68 g/L, 2.69 g/L, 2.7 g/L, 2.71 g/L, 2.72 g/L, 2.73 g/L, 2.74 g/L, 2.75 g/L, 2.76 g/L, 2.77 g/L, 2.78 g/L, 2.79 g/L, 2.8 g/L, 2.81 g/L, 2.82 g/L, 2.83 g/L, 2.84 g/L, 2.85 g/L, 2.86 g/L, 2.87 g/L, 2.88 g/L, 2.89 g/L, 2.9 g/L, 2.91 g/L, 2.92 g/L, 2.93 g/L, 2.94 g/L, 2.95 g/L, 2.96 g/L, 2.97 g/L, 2.98 g/L, 2.99 g/L, or 3 g/L.

[00182] In one embodiment, beneficial fermentation results can be achieved by adding corn steep powder to the fermentation. In another embodiment a typical composition for corn steep powder is shown in Tables 1-2. The addition of the corn steep powder can result in increased ethanol titer in batch fermentation, improved productivity and reduced production of side products such as organic acids. In another embodiment beneficial results with corn steep powder can be achieved at usage levels of about 3 to about 20 g/L, about 5 to about 15 g/L, or about 8 to about 12 g/L. In another embodiment beneficial results with steep powder can be achieved at a level of about 3 g/L, 3.1 g/L, 3.2 g/L, 3.3 g/L, 3.4 g/L, 3.5 g/L, 3.6 g/L, 3.7 g/L, 3.8 g/L, 3.9 g/L, 4 g/L, 4.1 g/L, 4.2 g/L, 4.3 g/L, 4.4 g/L, 4.5 g/L, 4.6 g/L, 4.7 g/L, 4.8 g/L, 4.9 g/L, 5 g/L, 5.1 g/L, 5.2 g/L, 5.3 g/L, 5.4 g/L, 5.5 g/L, 5.6 g/L, 5.7 g/L, 5.8 g/L, 5.9 g/L, 6 g/L, 6.1 g/L, 6.2 g/L, 6.3 g/L, 6.4 g/L, 6.5 g/L, 6.6 g/L, 6.7 g/L, 6.8 g/L, 6.9 g/L, 7 g/L, 7.1 g/L, 7.2 g/L, 7.3 g/L, 7.4 g/L, 7.5 g/L, 7.6 g/L, 7.7 g/L, 7.8 g/L, 7.9 g/L, 8 g/L, 8.1 g/L, 8.2 g/L, 8.3 g/L, 8.4 g/L, 8.5 g/L, 8.6 g/L, 8.7 g/L, 8.8 g/L, 8.9 g/L, 9 g/L, 9.1 g/L, 9.2 g/L, 9.3 g/L, 9.4 g/L, 9.5 g/L, 9.6 g/L, 9.7 g/L, 9.8 g/L, 9.9 g/L, 10 g/L, 10.1 g/L, 10.2 g/L, 10.3 g/L, 10.4 g/L, 10.5 g/L, 10.6 g/L, 10.7 g/L, 10.8 g/L, 10.9 g/L, 11 g/L, 11.1 g/L, 11.2 g/L, 11.3 g/L, 11.4 g/L, 11.5 g/L, 11.6 g/L, 11.7 g/L, 11.8 g/L, 11.9 g/L, 12 g/L, 12.1 g/L, 12.2 g/L, 12.3 g/L, 12.4 g/L, 12.5 g/L, 12.6 g/L, 12.7 g/L, 12.8 g/L, 12.9 g/L, 13 g/L, 13.1 g/L, 13.2 g/L, 13.3 g/L, 13.4 g/L, 13.5 g/L, 13.6 g/L, 13.7 g/L, 13.8 g/L, 13.9 g/L, 14 g/L, 14.1 g/L, 14.2 g/L, 14.3 g/L, 14.4 g/L, 14.5 g/L, 14.6 g/L, 14.7 g/L, 14.8 g/L, 14.9 g/L, 15 g/L, 15.1 g/L, 15.2 g/L, 15.3 g/L, 15.4 g/L, 15.5 g/L, 15.6 g/L, 15.7 g/L, 15.8 g/L, 15.9 g/L, 16 g/L, 16.1 g/L, 16.2 g/L, 16.3 g/L, 16.4 g/L, 16.5 g/L, 16.6 g/L, 16.7 g/L, 16.8 g/L, 16.9 g/L, 17 g/L, 17.1 g/L, 17.2 g/L, 17.3 g/L, 17.4 g/L, 17.5 g/L, 17.6 g/L, 17.7 g/L, 17.8 g/L, 17.9 g/L, 18 g/L, 18.1 g/L, 18.2 g/L, 18.3 g/L, 18.4 g/L, 18.5 g/L, 18.6 g/L, 18.7 g/L, 18.8 g/L, 18.9 g/L, 19 g/L, 19.1 g/L, 19.2 g/L, 19.3 g/L, 19.4 g/L, 19.5 g/L, 19.6 g/L, 19.7 g/L, 19.8 g/L, 19.9 g/L, or 20 g/L.

[00183] In one embodiment corn steep powder can also be fed throughout the course of the entire fermentation or a portion of the fermentation, continuously or delivered at intervals. In another embodiment usage levels include maintaining a nitrogen concentration of about 0.05 g/L to about 3g/L (as nitrogen), where at least a portion of the nitrogen is supplied from corn steep powder; about 0.3g/L to 1.3g/L; or about 0.4 g/L to about 0.9 g/L. hi another embodiment the nitrogen level is about 0.05 g/L,

0.06 g/L, 0.07 g/L, 0.08 g/L, 0.09 g/L, 0.1 g/L, 0.11 g/L, 0.12 g/L, 0.13 g/L, 0.14 g/L, 0.15 g/L, 0.16 g/L, 0.17 g/L, 0.18 g/L, 0.19 g/L, 0.2 g/L, 0.21 g/L, 0.22 g/L, 0.23 g/L, 0.24 g/L, 0.25 g/L, 0.26 g/L, 0.27 g/L, 0.28 g/L, 0.29 g/L, 0.3 g/L, 0.31 g/L, 0.32 g/L, 0.33 g/L, 0.34 g/L, 0.35 g/L, 0.36 g/L, 0.37 g/L, 0.38 g/L, 0.39 g/L, 0.4 g/L, 0.41 g/L, 0.42 g/L, 0.43 g/L, 0.44 g/L, 0.45 g/L, 0.46 g/L, 0.47 g/L, 0.48 g/L, 0.49 g/L, 0.5 g/L, 0.51 g/L, 0.52 g/L, 0.53 g/L, 0.54 g/L, 0.55 g/L, 0.56 g/L, 0.57 g/L, 0.58 g/L, 0.59 g/L, 0.6 g/L, 0.61 g/L, 0.62 g/L, 0.63 g/L, 0.64 g/L, 0.65 g/L, 0.66 g/L, 0.67 g/L, 0.68 g/L, 0.69 g/L, 0.7 g/L, 0.71 g/L, 0.72 g/L, 0.73 g/L, 0.74 g/L, 0.75 g/L, 0.76 g/L, 0.77 g/L, 0.78 g/L, 0.79 g/L, 0.8 g/L, 0.81 g/L, 0.82 g/L, 0.83 g/L, 0.84 g/L, 0.85 g/L, 0.86 g/L, 0.87 g/L, 0.88 g/L, 0.89 g/L, 0.9 g/L, 0.91 g/L, 0.92 g/L, 0.93 g/L, 0.94 g/L, 0.95 g/L, 0.96 g/L, 0.97 g/L, 0.98 g/L, 0.99 g/L, 1 g/L, 1.01 g/L, 1.02 g/L, 1.03 g/L, 1.04 g/L, 1.05 g/L, 1.06 g/L, 1.07 g/L, 1.08 g/L, 1.09 g/L, 1.1 g/L, 1.11 g/L, 1.12 g/L, 1.13 g/L, 1.14 g/L, 1.15 g/L, 1.16 g/L, 1.17 g/L, 1.18 g/L, 1.19 g/L, 1.2 g/L, 1.21 g/L, 1.22 g/L, 1.23 g/L, 1.24 g/L, 1.25 g/L, 1.26 g/L, 1.27 g/L, 1.28 g/L, 1.29 g/L, 1.3 g/L, 1.31 g/L, 1.32 g/L, 1.33 g/L, 1.34 g/L, 1.35 g/L, 1.36 g/L, 1.37 g/L, 1.38 g/L, 1.39 g/L, 1.4 g/L, 1.41 g/L, 1.42 g/L, 1.43 g/L, 1.44 g/L, 1.45 g/L, 1.46 g/L, 1.47 g/L, 1.48 g/L, 1.49 g/L, 1.5 g/L, 1.51 g/L, 1.52 g/L, 1.53 g/L, 1.54 g/L, 1.55 g/L, 1.56 g/L, 1.57 g/L, 1.58 g/L, 1.59 g/L, 1.6 g/L, 1.61 g/L, 1.62 g/L, 1.63 g/L, 1.64 g/L, 1.65 g/L, 1.66 g/L, 1.67 g/L, 1.68 g/L, 1.69 g/L, 1.7 g/L, 1.71 g/L, 1.72 g/L, 1.73 g/L, 1.74 g/L, 1.75 g/L, 1.76 g/L, 1.77 g/L, 1.78 g/L, 1.79 g/L, 1.8 g/L, 1.81 g/L, 1.82 g/L, 1.83 g/L, 1.84 g/L, 1.85 g/L, 1.86 g/L, 1.87 g/L, 1.88 g/L, 1.89 g/L, 1.9 g/L, 1.91 g/L, 1.92 g/L, 1.93 g/L, 1.94 g/L, 1.95 g/L, 1.96 g/L, 1:97 g/L, 1.98 g/L, 1.99 g/L, 2 g/L, 2.01 g/L, 2.02 g/L, 2.03 g/L, 2.04 g/L, 2.05 g/L, 2.06 g/L, 2.07 g/L, 2.08 g/L, 2.09 g/L, 2.1 g/L, 2.11 g/L, 2.12 g/L, 2.13 g/L, 2.14 g/L, 2.15 g/L, 2.16 g/L, 2.17 g/L, 2.18 g/L, 2.19 g/L, 2.2 g/L, 2.21 g/L, 2.22 g/L, 2.23 g/L, 2.24 g/L, 2.25 g/L, 2.26 g/L, 2.27 g/L, 2.28 g/L, 2.29 g/L, 2.3 g/L, 2.31 g/L, 2.32 g/L, 2.33 g/L, 2.34 g/L, 2.35 g/L, 2.36 g/L, 2.37 g/L, 2.38 g/L, 2.39 g/L, 2.4 g/L, 2.41 g/L, 2.42 g/L, 2.43 g/L, 2.44 g/L, 2.45 g/L, 2.46 g/L, 2.47 g/L, 2.48 g/L, 2.49 g/L, 2.5 g/L, 2.51 g/L, 2.52 g/L, 2.53 g/L, 2.54 g/L, 2.55 g/L, 2.56 g/L, 2.57 g/L, 2.58 g/L, 2.59 g/L, 2.6 g/L, 2.61 g/L, 2.62 g/L, 2.63 g/L, 2.64 g/L, 2.65 g/L, 2.66 g/L, 2.67 g/L, 2.68 g/L, 2.69 g/L, 2.7 g/L, 2.71 g/L, 2.72 g/L, 2.73 g/L, 2.74 g/L, 2.75 g/L, 2.76 g/L, 2.77 g/L, 2.78 g/L, 2.79 g/L, 2.8 g/L, 2.81 g/L, 2.82 g/L, 2.83 g/L, 2.84 g/L, 2.85 g/L, 2.86 g/L, 2.87 g/L, 2.88 g/L, 2.89 g/L, 2.9 g/L, 2.91 g/L, 2.92 g/L, 2.93 g/L, 2.94 g/L, 2.95 g/L, 2.96 g/L, 2.97 g/L, 2.98 g/L, 2.99 g/L, or 3 g/L.

