A review of US patent trends in biomass fermentation, 2011—Part I

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The number of patent publications in the United States from the first half of 2011 indicates a growth trend in advances in research on biomass fermentation and fermenters. There are more patent publications in this area of biofuel production in the first half of 2011 than for the entirety of 2010. This Commentary column will present a two-part review (over this and the December 2011 issue of *Industrial Biotechnology*). The first part, presented herein, reviews several fermentation-related patent publications from summer 2011. Specifically, the following topics are discussed: enzyme modification and enzyme systems, process efficiency, and microorganism biofilms and membranes. A subsequent column will cover some of the more significant patent applications over the last 3–5 years and provide a roadmap for how the research reached this point.

Enzymatic modification and enzyme systems

US Patent Publication 2011/0171674, *Beta-glucosidase variants having improved activity and uses thereof* (inventors: Nicolas Lopes-Ferreira, Antoine Margeot, Hugues Mathis, Laurent Fourage; assignee: IFP Energies Nouvelles; 14 July 2011) teaches the optimization of enzymes to break down lignocellulose. The enzymes and the process of modifying the enzymes are the patentable subject matter, in that the enzymes, specifically beta-glucosidase variants, contain modified amino acids that aid in the optimization of the lignocellulose biomass conversion. According to the inventors, the inventiveness of the publication relates to the fact that the proportion of the enzymes in the mixture need not be modified, which is otherwise often required in conventional formulations to convert cellulose to biofuel.

US Patent