[00184] In another embodiment, other related products can be used, such as dried brewer's yeast (DBY) or spent brewer's yeast, corn steep liquor or corn steep powder. When corn steep liquor is used, the usage rate would be approximately the same as for corn steep powder on a solids basis. In another embodiment, the corn steep powder (or solids or liquor) is added in relation to the amount of carbon substrate that is present or that will be added. When added in this way, beneficial amounts of corn steep powder (or liquor or solids) can include about 1:1 to about 1:6 g/g carbon, about 1:1 to about 1:5 g/g carbon, or about 1:2 to about 1:4 g/g carbon. In another embodiment ratios as high as about 1.5:1 g/g carbon or about 3:1 g/g carbon or as low as about 1: 8 g/g carbon or about 1:10 g/g carbon are used. In another embodiment the ratio is 2:1 g/g carbon, 1.9:1 g/g carbon, 1.8:1 g/g carbon, 1.7:1 g/g carbon, 1.6:1 g/g carbon, 1.5:1 g/g carbon, 1.4:1 g/g carbon, 1.3:1 g/g carbon, 1.2:1 g/g carbon, 1.1:1 g/g carbon, 1:1 g/g carbon, 1:1.1 g/g

carbon, 1:1.2 g/g carbon, 1:1.3 g/g carbon, 1:1.4 g/g carbon, 1:1.5 g/g carbon, 1:1.6 g/g carbon, 1:1.7 g/g carbon, 1:1.8 g/g carbon, 1:1.9 g/g carbon, 1:2 g/g carbon, 1:2.1 g/g carbon, 1:2.2 g/g carbon, 1:2.3 g/g carbon, 1:2.4 g/g carbon, 1:2.5 g/g carbon, 1:2.6 g/g carbon, 1:2.7 g/g carbon, 1:2.8 g/g carbon, 1:2.9 g/g carbon, 1:3 g/g carbon, 1:3.1 g/g carbon, 1:3.2 g/g carbon, 1:3.3 g/g carbon, 1:3.4 g/g carbon, 1:3.5 g/g carbon, 1:3.6 g/g carbon, 1:3.7 g/g carbon, 1:3.8 g/g carbon, 1:3.9 g/g carbon, 1:4 g/g carbon, 1:4.1 g/g carbon, 1:4.2 g/g carbon, 1:4.3 g/g carbon, 1:4.4 g/g carbon, 1:4.5 g/g carbon, 1:4.6 g/g carbon, 1:4.7 g/g carbon, 1:4.8 g/g carbon, 1:4.9 g/g carbon, 1:5 g/g carbon, 1:5.1 g/g carbon, 1:5.2 g/g carbon, 1:5.3 g/g carbon, 1:5.4 g/g carbon, 1:5.5 g/g carbon, 1:5.6 g/g carbon, 1:5.7 g/g carbon, 1:5.8 g/g carbon, 1:5.9 g/g carbon, 1:6 g/g carbon, 1:6.1 g/g carbon, 1:6.2 g/g carbon, 1:6.3 g/g carbon, 1:6.4 g/g carbon, 1:6.5 g/g carbon, 1:6.6 g/g carbon, 1:6.7 g/g carbon, 1:6.8 g/g carbon, 1:6.9 g/g carbon, 1:7 g/g carbon, 1:7.1 g/g carbon, 1:7.2 g/g carbon, 1:7.3 g/g carbon, 1:7.4 g/g carbon, 1:7.5 g/g carbon, 1:7.6 g/g carbon, 1:7.7 g/g carbon, 1:7.8 g/g carbon, 1:7.9 g/g carbon, 1:8 g/g carbon, 1:8.1 g/g carbon, 1:8.2 g/g carbon, 1:8.3 g/g carbon, 1:8.4 g/g carbon, 1:8.5 g/g carbon, 1:8.6 g/g carbon, 1:8.7 g/g carbon, 1:8.8 g/g carbon, 1:8.9 g/g carbon, 1:9 g/g carbon, 1:9.1 g/g carbon, 1:9.2 g/g carbon, 1:9.3 g/g carbon, 1:9.4 g/g carbon, 1:9.5 g/g carbon, 1:9.6 g/g carbon, 1:9.7 g/g carbon, 1:9.8 g/g carbon, 1:9.9 g/g carbon, or 1:10 g/g carbon.

[00185] In one embodiment, beneficial fermentation results can be achieved by adding corn steep powder in combination with yeast extract to the fermentation. Beneficial results with corn steep powder in combination with yeast extract can be achieved at corn steep powder usage levels of about 3 to about 20 g/L, about 5 to about 15 g/L, or about 8 to about 12 g/L and yeast extract usage levels of about 3 to 50 g/L, about 5 to about 30 g/L, or about 10 to about 30 g/L. The corn steep powder and yeast extract can also be fed throughout the course of the entire fermentation or a portion of the fermentation, continuously or delivered at intervals.

[00186] In one embodiment, the beneficial compounds from corn steep powder and/or yeast extract, such as glycine, histidine, isoleucine, proline, or phytate as well as combinations of these compounds can be added to the medium or broth to obtain a beneficial effect.

[00187] Various embodiments offer benefits relating to improving the titer and/or productivity of alcohol production by Q.12 or Q.13 by culturing the microorganism in a medium comprising one or more compounds comprising particular fatty acid moieties and/or culturing the microorganism under conditions of controlled pH. Both Q.12 and Q.13 are tolerant of high ethanol levels compared to the naturally-occurring species. Q.13 is more tolerant and tends to grow more rapidly than Q.12 in media with a cellobiose carbon substrate.

[00188] In one embodiment, production of high levels of alcohol uses an microorganism with the ability to thrive in the presence of elevated alcohol levels and the ability to continue to produce alcohol without undue inhibition or suppression by the alcohol and/or other components present. Frequently, different metabolic pathways will be implicated for each of these. For example, pathways related to cell growth generally include those related to protein production, membrane production as well as the production of all of the cellular subsystems involved in cell survival. Pathways related to alcohol

production can be more specific, such as those pathways related to the metabolism of sugars leading to production of alcohol and the enzymes that are involved in the production of alcohol and intermediates. The pathway for one alcohol, *e.g.*, ethanol, can share some similar enzymes, *etc.*, but can also have enzymes and substrates specific to that pathway. While there can be some overlap between these sets of pathways, it is not expected that enhancement of one will automatically result in the enhancement of the other.

[00189] In some cases, alcohol intolerance or alcohol-induced toxicity can be related to permeabilization of the cell membrane by elevated levels of alcohol, leading to leakage of intracellular enzymes and nutrients. In some other cases, alcohol tolerance and the ability to produce high alcohol titers is related to the ability of intracellular enzymes to withstand denaturing by the alcohol present, *e.g.*, within the cell, whether due to production by the cell itself or from transport across the cell membrane. In some cases, a more robust membrane will allow a higher alcohol gradient to be present across the membrane, thus allowing the cells to grow and/or continue to produce alcohol at higher external alcohol concentrations.

[00190] In one embodiment, Q.12 or Q.13 is fermented with a substrate at about pH 5-8.5 In one embodiment a Q.12 or Q.13 is fermented at pH of about 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, or 8.5.

[00191] Acidic Culture Conditions

[00192] In another aspect, methods of producing alcohol; *e.g.*, ethanol, comprising culturing Q.12 or Q.13 in a medium under conditions of controlled pH. In one embodiment, a culture of Q.12 or Q.13 can be grown at an acidic pH are provided herein. The medium that the culture is grown in can include a carbon source such as agricultural crops, algae, crop residues, modified crop plants, trees, wood chips, sawdust, paper, cardboard, or other materials containing cellulose, hemicellulosic, lignocellulose, pectin, polyglucose, polyfructose, and/or hydrolyzed forms of these (collectively, "Feedstock"). Additional nutrients can be present including sulfur-and nitrogen-containing compounds such as amino acids, proteins, hydrolyzed proteins, ammonia, urea, nitrate, nitrite, soy, soy derivatives, casein, casein derivatives, milk powder, milk derivatives, whey, yeast extract, hydrolyzed yeast, autolyzed yeast, dried brewer's yeast, corn steep liquor, corn steep solids, monosodium glutamate, and/or other fermentation nitrogen sources, vitamins, cofactors and/or mineral supplements. The Feedstock can be pretreated or not, such as described in U.S. Patent Application No. 12/919,750, filed August 26, 2010 or PCT Application No. PCT/US 10/40502, filed on June 29, 2010, which are herein incorporated by reference in their entireties. The procedures and techniques for growing the microorganism to produce a fuel or other desirable chemical such as is described in incorporated U.S. Patent Application No. 12/720,574 which is herein incorporated by reference in its entirety.

[00193] In one embodiment, the pH of the medium is controlled at less than about pH 7.2 for at least a portion of the fermentation. In one embodiment, the pH is controlled within a range of about pH 3.0 to about 7.1 or about pH 4.5 to about 7.1, or about pH 5.0 to about 6.3, or about pH 5.5 to about 6.3, or

about pH 6.0 to about 6.5, or about pH 5.5 to about 6.9 or about pH 6.2 to about 6.7. The pH can be controlled by the addition of a pH modifier. In one embodiment, a pH modifier is an acid, a base, a buffer, or a material that reacts with other materials present to serve to raise of lower the pH. In one embodiment, more than one pH modifier can be used, such as more than one acid, more than one base, one or more acid with one or more bases, one or more acids with one or more buffers, one or more bases with one or more buffers, or one or more acids with one or more bases with one or more buffers. When more than one pH modifiers are utilized, they can be added at the same time or at different times. In one embodiment, one or more acids and one or more bases can be combined, resulting in a buffer. In one embodiment, media components, such as a carbon source or a nitrogen source can also serve as a pH modifier; suitable media components include those with high or low pH or those with buffering capacity. Exemplary media components include acid- or base-hydrolyzed plant polysaccharides having with residual acid or base, AFEX treated plant material with residual ammonia, lactic acid, corn steep solids or liquor.

[00194] In one embodiment, the pH modifier can be added as a part of the medium components prior to inoculation with Q.12 or Q.13. In one embodiment, the pH modifier can also be added after inoculation with the Q.12 or Q.13. In one embodiment, sufficient buffer capacity can be added to the seed fermentation by way of various pH modifiers and/or other medium components and/or metabolites to provide adequate pH control during the final fermentation stage. In one embodiment, a pH modifier is added only to the final fermentation stage. In one embodiment, pH modifier is added to both the seed stage and the final stage. In one embodiment, the pH is monitored throughout the fermentation and is adjusted in response to changes in the fermentation. In one embodiment, the pH modifier is added whenever the pH of the fermentation changes by a pH value of about 0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 or more at any stage of the fermentation. In one embodiment, the pH modifier is added whenever the alcohol content of the fermentation is about 0.5 g/L, 1.0 g/L, 2.0 g/L, or 5.0 g/L or more. In one embodiment, different types of pH modifiers are utilized at different stages or points in the fermentation, such as a buffer being used at the seed stage, and base and/or acid added in the final fermenter, or an acid being used at one time and a base at another time.

[00195] In one embodiment, a constant pH can be utilized throughout the fermentation. In one embodiment, the timing and/or amount of pH reduction can be related to the growth conditions of the cells, such as in relation to the cell count, the alcohol produced, the alcohol present, or the rate of alcohol production. In one embodiment, the pH reduction can be made in relation to physical or chemical properties of the fermentation, such as viscosity, medium composition, gas production, off gas composition, *etc.*

[00196] Non-limiting examples of suitable buffers include salts of phosphoric acid, including monobasic, dibasic, and tribasic salts, mixtures of these salts and mixtures with the acid; salts of citric acid, including the various basic forms, mixtures and mixtures with the acid; and salts of carbonate.

[00197] Suitable acids and bases that can be used as pH modifiers include any liquid or gaseous acid or base that is compatible with the microorganism. Examples include ammonia, ammonium hydroxide,

sulfuric acid, lactic acid, citric acid, phosphoric acid, sodium hydroxide, and HC1. In some cases, the selection of the acid or base can be influenced by the compatibility of the acid or base with equipment being used for fermentation. In some cases, both an acid addition, to lower pH or consume base, and a base addition, to raise pH or consume acid, can be used in the same fermentation.

[00198] The timing and amount of pH modifier to add can be determined from a measurement of the pH of the contents of the fermentor, such as by grab sample or by a submerged pH probe, or it can be determined based on other parameters such as the time into the fermentation, gas generation, viscosity, alcohol production, titration, *etc.* In one embodiment, a combination of these techniques can be used.

[00199] In one embodiment, the pH of the fermentation is initiated at a neutral pH and then is reduced to an acidic pH when the production of alcohol is detected. In another embodiment, the pH of the fermentation is initiated at an acidic pH and is maintained at an acidic pH until the fermentation reaches a stationary phase of growth.

[00200] Fatty Acid Medium Component and Acidic Culture Conditions

[00201] In another embodiment, a combination of adding a fatty acid comprising compound to the medium and fermenting at reduced pH can be used. In one embodiment, addition of a fatty acid, such as a free fatty acid fulfills both techniques: adding a fatty acid compound and lowering the pH of the fermentation. In one embodiment, different compounds can be added to accomplish each technique. For example, a vegetable oil can be added to the medium to supply the fatty acid and then a mineral acid or an organic acid can be added during the fermentation to reduce the pH to a suitable level, as described above. When the fermentation includes both operation at reduced pH and addition of fatty acid comprising compounds, the methods and techniques described herein for each type of operation separately can be used together. In one embodiment, the operation at low pH and the presence of the fatty acid comprising compounds will be at the same time. In one embodiment, the presence of fatty acid comprising compounds will precede operation at low pH, and in one embodiment, operation at low pH will precede the addition of fatty acid comprising compounds. In one embodiment, the operation at low pH and the presence of the fatty acid will be prior to inoculation with Q.12 or Q.13. In one embodiment, the operation at low pH will be prior to inoculation with Q.12 or Q.13 and the presence of the fatty acid will occur after or during inoculation with Q.12 or Q.13. In one embodiment, the presence of the fatty acid will be prior to inoculation with Q.12 or Q.13 and the operation at low pH will occur after or during the inoculation with Q.12 or Q.13. In one embodiment, the operation at low pH and the presence of the fatty acid will be after inoculation with Q.12 or Q.13. In one embodiment, the operation at low pH and the presence of the fatty acid will be at other stages of fermentation.

[00202] Genetic Modification of Q.12 or Q.13

[00203] In another aspect, compositions and methods to produce a fuel such as one or more alcohols, *e.g.*, ethanol, by the creation and use of a genetically modified Q.12 or Q.13 are provided. In one embodiment, regulating fermentative biochemical pathways, expression of saccharolytic enzymes, or increasing tolerance of environmental conditions during fermentation of Q.12 or Q.13 is provided. One

example of methods that can be used to enhance expression of saccharolytic enzymes can be found in U.S. patent application No. 12/630,784 filed December 3, 2009. In one embodiment, Q.12 or Q.13 is transformed with heterologous polynucleotides encoding one or more genes for the pathway, enzyme, or protein of interest. In another embodiment, Q.12 or Q.13 is transformed to produce multiple copies of one or more genes for the pathway, enzyme, or protein of interest. In one embodiment, Q.12 or Q.13 is transformed with heterologous polynucleotides encoding one or more genes encoding enzymes for the hydrolysis and/or fermentation of a hexose, wherein said genes are expressed at sufficient levels to confer upon said Q.12 or Q.13 transformant the ability to produce ethanol at increased concentrations, productivity levels or yields compared to Q.12 or Q.13 that is not transformed. In such ways, an enhanced rate of ethanol production can be achieved.

[00204] In another embodiment, Q.12 or Q.13 is transformed with heterologous polynucleotides encoding one or more genes encoding saccharolytic enzymes for the saccharification of a polysaccharide, wherein said genes are expressed at sufficient levels to confer upon said Q.12 or Q.13 transformant the ability to saccharify a polysaccharide to mono-, di- or oligosaccharides at increased concentrations, rates of saccharification or yields of mono-, di- or oligosaccharides compared to Q.12 or Q.13 that is not transformed. The production of a saccharolytic enzyme by the host, and the subsequent release of that saccharolytic enzyme into the medium, can reduce the amount of commercial enzyme used to degrade biomass or polysaccharides into fermentable monosaccharides and oligosaccharides. The saccharolytic DNA can be native to the host, although more often the DNA will be foreign, and heterologous. Advantageous saccharolytic genes include cellulolytic, xylanolytic, and starch-degrading enzymes such as cellulases, xylanases, and amylases. The saccharolytic enzymes can be at least partially secreted by the host, or it can be accumulated substantially intracellularly for subsequent release. Advantageously, intracellularly-accumulated enzymes which are thermostable, can be released when desired by heat-induced lysis. Combinations of enzymes can be encoded by the heterologous DNA, some of which are secreted, and some of which are accumulated.

[00205] Other modifications can be made to enhance the ethanol production of the recombinant bacteria. For example, the host can further comprise an additional heterologous DNA segment, the expression product of which is a protein involved in the transport of mono- and/or oligosaccharides into the recombinant host. Likewise, additional genes from the glycolytic pathway can be incorporated into the host to redirect the bioenergetics of the ethanolic production pathways. In such ways, an enhanced rate of ethanol production can be achieved.

[00206] In order to improve the production of biofuels *(e.g.,* ethanol), modifications can be made in transcriptional regulators, genes for the formation of organic acids, carbohydrate transporter genes, sporulation genes, genes that influence the formation/regenerate of enzymatic cofactors, genes that further influence ethanol tolerance, genes that influence salt tolerance, genes that influence growth rate, genes that influence oxygen tolerance, genes that influence catabolite repression, genes that influence hydrogen

production, genes that influence resistance to heavy metals, genes that influence resistance to acids or genes that influence resistance to aldehydes.

[00207] Those skilled in the art will appreciate that a number of modifications can be made to the methods exemplified herein. For example, a variety of promoters can be utilized to drive expression of the heterologous genes in the recombinant *Clostridium* sp. host. The skilled artisan, having the benefit of the instant disclosure, will be able to readily choose and utilize any one of the various promoters available for this purpose. Similarly, skilled artisans, as a matter of routine preference, can utilize a higher copy number plasmid. In another embodiment, constructs can be prepared for chromosomal integration of the desired genes. Chromosomal integration of foreign genes can offer several advantages over plasmid-based constructions, the latter having certain limitations for commercial processes. Ethanologenic genes have been integrated chromosomally in *E. coli* B; see Ohta et al. (1991) Appl. Environ. Microbiol. 57:893-900. In general, this is accomplished by purification of a DNA fragment containing (1) the desired genes upstream from an antibiotic resistance gene and (2) a fragment of homologous DNA from the target microorganism. This DNA can be ligated to form circles without replicons and used for transformation. Thus, the gene of interest can be introduced in a heterologous host such as *E. coli,* and short, random fragments can be isolated and ligated in *Clostridium* sp. to promote homologous recombination.

[00208] Identification of *Clostridium phytofermentans* variants, mutants, recombinants, and strains

[00209] Methods of identifying microbial species have been based on culture methods coupled with biochemical tests, which are generally unreliable because bacteria can vary in appearance depending or growth stage and environmental conditions such as pH, temperature and food supply. *Clostridium phytofermentans* has a genome that can be distinguished from other microbial species through variations in its DNA. One aspect of this disclosure is a species-specific PCR assay for the direct detection of *Clostridium phytofermentans* in a sample. Table 3 and Figure 3 depicts a series of PCR oligonucleotide primers developed from genomic DNA that can be used to determine whether a microorganism is a variant or strain of *Clostridium phytofermentans.* These primers can amplify all the strains, producing species-specific profiles for *Clostridium phytofermentans* microorganisms.

[00210] PCR technology is simple, rapid, and, in the absence of specific nucleotide sequence information for many microbial species, able to generate species-specific or strain-specific DNA polymorphisms on the basis of characteristic band patterns detected by agarose gel electrophoresis. It is a rapid and simple technique in comparison with other molecular biology methods, especially due to the speed of the DNA extraction, and it can be applied to identify strains not presenting typical morphological characteristics.

[00211] In one embodiment, a fixed volume is extracted from a culture containing a microorganism. The total RNA or genomic DNA can be isolated from microbial cells using standard RNA or DNA purification techniques, *e.g.*, detergent or alkaline lysis, guanidium isothiocyanate, CsCl gradients, Phenol/SDS, Phenol/Chloroform, glass- or silica-based chromatography or other methods, including

readily available commercial kits from a variety of manufacturers. Enzymatic treatment with proteases and ribonucleases can be used to remove proteins and RNA, respectively. DNA can be precipitated from the sample using alcohol. (Maniatis, Cold Spring Harbor Laboratory Press; 2nd edition (December 1989)).

[00212] "PCR" refers to a process of amplifying one or more specific nucleic acid sequences, wherein 1) oligonucleotide primers which determine the ends of the sequences to be amplified are annealed to single-stranded nucleic acid in a test sample, 2) a nucleic acid polymerase extends the 3' ends of the annealed primers to create a nucleic acid strand complementary in sequence to the nucleic acid to which the primers were annealed, 3) the resulting double-stranded nucleic acid is denatured to yield two single-stranded nucleic acids, and 4) the processes of primer annealing, primer extension, and product denaturation are repeated enough times to generate easily identified and measured amounts of the sequences defined by the primers. Practical control of the sequential annealing, extension, and denaturation steps is exerted by varying the temperature of the reaction container, normally in a repeating cyclical manner. Annealing and extension can occur between about the 35°C to 80°C. In another embodiment, annealing and extension can occur between about 40°C to 75°C. Denaturation can be performed at temperatures in about the 80°C to 100°C range.

[00213] While a single primer pair is most often employed in PCR, a single primer ("one-sided PCR"), multiple primers ("multiplex PCR"), degenerate primers, and nested primers can also be employed in the methods disclosed herein. Moreover, in addition to amplification of DNA, the method can be employed for RT-PCR, *e.g.,* reverse transcription of an RNA molecule to produce a single stranded cDNA with subsequent PCR of the cDNA.

[00214] PCR specificity can be increased by omitting at least one reagent until the sample temperature is between 50-80 °C ("Hot StartTM."), the addition of a reagent which interferes with nonspecific polymerase reactions *(e.g.,* SSB), or the addition of a modified nucleotide *(e.g.,* dUTP) and the corresponding glycosylase *(e.g.,* UNG) into the reaction mixture. See U.S. Pat. No. 5,538,871, the disclosure of which is incorporated by reference herein.

[00215] "Thermal cycling" commonly is automated by a "thermal cycler," an instrument which rapidly (on the time scale of one to several minutes) heats and cools a "sample compartment," a partly or completely enclosed container holding the vessel, *e.g.,* a microcentrifuge tube, or flat substrate, a microscope slide, on which nucleic acid amplification occurs and the heat-transfer medium directly contacting the PCR vessel or flat substrate. Most commonly, the sample compartment is a "sample block," which can be temperature controlled.

[00216] "PCR reagents" refers to the chemicals, apart from the biological sample, needed to make nucleic acid amplification work. For example, the, reagents can consist of five classes of components: (1) an aqueous buffer, (2) a water-soluble magnesium salt, (3) at least four deoxyribonucleoside triphosphates (dbNTPs), although these can be augmented or sometimes replaced by dNTPs containing base analogues which Watson-Crick base-pair like the conventional four bases, such as the analog deoxyuridine

triphosphate (dUTP) and dUTP carrying molecular tags such as biotin and digoxigenin, covalently attached to the uracil base via spacer arms, (4) oligonucleotide primers (normally two for each target sequence, with sequences which define the 5' ends of the two complementary strands of the double-stranded target sequence), and (5) a polynucleotide polymerase, *e.g.,* a DNA polymerase, *e.g.,* a thermostable DNA polymerase, which can tolerate temperatures between 90 °C and 100 °C for a total elapsed time of at least 10 minutes without losing more than about half of its activity.

[00217] "Detection" of PCR-amplified nucleic acid refers to the process of observing, locating, or quantitating an analytical signal which is inferred to be specifically associated with the product of PCR amplification, as distinguished from PCR reactants. The analytical signal can result from visible or ultraviolet absorbance or fluorescence, chemiluminescence, or the photographic or autoradiographic image of absorbance, fluorescence, chemiluminescence, or ionizing radiation. Detection of in situ PCR products involves microscopic observation or recording of such signals. The signal derives directly or indirectly from a molecular "tag" attached to a PCR primer or dNTP or to a nucleic acid probe, which tag can be a radioactive atom, a chromophore, a fluorophore, a chemiluminescent reagent, an enzyme capable of generating a colored, fluorescent, or chemiluminescent product, or a binding moiety capable of reaction with another molecule or particle which directly carries or catalytically generates the analytical signal. Common binding moieties are biotin, which binds tightly to streptavidin or avidin, digoxigenin, which binds tightly to anti-digoxigenin antibodies, and fluorescein, which binds tightly to anti-fluorescein antibodies. Intercalators, such as ethidium bromide, which bind double stranded DNA can also be used to detect DNA. The avidin, streptavidin, and antibodies are easily attached to chromophores, fluorophores, radioactive atoms, and enzymes capable of generating colored, fluorescent, or chemiluminescent signals.

[00218] The methods of the present disclosure are also amenable to other amplifications techniques, including, for example, methods broadly classified as thermal cycling amplification methods and isothermal amplification methods. Suitable thermal cycling methods include, for example, ligase chain reaction (Wu and Wallace, Genomics 4:560, (1989); and Landergren et al., Science 241: 1077 (1988)). Isothermal amplification methods useful in the present disclosure include, for example, Strand Displacement Amplification (SDA) (Walker et al, Proc. Nat. Acad. Sci. USA 89:392-396 (1992)), Q-beta-replicase (Lizardi et al., Bio/Technology 6:1197-1202 (1988)); nucleic acid-based Sequence Amplification (NASBA) (Sooknanan, R., et al., Bio/Technology 13:563-565 (1995)); and Self-Sustained Sequence Replication (Guatelli, et al., Proc. Nat. Acad. Sci. USA 87:1874-1878 (1990)).

[00219] For the sake of identifying the presence of a microorganism in a culture, a variety of methods can be used to determine if a PCR product has been produced. One way to determine if a PCR product has been produced in the reaction is to analyze a portion of the PCR reaction by agarose gel electrophoresis. For example, a horizontal agarose gel of from 0.6 to 3.0% agarose is made and a portion of the PCR reaction mixture is electrophoresed through the agarose gel. After electrophoresis, the gel is stained with ethidium bromide. PCR products are visible when the gel is viewed during illumination with ultraviolet light. By comparison to standardized size markers, it is determined if the PCR product is of the

correct expected size. In one embodiment, the PCR product DNA is analyzed through restriction enzyme digest or sequencing analysis to confirm the microorganism strain present in the culture. In one embodiment, the microorganism detected after PCR is comprises a *Clostridium* species. In one embodiment, the microorganism detected after PCR is comprises *Clostridium phytofermentans* Q.13 or *Clostridium phytofermentans* Q.12. When multiple microorganisms are present, or when knowing the amount or relative amount of microorganism present is desired, more quantitative amplification methods can be utilized.

[00220] The PCR procedure can be done in such a way that the amount of PCR products can be quantified. In one embodiment, quantitative PCR is used to determine the quantity of microorganism in a culture. In one embodiment, quantitative PCR is used to determine the quantity of a *Clostridium* species in a culture. In one embodiment, quantitative PCR is used to determine the quantity of *Clostridium phytofermentans* Q.13 or *Clostridium phytofermentans* Q.12 species in a culture. _Such "quantitative PCR" procedures normally involve comparisons of the amount of PCR product produced in different PCR reactions. A number of such quantitative PCR procedures, and variations thereof, are well known to those skilled in the art. One inherent property of such procedures, however, is the ability to determine relative amounts of a sequence of interest within the template that is amplified in the PCR reaction.

[00221] One method of quantitative PCR that can be used to quantify copy numbers of sequences within the PCR template is a modification of the standard PCR called "real-time PCR." Real-time PCR utilizes a thermal cycler *{e.g.,* an instrument that provides the temperature changes during the PCR) that incorporates a fluorimeter *{e.g.,* an instrument that measures fluorescence). In one embodiment, the real-time PCR reaction mixture also contains a reagent whose incorporation into a PCR product can be quantified and whose quantification is indicative of copy number of that sequence in the template. One such reagent is a fluorescent dye, called SYBR Green I (Molecular Probes, Inc.; Eugene, Oreg.) that preferentially binds double-stranded DNA and whose fluorescence is greatly enhanced by binding of double-stranded DNA. When a PCR reaction is performed in the presence of SYBR Green I, resulting DNA products bind SYBR Green I and fluoresce. The fluorescence is detected and quantified by the fluorimeter. After each cycle, the levels of fluorescence are measured with a detector; the dye only fluoresces when bound to the dsDNA *{e.g.,* the PCR product). With reference to a standard dilution, the dsDNA concentration in the PCR can be determined. Such technique is particularly useful for quantification of the amount of template in a PCR reaction. Relative concentrations of DNA present during the exponential phase of the reaction are determined by plotting fluorescence against cycle number on a logarithmic scale. Amounts of RNA or DNA are determined by comparing the results to a standard curve produced by real-time PCR of serial dilutions *(e.g.,* undiluted, 1:4, 1:16, 1:64) of a known amount of RNA or DNA.

[00222] In one embodiment, fluorescence can be detected after a laser light source excites each a well containing the PCR product, and a CCD camera measures the fluorescence spectrum and intensity from each well to generate real-time data during PCR amplification. Multiple commercial providers have

software available to process the data generated (Applied Biosystems and Cephied) and examine the fluorescence intensity collected and calculate the increase in normalized reporter emission intensity over the course of the amplification. The results can then be plotted versus time, represented by cycle number, to produce a continuous measure of PCR amplification. To provide precise quantification of initial target in each PCR reaction, the amplification plot is examined at a point during the early log phase of product accumulation. This is accomplished by assigning a fluorescence threshold above background and determining the time point at which each sample's amplification plot reaches the threshold (defined as the threshold cycle number or CT). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube, and therefore the relative amount of starting template, which is indicative of the relative amount of microorganism of interest present.

[00223] In one embodiment, polynucleotide sequences that hybridize to a fragment of a target nucleic acid sequence found in the genome of *Clostridium phytofermentans* and variants thereof are provided. In one embodiment, the polynucleotide sequences are between about 10 nucleotides and about 50 nucleotides long; for example, about 10-50, 10-35, 10-25, 10-20, 10-15, 15-50, 15-35, 15-25, 15-20, 20-50, 20-35, 20-25, 25-50, 25-35, 35-50, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides long. In another embodiment, the fragment of the target nucleotide sequence is the same length as the polynucleotide sequence. In one embodiment, the target nucleic acid sequence has about 50%, 60%, 70%, 80%, 90%, 99%, or 100% identity to SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, or SEQ ID NO:20. In one embodiment, the polynucleotide sequences are SEQ ID NO:l, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO: 10.

[00224] In another embodiment, a pair of polynucleotide sequences that hybridize to fragments of a target nucleic acid sequence can be used to amplify all or a part of the target nucleotide sequence. In one embodiment, a polymerase chain reaction (PCR) is used in the amplifying. In one embodiment, the target nucleic acid sequence has about 50%, 60%, 70%, 80%, 90%, 99%, or 100% identity to SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, or SEQ ID NO:20. In one embodiment, one of the pair of polynucleotide sequences comprises SEQ ID NO:l, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9. In another embodiment, one of the pair of polynucleotide sequences comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO: 10. In one embodiment, the primer pair comprises SEQ ID NO:l/SEQ ID NO:2, SEQ ID NO:3/SEQ ID NO:4, SEQ ID NO:5/SEQ ID NO:6, SEQ ID NO:7/SEQ ID NO:8, or SEQ ID NO:9/SEQ ID NO: 10. In one embodiment, a level of amplified nucleotide after the PCR indicates the presence of a *Clostridium phytofermentans* species or variant in a sample. In one embodiment, the sample is an environmental sample. In another embodiment, the sample is from a fermentation reaction. In another embodiment, the sample is from a culture.

[00225] In one aspect, the primer pairs, SEQ ID NO:l/SEQ ID NO:2, SEQ ID NO:3/SEQ ID NO:4, SEQ ID NO:5/SEQ ID NO:6, SEQ ID NO:7/SEQ ID NO:8, or SEQ ID NO:9/SEQ ID NO:10, can be used alone or in combination with each other to identify variants or strains of *C. phytofermentans.* These variants can include recombinants and mutants as well. In other aspect, the primers can be used alone or in combinations of one or more primers to identify or exclude contaminants or other microorganisms from a culture. In another aspect, PCR primers obviate the need for cloning in order to compare the sequences of genes from related microorganisms, allowing rapid construction of DNA sequence-based phylogenies.

[00226] In general, the PCR amplification process can be carried out using procedures known to those skilled in the art, namely, denaturing, annealing, and elongation, which can be repeated any number of times to produce the desired amount of the target nucleic acid. While the number of cycles can be affected by any of a number of factors, such as the nature of the sample, the number of cycles required to achieve the desired amplification can be in the range of about 10 to about 50. The number of cycles can be 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49. However, cycles numbers outside of this range can be used and, thus, the above stated range is in no way intended to limit the scope of this disclosure.

[00227] As used herein, the term "amplification" refers to any *in vitro* method for increasing the number of copies of a nucleotide sequence with the use of a DNA polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA molecule or primer, thereby forming a new DNA molecule complementary to a DNA template. The formed DNA molecule and its template can be used as templates to synthesize additional DNA molecules. As used herein, one amplification reaction can consist of many rounds of DNA replication. DNA amplification reactions include polymerase chain reactions, PCR, which can consist of 10 to 50 cycles of denaturization and synthesis of a DNA molecule.

[00228] Thus, these primer pairs or other primer pairs specific to *C. phytofermentans* can be used to identify or fingerprint the *C. phytofermentans* strain and provide a tracking system for the strains in a culture or industrial process. See, for example, U.S. Patent No. 7,276,358. The tracking system can also be expanded into a quantitative method to detect the cell density of *C. phytofermentans* cells in a complex fermentation media containing insoluble substrates such as lignocelluloses, polysaccharides and hemicelluloses, as well as contaminants. For example, samples can be extracted from a fermentation broth (or culture) and a fixed volume of DNA preparations prepared. These preparations contain DNA from C. *phytofermentans* cells and any contaminants in the fermentations. The samples are used as a template with *C. phytofermentans* primers in a PCR reaction to quantify the concentration of initial C. *phytofermentans* DNA. The same template can also be used with generic 16S RNA primers to quantify contaminating species DNA. A correlation between gene copy (template copy) and cell density can be used to calculate *C. phytofermentans* and other species densities in a complex lignocellulosic fermentation broth. This is a great advantage over traditional quantification methods because it provides a more rapid analysis of the fermentation process rather than waiting for growth on plate cultures.

[00229] Biofuel plant and process of producing biofuel

[00230] Large Scale Ethanol Production from Biomass

[00231] Generally, there are two basic approaches to producing fuel grade ethanol from biomass on a large scale utilizing of microbial cells, especially Q.12 or Q.13 cells. In the first method, one first hydrolyzes a biomass material that includes high molecular weight carbohydrates to lower molecular weight carbohydrates, and then ferments the lower molecular weight carbohydrates utilizing of microbial cells to produce ethanol. In the second method, one ferments the biomass material itself without chemical and/or enzymatic pretreatment. In the first method, hydrolysis can be accomplished using acids, *e.g.*, Bronsted acids *(e.g.*, sulfuric or hydrochloric acid), bases, *e.g.*, sodium hydroxide, hydrothermal processes, ammonia fiber explosion processes ("AFEX"), lime processes, enzymes, or combination of these. Hydrogen, and other products of the fermentation can be captured and purified if desired, or disposed of, *e.g*, by burning. For example, the hydrogen gas can be flared, or used as an energy source in the process, *e.g*, to drive a steam boiler, *e.g.*, by burning. Hydrolysis and/or steam treatment of the biomass can, *e.g.*, increase porosity and/or surface area of the biomass, often leaving the cellulosic materials more exposed to the biocatalyst cells, which can increase fermentation rate and yield. Removal of lignin can, *e.g.*, provide a combustible fuel for driving a boiler, and can also, *e.g.*, increase porosity and/or surface area of the biomass, often increasing fermentation rate and yield. Generally, in any of the below described embodiments, the initial concentration of the carbohydrates in the medium is greater than 20 mM, *e.g.*, greater than 30 mM, 50 mM, 75 mM, 100 mM, 150 mM, 200 mM, or even greater than 500 mM.

[00232] Biomass Processing Plant and Process of Producing Products from Biomass

[00233] In one aspect, a fuel plant that includes a hydrolysis unit configured to hydrolyze a biomass material that includes a high molecular weight carbohydrate, a fermentor configured to house a medium with Q.12 or Q.13 cells or another C5/C6 hydrolyzing microorganism dispersed therein, and one or more product recovery system(s) to isolate a product or products and associated by-products and co-products is provided.

[00234] In another aspect, methods of making a product or products that include combining Q.12 or Q.13

cells or another C5/C6 hydrolyzing microorganism and a biomass feed in a medium, and fermenting the biomass material under conditions and for a time sufficient to produce a biofuel, chemical product or fermentation end-products, *e.g.*, ethanol, propanol, hydrogen, lignin, terpenoids, and the like as described above, is provided.

[00235] In another aspect, products made by any of the processes described herein are also provided.

[00236] Large Scale Chemical Production From Biomass

[00237] Generally, there are two basic approaches to producing chemical products from biomass on a large scale utilizing microorganisms such as *Q.12 or Q.13* or other C5/C6 hydrolyzing microorganisms. In all methods, depending on the type of biomass and its physical manifestation, one of the processes can

comprise a milling of the carbonaceous material, via wet or dry milling, to reduce the material in size and increase the surface to volume ratio (physical modification).

[00238] In a first method, one first hydrolyzes a biomass material that includes high molecular weight carbohydrates to delignify it or to separate the carbohydrate compounds from noncarbohydrate compounds. Using any combination of heat, chemical, and/or enzymatic treatment, the hydrolyzed material can be separated to form liquid and dewatered streams, which can be separately treated and kept separate or recombined, and then ferments the lower molecular weight carbohydrates utilizing Q.12 or Q.13 cells or another C5/C6 hydrolyzing biocatalyst to produce one or more chemical products. In the second method, one ferments the biomass material itself without heat, chemical, and/or enzymatic pretreatment. In the first method, hydrolysis can be accomplished using acids *(e.g.,* sulfuric or hydrochloric acids), bases *(e.g.,* sodium hydroxide), hydrothermal processes, ammonia fiber explosion processes ("AFEX"), lime processes, enzymes, or combination of these. Hydrolysis and/or steam treatment of the biomass can, *e.g.,* increase porosity and/or surface area of the biomass, often leaving the cellulosic materials more exposed to any C5/C6 hydrolyzing microorganism, such as Q.12 or Q.13 or C. *phytofermentans,* which can increase fermentation rate and yield. Hydrolysis and/or steam treatment of the biomass can, *e.g.,* produce by-products or co-products which can be separated or treated to improve fermentation rate and yield, or used to produce power to run the process, or used as products with or without further processing. Removal of lignin can, *e.g.,* provide a combustible fuel for driving a boiler. Gaseous, *e.g.,* hydrogen and CO_2, liquid, *e.g.,* ethanol and organic acids, and solid, *e.g.,* lignin, products of the fermentation can be captured and purified if desired, or disposed of, *e.g.,* by burning. For example, the hydrogen gas can be flared, or used as an energy source in the process, *e.g.,* to drive a steam boiler, *e.g.,* by burning. Products exiting the fermentor can be further processed, *e.g.,* ethanol can be transferred to distillation and rectification, producing a concentrated ethanol mixture or solids can be separated for use to provide energy or as chemical products. It is understood that other methods of producing fermentation end products or biofuels can incorporate any and all of the processes described as well as additional or substitute processes that can be developed to economically or mechanically streamline these methods, all of which are meant to be incorporated in their entirety within the scope of this disclosure.

[00239] Figure 4 is an example of a method for producing chemical products from biomass by first treating biomass with an acid at elevated temperature and pressure in a hydrolysis unit. The biomass can first be heated by addition of hot water or steam. The biomass can be acidified by bubbling gaseous sulfur dioxide through the biomass that is suspended in water, or by adding a strong acid, *e.g.,* sulfuric, hydrochloric, or nitric acid with or without preheating/presteaming/water addition. During the acidification, the pH is maintained at a low level, *e.g.,* below about 5. The temperature and pressure can be elevated after acid addition. In addition to the acid already in the acidification unit, optionally, a metal salt such as ferrous sulfate, ferric sulfate, ferric chloride, aluminum sulfate, aluminum chloride, magnesium sulfate, or mixtures of these can be added to aid in the hydrolysis of the biomass. The acid-impregnated biomass is fed into the hydrolysis section of the pretreatment unit. Steam is injected into the

hydrolysis portion of the pretreatment unit to directly contact and heat the biomass to the desired temperature. The temperature of the biomass after steam addition is, *e.g.*, between about 130° C and 220° C. The hydrolysate is then discharged into the flash tank portion of the pretreatment unit, and is held in the tank for a period of time to further hydrolyze the biomass, *e.g.*, into oligosaccharides and monomeric sugars. In one embodiment, the level of oligosaccharides is higher than the level of monomeric sugars. Steam explosion can also be used to further break down biomass. Alternatively, the biomass can be subject to discharge through a pressure lock for any high-pressure pretreatment process. Hydrolysate is then discharged from the pretreatment reactor, with or without the addition of water, *e.g.*, at solids concentrations between about 15% and 60%.

[00240] After pretreatment, the biomass can be dewatered and/or washed with a quantity of water, *e.g.*, by squeezing or by centrifugation, or by filtration using, *e.g.*, a countercurrent extractor, wash press, filter press, pressure filter, a screw conveyor extractor, or a vacuum belt extractor to remove acidified fluid. The acidified fluid, with or without further treatment, *e.g.*, addition of alkali *(e.g* lime) and or ammonia *(e.g.*, ammonium phosphate), can be re-used, *e.g.*, in the acidification portion of the pretreatment unit, or added to the fermentation, or collected for other use/treatment. Products can be derived from treatment of the acidified fluid, *e.g.*, gypsum or ammonium phosphate. Enzymes or a mixture of enzymes can be added during pretreatment to assist, *e.g.*, endoglucanases, exoglucanases, cellobiohydrolases (CBH), beta-glucosidases, glycoside hydrolases, glycosyltransferases, lyases, and esterases active against components of cellulose, hemicelluloses, pectin, and starch, in the hydrolysis of high molecular weight components.

[00241] The fermentor is fed with hydrolyzed biomass, any liquid fraction from biomass pretreatment, an active seed culture of *Clostridium* sp. cells, if desired a co-fermenting microorganism, *e.g.*, yeast or *E. coli*, and, optionally, nutrients to promote growth of *Clostridium* sp. or other microorganisms. Alternatively, the pretreated biomass or liquid fraction can be split into multiple fermentors, each containing a different strain of *Clostridium* sp.and/or other microorganisms, and each operating under specific physical conditions. Fermentation is allowed to proceed for a period of time, *e.g.*, between about 15 and 150 hours, while maintaining a temperature of, *e.g.*, between about 25° C and 50° C. Gas produced during the fermentation is swept from fermentor and is discharged, collected, or flared with or without additional processing, *e.g.*, hydrogen gas can be collected and used as a power source or purified as a co-product.

[00242] After fermentation, the contents of the fermentor are transferred to product recovery. Products are extracted, *e.g.*, ethanol is recovered through distilled and rectification.

[00243] Chemical production from biomass without pretreatment

[00244] Fig. 5 depicts a method for producing chemicals from biomass by charging biomass to a fermentation vessel. The biomass can be allowed to soak for a period of time, with or without addition of heat, water, enzymes, or acid/alkali. The pressure in the processing vessel can be maintained at or above atmospheric pressure. Acid or alkali can be added at the end of the pretreatment period for neutralization. At the end of the pretreatment period, or at the same time as pretreatment begins, an active seed culture of

Clostridium sp. cells or another C5/C6 hydrolyzing microorganism and, if desired, a co-fermenting microorganism, *e.g.*, yeast or *E. coli*, and, optionally, nutrients to promote growth of *Clostridium* sp. or other microorganisms are added. Fermentation is allowed to proceed as described above. After fermentation, the contents of the fermentor are transferred to product recovery as described above.

[00245] Any combination of the chemical production methods and/or features can be utilized to make a hybrid production method. In any of the methods described herein, products can be removed, added, or combined at any step. *Clostridium* sp. can be used alone, or synergistically in combination with one or more other microorganisms *(e.g.*, yeasts, fungi, or other bacteria). Different methods can be used within a single plant to produce different products.

[00246] In another aspect, a fuel plant that includes a hydrolysis unit configured to hydrolyze a biomass material that includes a high molecular weight carbohydrate, and a fermentor configured to house a medium and contains *Clostridium* cells dispersed therein, is provided.

[00247] In another aspect, methods of making a fuel or fuels that include combining *Clostridium* sp. cells and a hgnocellulosic material (and/or other biomass material) in a medium, and fermenting the hgnocellulosic material under conditions and for a time sufficient to produce a fuel or fuels, *e.g.*, ethanol, propanol and/or hydrogen or another chemical compound is provided herein.

[00248] In one embodiment, a process for producing ethanol and hydrogen from biomass using acid hydrolysis pretreatment is provided. In one embodiment, a process for producing ethanol and hydrogen from biomass using enzymatic hydrolysis pretreatment is provided. In one embodiment, the process for producing ethanol and hydrogen from biomass is by using biomass that has not been enzymatically pretreated. Still in another embodiment, the process for producing ethanol and hydrogen from biomass is by using biomass that has not been chemically or enzymatically pretreated, but is optionally steam treated.

[00249] Genetic Modification of *Clostridium phytofermentans* Q.12 or Q.13

[00250] In one embodiment, *Clostridium phytofermentans* Q.12 or Q.13 can be modified to enhance enzyme activity of one or more enzymes, including but not limited to hydrolytic enzymes (such as cellulase(s), hemice//ulase(s), or pectinases *etc.).* In another embodiment, an enzyme can be selected from the annotated genome of *C. phytofermentans,* another bacterial species, such as ***B. subtilis,*** *E. coli,* various *Clostridium* species, such as *C. cellulolyticum* or C. sp. Q.D., or yeasts such as *S. cerevisiae* for utilization in products and processes described herein. Examples include enzymes such as L-butanediol dehydrogenase, acetoin reductase, 3-hydroxyacyl-CoA dehydrogenase, cis-aconitate decarboxylase or the like, to create pathways for new products from biomass.

[00251] Examples of such modifications include modifying endogenous nucleic acid regulatory elements to increase expression of one or more enzymes *(e.g.*, operably linking a gene encoding a target enzyme to a strong promoter), introducing into a microorganism additional copies of endogenous nucleic acid molecules to provide enhanced activity of an enzyme by increasing its production, and operably linking genes encoding one or more enzymes to an inducible promoter or a combination thereof. A

detailed description of methods of genetic modification of microorganisms, including *Clostridia* species, is disclosed in the International Application Publication WO201 1008564, which is hereby incorporated by reference in its entirety.

[00252] In another embodiment, *Clostridium phytofermentans* Q.12 or Q.13 can be modified to enhance an activity of one or more cellulases, or enzymes associated with cellulose processing. The classification of cellulases is usually based on grouping enzymes together that form a family with similar or identical activity, but not necessarily the same substrate specificity. One of these classifications is the CAZy system (CAZy stands for Carbohydrate-Active enZymes), for example, where there are 115 different Glycoside Hydrolases (GH) listed, named GHl to GHl 55. Each of the different protein families usually has a corresponding enzyme activity. This database includes both cellulose and hemicellulase active enzymes. Furthermore, the entire annotated genome of *C phytofermentans* is available on the worldwideweb at www.ncbi.nlm.nih.gov/sites/entrez. Detailed methods for genetic modification

[00253] Several examples of cellulase enzymes whose function can be enhanced for expression endogenously or for expression heterologously in a microorganism include one or more of the genes disclosed in Table 2.

[00254] Table 2: Cellulase enzymes

Cellulase Protein ID	Description (on www.ncbi.nlm.nih.gov/sites/entrez)
ABX43556	Cellulase [*Clostridium phytofermentans* ISDg] gi|160429993|gb|ABX43556.1|[160429993] Cphy_3302
ABX42426	Cellulase [*Clostridium phytofermentans* ISDg] gi|160428863|gb|ABX42426.1|[160428863] Cphy_2058
ABX41541	Cellulase [*Clostridium phytofermentans* ISDg] gi|160427978|gb|ABX41541.1|[160427978] Cphy_1163
ABX43720	Cellulose 1,4-beta-cellobiosidase [*Clostridium phytofermentans* ISDg] gi|160430157|gb|ABX43720.1|[160430157] Cphy_3367
ABX41478	Cellulase M Cphy_1100
ABX41884	Endo-1,4-beta-xylanase Cphy_1510
ABX43721	Cellulase 1,4-beta-cellobiosidase Cphy_3368
ABX42494	Mannan endo-1,4-beta-mannosidase, Cellulase 1,4-beta-cellobiosidase Cphy_2128

[00255] Directed Evolution

[00256] Various methods can be used to produce and select mutants that differ from wild-type cells. In some instances, bacterial populations are treated with a mutagenic agent, for example, nitrosoguanidine (N-methyl-N'-nitro-N-nitrosoguanidine) or the like, to increase the mutation frequency above that of spontaneous mutagenesis. This is induced mutagenesis. Techniques for inducing mutagenesis include, but are not limited to, exposure of the bacteria to a mutagenic agent, such as x-rays or chemical mutagenic

agents. More sophisticated procedures involve isolating the gene of interest and making a change in the desired location, then reinserting the gene into bacterial cells. This is site-directed mutagenesis.

[00257] Directed evolution is usually performed as three steps which can be repeated more than once. First, the gene encoding a protein of interest is mutated and/or recombined at random to create a large library of gene variants. The library is then screened or selected for the presence of mutants or variants that show the desired property. Screens enable the identification and isolation of high-performing mutants by hand; selections automatically eliminate all non functional mutants. Then the variants identified in the selection or screen are replicated, enabling DNA sequencing to determine what mutations occurred. Directed evolution can be carried out *in vivo* or *in vitro.* See, for example, Otten, L.G.; Quax, W.J. (2005). Biomolecular Engineering 22 (1-3): 1-9; Yuan, L., *et al.* (2005) Microbiol. Mol. Biol. Rev. 69 (3): 373-392.

[00258] In another aspect, the products made by any of the processes described herein is provided.

EXAMPLES

[00259] The following examples serve to illustrate certain embodiments and aspects and are not to be construed as limiting the scope thereof

[00260] **Isolation of *Clostridium phytofermentans* Q.12 and Q.13**

[00261] Example 1. Methodology

[00262] Selection of ethanol tolerant *Clostridium phytofermentans* mutants was carried out either through adaptation or by spontaneous mutation in ethanol-containing growth medium. This normally produces strains that can grow in the presence of increased ethanol concentration compared to parent strain, but display an increase the production of organic acids at the expense of ethanol. To isolate an ethanol tolerant, but increased ethanol-producing strain: random saturation mutations were introduced using a mutagen to create a large ($>10^7$) diverse mutant pool; followed by selection for tolerant strains by selective enrichment; and followed by secondary selection agar plates then followed by screening of "tolerant" strains for improved growth rates. In these experiments the mutagen was N-methyl- N'-nitro-N-nitroso-guanidine (NTG). Promising mutants were sub-cultured and subjected to a fitness test to assess the ethanol production and tolerance improvement over the parent strain.

[00263] Example 2. Bacterial Strains and Media

[00264] All bacterial strains used were derived from genus *Clostridium,* species *phytofermentans.* Growth conditions were anaerobic (Oxygen maintained at 0 to less than 1 ppm), at 35°C. The growth medium QM contained (per liter): 10.6 g K_2HP0_4, 1.92 g KH_2P0_4, 4.6 g (NH4)2S0 $_4$, 3 g $Na_3C_6H_50_7$, 6 g Bacto yeast extract , 1g Cysteine'HCl adjusted to pH 7.5 with NaOH. Cellobiose (20% w/v) stock solution in ddH_20 , filter sterilized, was added post autoclaving to a final concentration. Salts solution (see *infra)* was prepared at lOOx in ddH20, and added to the QM medium post autoclaving. Agar (1.5% w/v) was used as needed. Solvent was added to previously autoclaved media cooled to at least 45 to 50°C (agar

medium) or room temperature (liquid medium). Fermentation medium FM contained (per liter): 20g Bacto yeast extract, 1.5g Corn Steep Powder, 1.36g KH_2P0_4, 2g$Na_3C_6H_50_7$ 1.2g $C_6H_80_7$'1120, 0.5g $(NH4)_2S0_4$, 1g NaCl, 1g Cysteine-FICl, 11.45 g TES (Tris EthaneSulfonic acid) adjusted to pH 8.0 with NaOH.

[00265] Composition of lOOx Salts solution

100X salt components	Gram per Liter
$Na_3C_6H_5O_7$	10
$CaCl_2$'$2H_20$	0.5
$MgSO_4$'$7H_20$	6
$FeSO_4$'$7H_20$	0.4
$CoSO_4$•H_20	0.2
$ZnSO_4$•$7H_20$	0.2
$NiCl_2$	0.2
$MnSO_4$•H_20	0.5
$CuSO_4$•$5H_20$	0.04
$KAI(SO_4)_2$•$12H_20$	0.04
H_3BO_3	0.04
$(NH_4)_6Mo_7O_{24}$·$4H_20$	0.04
Na_2Se0_3	0.04

Each salt is added in order (allowing each to dissolve prior to next addition) to 1000 g ddH_20 and mixed

[00266] Example 3. Mutation protocol

[00267] A single colony (not more than 48h old) of *C. phytofermentans* was re-suspended in 4 mL QM medium. The resuspended cells were inoculated into QM medium — Cellobiose (2% w/v) at 1:100 (v/v) and grown to mid log phase ($0D_{660}$= 0.4 to 0.5), then centrifuged 2500 x g for 10 min to pellet the cells. While the cells were growing the NTG solution was prepared at 200 mg/mL in 0.1 M phosphate buffer (pH 7.4). The solution was held anaerobically for at least 16h to equilibrate. 1-2 mL aliquots (about 10^9 cells) of exponential phase cells were centrifuged at 2500 x g for 10 min and the supernatant discared. 1 mL of equilibrated NTG solution was added and the suspension incubated at 35°C for 60 minutes. The time and dose of NTG exposure was determined by identifying the conditions that achieve a 99% kill of viable *C. phytofermentans* cells. Following NTG exposure, the cell pellet was centrifuged to remove the NTG solution. The cells were re-suspended in QM medium containing 15%> (w/v) glycerol and frozen at -80°C for storage.

[00268] Example 4. Screening and selection

[00269] NTG mutated pools were inoculated into QM medium containing 2% (w/v) cellobiose with or without ethanol (ethanol as selective pressure to favor the growth of tolerant mutants). Ethanol concentrations were selected by determining the Minimum Inhibitory Concentration (MIC) of ethanol

that would prevent growth of the parent strain in QM (containing *2%* w/v cellobiose) medium for at least 7 days as monitored by increase in OD_{660}. Enriched mutant pools were transferred when OD_{660} reached >0.2 in less than 7 days. Enriched mutant pools were sub-cultured in the same ethanol concentration until an OD_{660} >0.2 to 0.3 was reached in 24-48h. Following enrichment, a sample of the enriched pool (about 1%) was placed on QM cellobiose (2% w/v) agar plates containing 4% (v/v) 2-propanol (IPA plates). Colonies that appear within 72 h were picked and inoculated into QM cellobiose medium containing 3% (w/v) ethanol (in a multi-well plate). Cultures that grew to an OD_{660} > 0.3 in 48 h were considered "hits" and re-streaked to single colonies.

[00270] An example of the process is as follows: 0.75% ethanol ^1% ethanol ➔ 1.5% ethanol ^IPA plate ➔3% ethanol screen.

[00271] A single colony from a "hit" was evaluated in a shake flask fermentation or "fitness test" in FM medium containing 8% (w/v) cellobiose. Cultures that fermented faster or to higher ethanol titer than the parent strain were considered positive and given a new strain designation. See Figure 1 and Figure 2.

[00272] Example 5. Strain Isolation sequence

[00273] For Q.12 isolation, *Clostridium phytofermentans* Q.8 (NRRL B-50351) was used as the initial strain, mutated with NTG and inoculated in ethanol-containing QM as follows:

0.75% ➔ 1.0% ➔ 1.5% ➔ IPA Plates ➔ Fitness Test ➔ Q.12
10 transfers 2 transfers 4 transfers (OD_{660}=0.22)

[00274] Q.13 was isolated from Q.12 as follows:

2.0% ➔ 3.0% ➔ IPA Plates ➔ Fitness Test ➔ Q.13
3 transfers 4 transfers (OD_{660}=0.22)

[00275] Example 6. Identification of genomic regions of *C. phytofermentans*

[00276] Coding regions of the *Clostridium phytofermentans* genome having no homology to any previously sequenced microorganism were identified using the NR (non-redundant) sequence database at NCBI (http://blast.ncbi.nlm.nih.gov). Five of these regions (primer sequences and ORF sequences are listed in Tables 3 and 4 respectively) were chosen and primers designed to amplify a 250-350 base pair (bp) fragment within the selected regions. Amplification of any one of these targeted sites is sufficient to confirm the presence of *C. phytofermentans.* Melting temperatures were standardized between 58-62°C. Assay conditions were as follows:

[00277] The following reagents were mixed in a PCR tube prior to thermocycling:

12.5 µL GoTAQ ready mixed TAQ polymerase
0.125 µL Fwd (forward) primer (100 µM stock)
0.125 µL Rvs (reverse) primer (100 µM stock)
0.25 µL DMSO (dimethyl sulfoxide)

0.5 µL Template (template can be a single colony resuspended in H_20, a growing liquid bacterial culture, or a total DNA preparation obtained from a growing bacterial culture.

H_20 to a total volume of 25 µL.

Standard thermocycling parameters were applied using a BioRad iCycler

Step	Temp (°C)	Duration (sec)
1. Melt	94	142
2. Melt	94	22
3. Anneal	50	22
4. Amplify	68	44

Cycle to step 2 for 33 cycles.

PCR reactions are analyzed via agarose gel electrophoresis (0.8% agarose) and Ethidium Bromide staining to detect DNA bands resulting from PCR amplification. The assay is considered positive for *Clostridium phytofermentans* if the assay yields a DNA fragment corresponding to the DNA sequence targeted by the selected primer pair. The assay is considered negative if the PCR reaction fails to yield a DNA product corresponding to the DNA sequence targeted by the selected primer pair.

Table 3. Primer pairs for identification of *Clostridium phytofermentans*

Genomic ORF target and primer sequences used to amplify.
Cphy_0389 Fwd - GAAAGAAGAACTTCGGTGGACGC (SEQ ID NO: 1) Rvs - GAGAACCTGCTGCTAAGAACAAG (SEQ ID NO: 2)
Cphy_0451 Fwd - GTATGCTTGTGCTTTCTGCCTGTG (SEQ ID NO: 3) Rvs — GGAAGCATAACTGCATATTCCTG (SEQ ID NO: 4)
Cphy_0727 Fwd - GGAGTTGGAATCGGTTTTAATGCTG (SEQ ID NO: 5) Rvs - CTCCCACATAGATTAGTAGGCAACC (SEQ ID NO: 6)
Cphy_2681 Fwd - CGATGGGAGTGGAGTGGAAAGC (SEQ ID NO: 7) Rvs - GGTACGAATCTTGCCAGCATAACC (SEQ ID NO: 8)
Cphy_2804 Fwd - CCGAATTAGAAGGTTTCCTTGCCC (SEQ ID NO: 9) Rvs - GCCGCACTATCTCATATTCTTCACG (SEQ ID NO: 10)

Table 4. Open reading frame polynucleotide sequences

Cphy_0389 (reverse_complement) Polynucleotide SEQ ID NO: 11	ctaaacgttaataaacacaatagcaagtactcctagaaggatac caattttaccacggttagataacttctctttaaaaaatagcgtt gaaattatggtaatgccgaggataatactgccttgtacgagtgg aaacagataactgctgggaattttccctgctaagcttgttaata gcaaatttccaagtagagaacctgctgctaagaacaagattggt

	aataagttagtttttagttgctttacagaggatgaggtcgtgat ttttacctcgcttgcctttgacttagcaaccggataagcaataa agtagcaaagagaagcaaaggcaaatccaaggagtattaaccca gaagcttccgtgccgtgttgaagctgttgatgaagttttgaac aatagcaaggcaaccattacctataaacgtgagaatacaataaa agagccattgctttttttgttttctatgagtgggggaaacagct tccttttttgattcttttgtaacattaaggaagtaaaatgccat caggaataaaatgatagcaataaggagcgtccaccgaagttctt ctttcaaaaagagaagaccaaaaaaggtaggaagcagcatgcta gaagaaaagtagaatgctgtgtaggaaaggggccgctgttaat tgccagattataaaataatatggtaaaagcaaataaaaatccaa aaagaataccaaacaaaatcgtgttcttacttagagaaaaggat tccctggagaacaataaaaagaaactcattccgatacctgccac gagtgtaaatactccatggaccagcatttttgcggaagcgtat ctgctttcacaaacttcattgagactgattgcaagatgaaaaag gtgatacttaaaaccaaaagtagagttatcat
Cphy_038 9 Polynucleotide SEQ ID NO: 12	atgataactctacttttggttttaagtatcacctttttcatctt gcaatcagtctcaatgaagtttgtgaaagcagatacgcttccgc aaaaaatgctggtccatggagtatttacactcgtggcaggtatc ggaatgagtttcttttattgttctccagggaatccttttctct aagtaagaacacgattttgtttggtattctttttggatttttat ttgcttttaccatattattttataatctggcaattaacagcggc ccccttttcctacacagcattctacttttcttctagcatgctgct tcctacctttttttggtcttctctttttgaaagaagaacttcggt ggacgctccttattgctatcatttttattcctgatggcattttac ttccttaatgttacaaaagaatcaaaaaaaggaagctgtttcccc cactcatagaaaacaaaaaaaagcaatggctcttttattgtattc tcacgtttataggtaatggttgccttgctattgttcaaaaactt catcaacagcttcaacacggcacggaagcttctgggttaatact ccttggatttgcctttgcttctctttgctactttattgcttatc cggttgctaagtcaaaggcaagcgaggtaaaaatcacgacctca tcctctgtaaagcaactaaaaactaacttattaccaatcttgtt cttagcagcaggttctctacttggaaatttgctattaacaagct tagcagggaaaattcccagcagttatctgtttccactcgtacaa ggcagtattatcctcggcattaccataatttcaacgctattttt taaagagaagttatctaaccgtggtaaaattggtatccttctag gagtacttgctattgtgtttattaacgtttag
Cphy_0 451 (reverse_complement) Polynucleotide SEQ ID NO: 13	atgaaaaaaatacaacttttttagtttctctattctgtatgct tgtgctttctgcctgtggtgctgaaatcacgacaacaacgaatt taaataaagacggttccggagagcttatcgtaactgcagtagct tcctcttcggatgagaagtaccttaatggcggttatgaggcatt aaatcaattattattggagaaggcgccagaaggattggagttat ctgttgtttatgatgaaaccaattctaattatacatattcttat aaaataggatttcaggatgcaaatgattataacaataagatagc agcacttttgggacgcaaggactagctgaattttcggatacca ccgataatttaactcatacaacgaaatatcaggatttgacggat aaggcacttttattgattgggctctaaatgcaatcgatgaatc aggaatatgcagttatgcttcctctgaaatctattcttgggggt accgctatttaaattacaatggagaagaagtaaattacggatat aaagacccttcgatgaagaagactacaacagtaggtgttttaaa ggttaagtgtttttctgagtatgatatttattcagatgccacaa agacgatagaaatttatcttaacacttcggatcttgtaggaata gaggaagaagacatactttcctactttcgtaaatttgatcaaga tgttgaatacaatgctacagcaggtacaattatttatcgattcg atagtttagagaaaatcaatcagtttttattatctgtaatgaat tcagatggagaaactaatataacggaatcaccagttaagttatc aataaccgattcctctatttttcgttcacctttgaattagagg

	aatattataacttgggaaatctctttaaaacattaagaatggat accgattatatcgatgtttatttatcgattccagaagatcttgc gtatggatggtaccattttacttcctataaagatgccagtgaag tagctgatgcaaaatacaattttcaaggtgcatgttattacgat agttcatttaacagtagctttaaagtaaatcagtccgttacggt taagaatatagatgttaagtgtattcttgaaaatgatatgtcgg gaaagctaactaccgagttttcttttctgccaaatggttgtaat attgatgaggccaaatgtaaggaatttataaagacctaggaca ggagttaacttatcgagagcaaggagacactgtagttgttgcct ttacgaaaaattttcataaaggagagagttatatcgataaatcc aatgtcattcaactaaaagagtcttccttgtcaaaccgaaaaac taagatctatacacttgatcatgtatttatgctatcgggattag ttcctatgaaagattgtgaagtgaattacaaaatcatggttccg gattccttaaaagtaactttcttgcattcgaatgacgatattct tacaggaaaaaagattgaaaagattcaaaaaaatggatattata cagagagcttatccacctatcgaggaaccgtatcagtttcggta gcaatagaaaaaacgaatgtattatattatgttattgtaattgc gatagttgtagcagcagtggctggtgttgcaattactactgtta ttataattaaaaataaaaaaggactaaaaagagaagcggctact acaagtgatactgcgcctactaatgaagttgcacctactgatga agctactcctacaaatgaagttgcacctactgatgaagctactc ctacaaatgaagttgcacctactgatgaagctactcctacaaat gaagttgcacctactgatgaagctactcctacaaatgaagtttc acctactcatgaagctacaactatgaatgaaactgcaacatcga atgaagctgcacctacgaaggaagcggcagttacgagtgatact gcaactacatatgaaactgtaattcctaatgaaactgtagctgc gaaagatactgctaataatccgtaa
Cphy_0451 Polynucleotide SEQ ID NO: 14	ttacggattattagcagtatctttcgcagctacagtttcattag gaattacagtttcatatgtagttgcagtatcactcgtaactgcc gcttccttcgtaggtgcagcttcattcgatgttgcagtttcatt catagttgtagcttcatgagtaggtgaaacttcatttgtaggag tagcttcatcagtaggtgcaacttcatttgtaggagtagcttca tcagtaggtgcaacttcatttgtaggagtagcttcatcagtagg tgcaacttcatttgtaggagtagcttcatcagtaggtgcaactt cattagtaggcgcagtatcacttgtagtagccgcttctcttttt agtccttttttatttttaattataataacagtagtaattgcaac accagccactgctgctacaactatcgcaattacaataacataat ataatacattcgtttttttctattgctaccgaaactgatacggtt cctcgataggtggataagctctctgtataatatccatttttttg aatcttttcaatctttttttcctgtaagaatatcgtcattcgaat gcaagaaagttacttttaaggaatccggaaccatgattttgtaa ttcacttcacaatctttcataggaactaatcccgatagcataaa tacatgatcaagtgtatagatcttagtttttcggtttgacaagg aagactcttttagttgaatgacattggatttatcgatataactc tctcctttatgaaaatttttcgtaaaggcaacaactacagtgtc tccttgctctcgataagttaactcctgtcctaggtctttataaa attccttacatttggcctcatcaatattacaaccatttggcaga aaagaaaactcggtagttagctttcccgacatatcattttcaag aatacacttaacatctatattcttaaccgtaacggactgattta ctttaaagctactgttaaatgaactatcgtaataacatgcacct tgaaaattgtattttgcatcagctacttcactggcatctttata ggaagtaaaatggtaccatccatacgcaagatcttctggaatcg ataaataaacatcgatataatcggtatccattcttaatgtttta aagagatttcccaagttataatattcctctaattcaaaggtgaa cgaaaaaatagaggaatcggttattgataacttaactggtgatt ccgttatattagtttctccatctgaattcattacagataataaa aactgattgattttctctaaactatcgaatcgataaataattgt

	acctgctgtagcattgtattcaacatcttgatcaaatttacgaa agtaggaaagtatgtcttcttcctctattcctacaagatccgaa gtgttaagataaatttctatcgtctttgtggcatctgaataaat atcatactcagaaaaacacttaacctttaaaacacctactgttg tagtcttcttcatcgaagggtctttatatccgtaatttacttct tctccattgtaatttaaatagcggtacccccaagaatagatttc agaggaagcataactgcatattcctgattcatcgattgcattta gagcccaatcaataaaaagtgccttatccgtcaaatcctgatat ttcgttgtatgagttaaattatcggtggtatccgaaaattcagc tagtccttgcgtcccaaaaagtgctgctatcttattgttataat catttgcatcctgaaatcctattttataagaatatgtataatta gaattggtttcatcataaacaacagataactccaatccttctgg cgccttctccaataataattgatttaatgcctcataaccgccat taaggtacttctcatccgaagaggaagctactgcagttacgata agctctccggaaccgtctttatttaaattcgttgttgtcgtgat ttcagcaccacaggcagaaagcacaagcatacagaatagagaaa ctaaaaaaagttgtatttttttcat
Cphy_0727 (reverse_complement) Polynucleotide SEQ ID NO: 15	atgaaaaagaacttctggattaaactagtacttgctttaattgg tattttatttgttggagttggaatcggttttaatgctgaatctt tatttggaaatgatcctgttgcattactttatgatggtgttaga aatgctttttctctaccattatcacggctagggatgatatcaaa tattgtgaatcttagtattatcgttttgctatttttttataggta gacattatataaatattggtacagtggtgtatatcctaccatta ggtacatttgtaaatcttggtatgtttttatttcccaagatatt tgtttcaaatacagttttaagccgaggaatcggaacttttttg gttgcctactaatctatgtgggagtatccatttttatcagcgta gatatcggtttagatccctttaccgggcttgtaatgacgattag tgaccaccttccatgggaatttggaataactaaaatatgctttg attttattcttattttaattggtttttttacttggaggcaatgtg ggtgtgattaccttcattaccgctttgtccgcaggacctagtat tcaattatttacaaaactaataaaacggctattaaagaaaatta agttaatataa
Cphy_0727 Polynucleotide SEQ ID NO: 16	ttatattaacttaattttctttaatagccgttttattagttttg taaataattgaatactaggtcctgcggacaaagcggtaatgaag gtaatcacacccacattgcctccaagtaaaaaaccaattaaaat aagaataaaatcaaagcatattttagttattccaaattcccatg gaaggtggtcactaatcgtcattacaagcccggtaaagggatct aaaccgatatctacgctgataaaaatggatactcccacatagat tagtaggcaaccaaaaaaagttccgattcctcggcttaaaactg tatttgaaacaaatatcttgggaaataaaaacataccaagattt acaaatgtacctaatggtaggatatacaccactgtaccaatatt tatataatgtctacctataaaaaatagcaaaacgataatactaa gattcacaatatttgatatcatccctagccgtgataatggtaga gaaaaagcatttctaacaccatcataaagtaatgcaacaggatc atttccaaataaagattcagcattaaaaccgattccaactccaa caaataaaataccaattaaagcaagtactagtttaatccagaag ttctttttcat
Cphy_2681 (reverse_complement) Polynucleotide SEQ ID NO: 17	ttagtttgcatacaaatctagtacaagtttcacttccccttgtc ctaatcctaactgtttcgatatatcacggatggaatattgcttc ttatagagattaataatttcatccttgtgctttttaactaagag gttagtatctgtcctttcagacttctttgcgctcttttccatat ggacaaaatccattgattctgcttcctcaagagctttcagtttc tcttctgcgctctcaaagtgttcctcactattcgtagttactgt ggttatcttatccctttcagccatgagtattgagagtttatcaa gcaattccttaacatccttacgtactgtttccatctttgagaat gttgatttcagttcttcttctttctcatttagcatctgaaataa gaagacaacttcactattgtttatttctatcttttcaagaaccc

	ctgtagaaaactcattaaccgccataatttttcattcgataac tgatttaacttatcttcagcatcgaagatgcgatcgctcatttc ttgttcgattactgcttgcacctgtcttcttattttattcttct cttcttcacttagttcataatttaaattatcaccgagactagaa gactgtccttttcctgcttttggtcgttcgacaagtaagaaact tagaaaaataaaagccaatccgacaatcagtaatattatttgtg gtgtactcat
Cphy_2681 Polynucleotide SEQ ID NO: 18	atgagtacaccacaaataatattactgattgtcggattggcttt tatttttctaagtttcttacttgtcgaacgaccaaaagcaggaa aaggacagtcttctagtctcggtgataatttaaattatgaacta agtgaagaagagaagaataaaataagaagacaggtgcaagcagt aatcgaacaagaaatgagcgatcgcatcttcgatgctgaagata agttaaatcagttatcgaatgaaaaaattatggcggttaatgag ttttctacaggggttcttgaaaagatagaaataaacaatagtga agttgtcttcttatttcagatgctaaatgagaaagaagaagaac tgaaatcaacattctcaaagatggaaacagtacgtaaggatgtt aaggaattgcttgataaactctcaatactcatggctgaaaggga taagataaccacagtaactacgaatagtgaggaacactttgaga gcgcagaagagaaactgaaagctcttgaggaagcagaatcaatg gattttgtccatatggaaaagagcgcaaagaagtctgaaaggac agatactaacctcttagttaaaaagcacaaggatgaaattatta atctctataagaagcaatattccatccgtgatatatcgaaacag ttaggattaggacaaggggaagtgaaacttgtactagatttgta tgcaaactaa
Cphy_2804 (reverse_complement) Polynucleotide SEQ ID NO: 19	ctagtcggaattttctatattatctatttcaataagttcacgtt gatttacatagatttgccgcactatctcatattcttcacgattc tttcgataaaattcaagtctaccatcttttaccttcttaacgta ttcacatgccattgtatcataatcagtacggatacgaaacatcg ggtcatatcttcttggaatccctgaatacctcaggatataaggc ggctttgttggagcagcaggaatttctgtcagtttacgaatcga tagactggagacaatggttactccaaccacaactaacataaaca cgattcttcgattcactttatttcgcttatttcttcggatatgt tcttcacaactacgaattttacgattcagttcctctgtaaaatt gttcgacatttccttatcttcgtctaataactttattgcagtta acaaggcataatatacctctaattcttctctacataccggacat tctttcacatgggcaaggaaaccttctaattcggtcatgctaag ttcatctttaatgaatggtgtaattaatctttgcgcattgaggc aatccat
Cphy_2804 Polynucleotide SEQ ID NO: 20	atggattgcctcaatgcgcaaagattaattacaccattcattaa agatgaacttagcatgaccgaattagaaggtttccttgcccatg tgaaagaatgtccggtatgtagagaagaattagaggtatattat gccttgttaactgcaataaagttattagacgaagataaggaaat gtcgaacaattttacagaggaactgaatcgtaaaattcgtagtt gtgaagaacatatccgaagaaataagcgaaataaagtgaatcga agaatcgtgtttatgttagttgtggttggagtaaccattgtctc cagtctatcgattcgtaaactgacagaaattcctgctgctccaa caaagccgccttatatcctgaggtattcagggattccaagaaga tatgacccgatgtttcgtatccgtactgattatgatacaatggc atgtgaatacgttaagaaggtaaaagatggtagacttgaatttt atcgaaagaatcgtgaagaatatgagatagtgcggcaaatctat gtaaatcaacgtgaacttattgaaatagataatatagaaaattc cgactag

[00278] The isolated strains disclosed herein have been deposited in the Agricultural Research Service culture Collection (NRRL), an International Depositary Authority, National Center for Agricultural

Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604 U.S.A. on March 9, 2010 *(Clostridium phytofermentans* Q.8) and November 3, 2010 *(Clostridium phytofermentans* Q.12 and *Clostridium phytofermentans* Q.13) in accordance with and under the provisions of the Budapest Treaty for the Deposit of Microorganisms; *e.g.*, they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposits, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures plus five years after the last request for a sample from the deposit. The strains were tested by the NRRL and determined to be viable. The NRRL has assigned the following NRRL deposit accession numbers to strains: *Clostridium phytofermentans* Q.8 (NRRL B-50351), *Clostridium phytofermentans* Q.12 (NRRL B-50436), and *Clostridium phytofermentans* Q.13 (NRRL B-50437). The depositor acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposits. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject matter disclosed herein in derogation of patent rights granted by governmental action.

[00279] All references cited herein, including but not limited to published and unpublished applications, patents, and literature references, and also including but not limited to the references listed in the Appendix, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

[00280] The term "comprising" as used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[00281] All numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that can vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of any claims in any application claiming priority to the present application, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[00282] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only.

Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure herein. It should be understood that various alternatives to the embodiments of the disclosure described herein can be employed in practicing the described subject matter. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A primer for amplifying a target polynucleotide sequence, wherein the target nucleotide sequence is from a *Clostridium phytofermentans* species or variant.

2. The primer of claim 1, wherein the target nucleotide sequence has at least about 80% identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

3. The primer of claim 1, wherein the target nucleotide sequence has at least about 90% identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

4. The primer of claim 1, wherein the target nucleotide sequence has at least about 99%> identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

5. The primer of claim 1, wherein the target nucleotide sequence comprises all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

6. The primer of claim 1, wherein the primer is between about 10 and about 50 nucleotides in length.

7. The primer of claim 1, wherein the primer is between about 15 and about 30 nucleotides in length.

8. The primer of claim 1, wherein the primer is SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO: 10.

9. A method of detecting a *Clostridium phytofermentans* species or variant in a sample comprising:

(a) providing a pair of primers, wherein each of the primers hybridize to a target nucleotide sequence from the *Clostridium phytofermentans* species or variant;

(b) performing a polymerase chain reaction (PCR), wherein the PCR comprises a derivative of the sample and the pair of primers; and,

(c) determining the presence or absence of the *Clostridium phytofermentans* species or variant based upon a level of a product of the PCR.

10. The method of claim 9, wherein the target nucleotide sequence has at least about 80%> identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

11. The method of claim 9, wherein the target nucleotide sequence has at least about 90%> identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

12. The method of claim 9, wherein the target nucleotide sequence has at least about 99% identity to all or a part of SEQ ID NO:l 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

13. The method of claim 9, wherein the target nucleotide sequence comprises all or a part of SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, or SEQ ID NO:20.

14. The method of claim 9, wherein the primer is between about 10 and about 50 nucleotides in length.

15. The method of claim 9, wherein the primer is between about 15 and about 30 nucleotides in length.

16. The method of claim 9, wherein the primer is SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO: 10.

17. The method of claim 9, wherein the sample is an environmental sample.

18. The method of claim 9, wherein the sample is from a fermentation reaction.

19. The method of claim 9, wherein the sample is from a culture.

20. The method of claim 9, wherein the sample is from a culture comprising two or more microorganisms.

2 1. The method of claim 9, wherein the derivative of the sample comprises polynucleotides isolated from the sample.

22. The method of claim 9, wherein the derivative of the sample comprises one or more microorganisms.

23. The method of claim 9, further comprising estimating a level of the *Clostridium phytofermentans* species or variant based upon the level of the product of the PCR.

24. An isolated bacterium, wherein the bacterium is *Clostridium phytofermentans* Q.13 or *Clostridium phytofermentans* Q.12.

25. The bacterium of claim 24, wherein the bacterium is deposited under NRRL Accession Number NRRL B50436 or NRRL Accession Number NRRL B-50437.

26. The bacterium of claim 24, wherein the bacterium can hydrolyze polysaccharides.

27. The bacterium of claim 24, wherein the bacterium can hydrolyze hexose or pentose sugars.

28. The bacterium of claim 24, wherein the bacterium can utilize cellulose or xylose as its sole carbon source.

29. The bacterium of claim 24, wherein the bacterium can hydrolyze and ferment hemicellulosic or lignocellulosic material.

30. The bacterium of claim 24, wherein the bacterium produces alcohol dehydrogenase, wherein the alcohol dehydrogenase reduces acetaldehyde into ethanol.

31. The bacterium of claim 24, wherein the bacterium can produce ethanol at greater than 90% theoretical yield from a biomass.

32. The bacterium of claim 24, wherein the bacterium can produce ethanol at greater than *90%* theoretical yield from a biomass comprising cellulosic, hemicellulosic, or lignocellulosic material.

33. A high-yielding mutant of *Clostridium phytofermentans* that produces ethanol at a rate of over 45 g/L from biomass.

34. A method of producing one or more fermentation end-products comprising:

(a) providing a biomass in a media;

(b) contacting the biomass with one or more microorganisms, wherein at least one of said microorganisms is *Clostridium phytofermentans* Q.12 or Q.13; and

(c) allowing sufficient time for the microorganisms to hydrolyze and ferment the biomass to produce the fermentation end-products.

35. The method of claim 34, wherein the biomass comprises hemicellulosic or lignocellulosic material.

36. The method of claim 34, wherein the biomass comprises corn, wheat, rice, barley, soybeans, bamboo, cotton, crambe, jute, sorghum, high biomass sorghum, oats, tobacco, grasses, miscanthus, switchgrass, trees, beans, rape/canola, alfalfa, flax, sunflowers, safflowers, millet, rye, sugarcane, sugar beets, cocoa, tea, Brassica sp., cotton, coffee, sweet potatoes, flax, peanuts, clover; lettuce, tomatoes, cucurbits, cassava, potatoes, carrots, radishes, peas, lentils, cabbages, cauliflower, broccoli, Brussels sprouts, grapes, peppers, apples, pears, peaches, apricots, walnuts, almonds, olives, avocadoes, bananas, coconuts, stillage, bagasse, leaves, pomace, distillers grains, Distillers Dried Solubles (DDS), Distillers Dried Grains (DDG), Condensed Distillers Solubles (CDS), Distillers Wet Grains (DWG), Distillers Dried Grains with Solubles (DDGS), peels, pits, fermentation waste, skins, straw, seeds, shells, beancake, sawdust, wood flour, wood pulp, paper pulp, paper pulp waste streams, rice or oat hulls, grass clippings, lumber, food leftovers, or a combination thereof.

37. The method of claim 34, wherein the biomass is pretreated by acid, steam explosion, hot water treatment, alkali, catalase, or a detoxifying or chelating agent.

38. The method of claim 34, wherein the fermentation end-products comprise one or more alcohols.

39. The method of claim 34, wherein the fermentation end-products comprise ethanol.

40. The method of claim 34, wherein the *Clostridium phytofermentans* Q.12 or Q.13 is deposited under NRRL Accession Numbers NRRL B-50436 or NRRL B-50437.

41. The method of claim 34, wherein the biomass is pretreated to make the polysaccharides more available to the microorganisms.

42. A method of hydrolyzing and fermenting a carbonaceous biomass wherein the biomass is contacted by a *C. phytofermentans* Q.12 or *C. phytofermentans* Q.13 bacterium for a period long enough to produce ethanol at greater than 90%> theoretical yield from the carbonaceous biomass.

43. The method of claim 42, wherein the contacting is at a temperature from about 30°C to about 40° C.

44. The method of claim 42, wherein the contacting is at a temperature from about 35°C to about 39°C.

45. The method of claim 42, wherein contacting is at a pH from about 5.5 to about 7.5.

46. The method of claim 42, wherein the bacterium uses biomass as a major carbon source.

47. The method of claim 42, wherein the bacterium is deposited under NRRL Accession Numbers NRRL B50436 or NRRL B-50437.

48. The method of claim 42, wherein the bacterium is genetically-modified.

49. A method for producing one or more fermentation end-product comprising:

(a) culturing a medium comprising a non-recombinant or recombinant *Q.12 or Q.13* bacterium for a period of time under conditions suitable for production of one or more fermentation end-products by said *Q.12 or Q.13* bacterium; and

(b) harvesting the fermentation end-products from the medium.

50. The method of claim 49, wherein the Q.12 or Q.13 bacterium is a mesophile.

51. The method of claim 49, wherein the fermentation end-products comprise an alcohol.

52. The method of claim 49, wherein the fermentation end-products comprise ethanol.

53. The method of claim 49, wherein the medium comprises a cellulosic and/or lignocellulosic material.

54. The method of claim 53, wherein the cellulosic or lignocellulosic material is not enzymatically treated with a sufficient quantity of enzymes to convert more than 15% of the cellulosic or lignocellulosic material to simple sugars within 24 hours.

55. The method of claim 53, wherein the cellulosic or lignocellulosic material is pretreated with acid, hot water, steam explosion, alkali, a chelating agent and/or a detoxifying agent.

56. The method of claim 53, wherein a second microorganism is added to the medium.

57. A fuel plant comprising a fermenter configured to house a medium and a strain of *Q.12 or Q.13* bacteria, wherein said fermenter comprises a cellulosic or lignocellulosic material.

58. The fuel plant of claim 57, wherein said cellulosic or lignocellulosic material is pretreated.

59. A set of primer pairs for amplifying a polynucleotide of a *C. phytofermentans* bacteria, wherein the primer pairs are SEQ ID NO:l/SEQ ID NO:2, SEQ ID NO:3/SEQ ID NO:4, SEQ ID NO:5/SEQ ID NO:6, SEQ ID NO:7/SEQ ID NO:8, or SEQ ID NO:9/SEQ ID NO: 10.

60. The set of primer pairs of claim 59, wherein the bacteria are mutants, variants, or recombinant.

61. A method for detecting a *Clostridium phytofermentans* bacteria in a sample comprising: performing a PCR reaction comprising the sample in the presence of forward primers selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, and SEQ ID NO: 9

and mixtures thereof and reverse primers selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 and mixtures thereof to amplify a PCR product, wherein the presence or absence of the PCR product indicates the presence or absence of the *Clostridium phytofermentans* bacteria in the sample.

62. The method of claim 61, wherein the sample is an environmental sample.

63. The method of claim 61, wherein the sample is taken from a fermentation production operation.

64. The method of claim 61, wherein the sample is taken from a culture operation.

65. An assay to detect a *C phytofermentans* bacteria in a culture of microorganisms comprising:

(a) extracting a fixed volume from the culture of microorganisms;

(b) performing a PCR reaction comprising the sample in the presence of any of the primer pairs selected from the group consisting of SEQ ID NO:l / SEQ ID NO:2 , SEQ ID NO: 3/ SEQ ID NO:4, SEQ ID NO: 5/ SEQ ID NO:6, SEQ ID NO:7 / SEQ ID NO:8, and SEQ ID NO:9 / SEQ ID NO: 10 to amplify a PCR product; and

(c) determining the presence of the *C. phytofermentans* in the culture based on the level of the PCR product.

66. An assay to identify *C. phytofermentans* in a culture of microorganisms comprising:

(a) extracting a fixed volume from the culture of microorganisms;

(b) amplifying a polynucleotide in the fixed volume with any of the primer pairs selected from the group consisting of SEQ ID NO:l / SEQ ID NO:2 , SEQ ID NO: 3/ SEQ ID NO:4, SEQ ID NO: 5/ SEQ ID NO:6, SEQ ID NO:7 / SEQ ID NO:8, and SEQ ID NO:9 / SEQ ID NO: 10; and

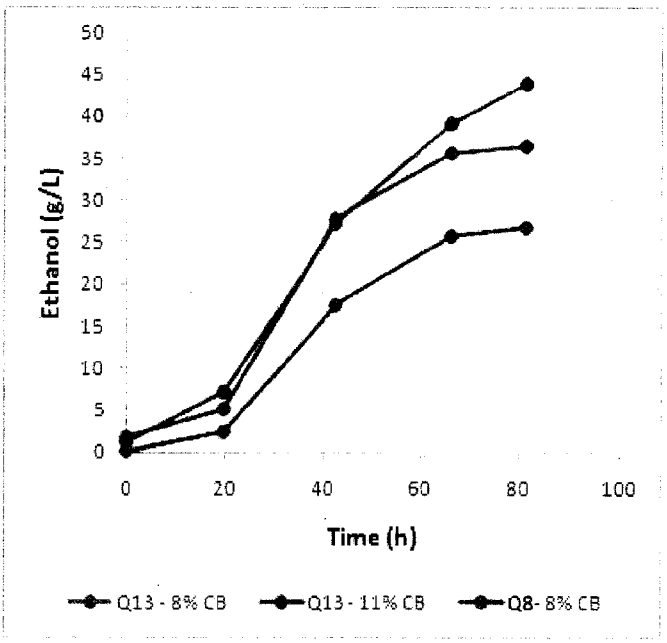
(c) calculating the correlation between the amount of the amplified polynucleotide and the cell density to determine the *C. phytofermentans* and non-C. *phytofermentans* cell densities in the culture.

67. A method for quantification of a *Clostridium* bacteria comprising hybridizing an oligonucleotide of said *Clostridium* to any of SEQ ID NO: 1 through 10.

68. The method of claim 66, wherein said method comprises PCR amplification.

69. The method of claim 67, wherein said method comprises array hybridization.

FIGURE 1



45.7 g/L (Q13) with 11% sugar

36.5 g/L (Q13) with 8% sugar

26.8 g/L (Q8) with 8% sugar

FIGURE 2

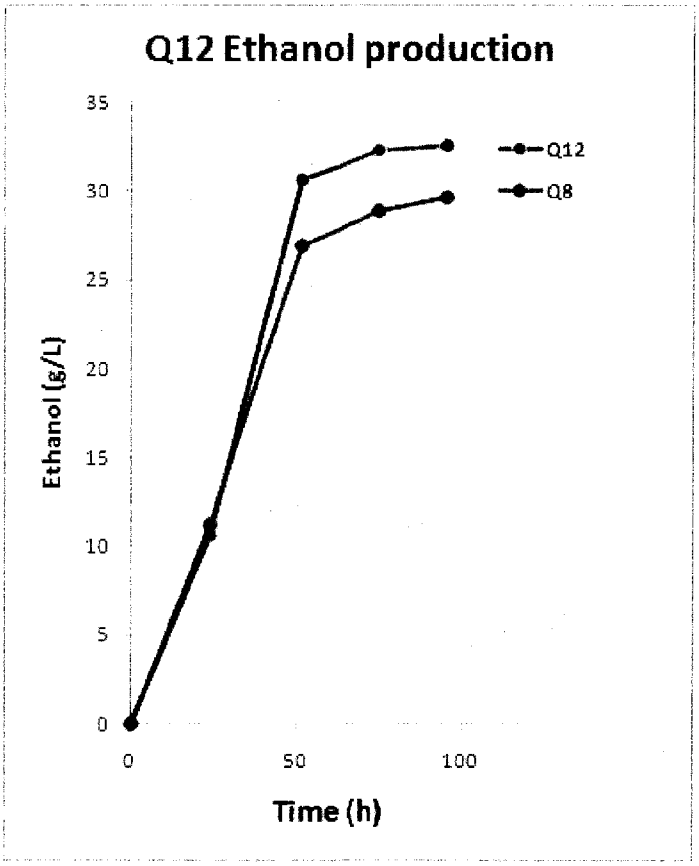

Q12 Ethanol production
35
30
25
20
15
10
5
0
Ethanol (g/L)
0
50
100
Time (h)
Q12
Q8

FIGURE 3

Genomic ORF target and primer sequences used to amplify.

ORF 0389
Fwd - GAAAGAAGAACTTCGGTGGACGC (SEQ ID NO: 1)
Rvs - GAGAACCTGCTGCTAAGAACAAG (SEQ ID NO: 2)
ORF 0451
Fwd - GTATGCTTGTGCTTTCTGCCTGTG (SEQ ID NO:3)
Rvs – GGAAGCATAACTGCATATTCCTG (SEQ ID NO:4)
ORF 0727
Fwd - GGAGTTGGAATCGGTTTTAATGCTG (SEQ ID NO: 5)
Rvs - CTCCCACATAGATTAGTAGGCAACC (SEQ ID NO: 6)
ORF 2681
Fwd - CGATGGGAGTGGAGTGGAAAGC (SEQ ID NO:7)
Rvs - GGTACGAATCTTGCCAGCATAACC (SEQ ID NO: 8)
ORF 2804
Fwd - CCGAATTAGAAGGTTTCCTTGCCC (SEQ ID NO: 9)
Rvs - GCCGCACTATCTCATATTCTTCACG (SEQ ID NO: 10)

FIGURE 4

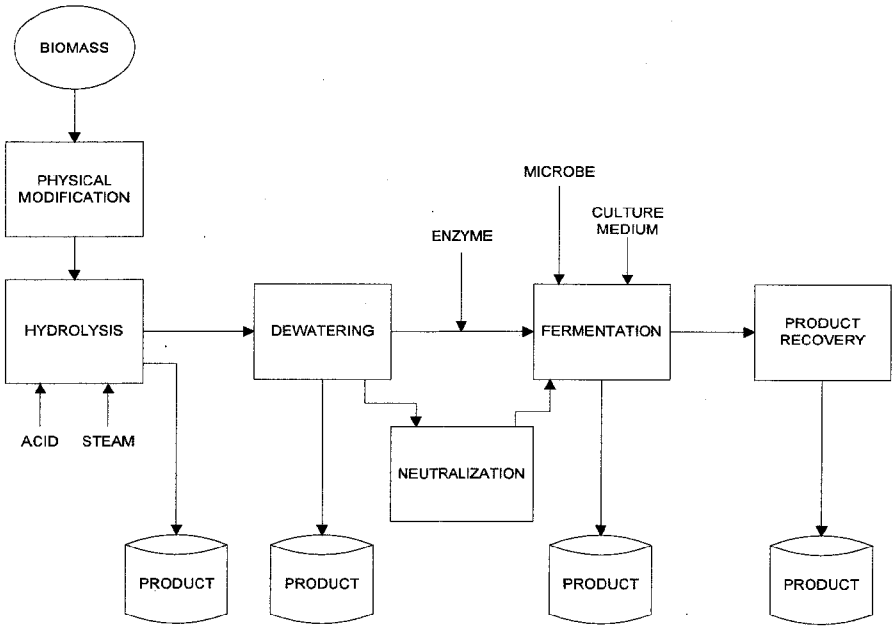

BIOMASS
PHYSICAL MODIFICATION
HYDROLYSIS
ACID
STEAM
DEWATERING
ENZYME
MICROBE
CULTURE MEDIUM
FERMENTATION
PRODUCT RECOVERY
NEUTRALIZATION
PRODUCT
PRODUCT
PRODUCT
PRODUCT

FIGURE 5

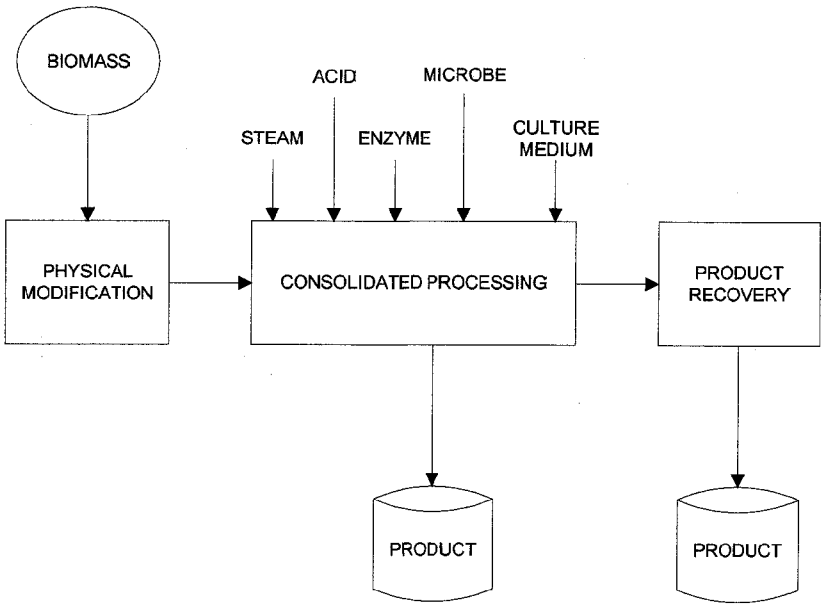
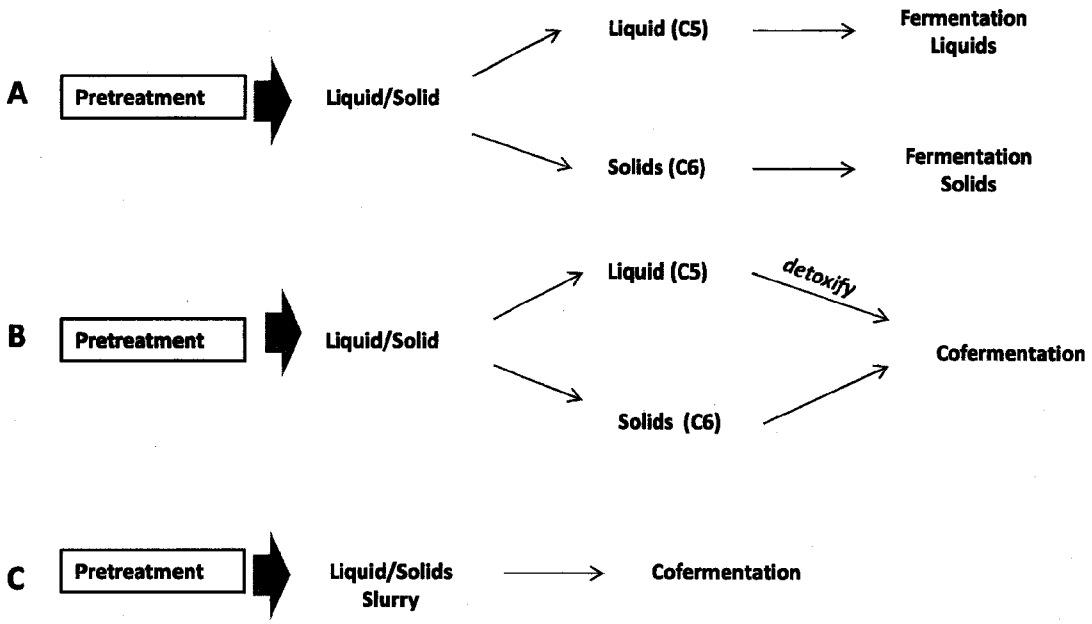

BIOMASS
ACID
MICROBE
STEAM
ENZYME
CULTURE MEDIUM
PHYSICAL MODIFICATION
CONSOLIDATED PROCESSING
PRODUCT RECOVERY
PRODUCT
PRODUCT

FIGURE 6


A
Pretreatment
Liquid/Solid
Liquid (C5)
Fermentation Liquids
Solids (C6)
Fermentation Solids
B
Pretreatment
Liquid/Solid
Liquid (C5)
detoxify
Cofermentation
Solids (C6)
C
Pretreatment
Liquid/Solids Slurry
Cofermentation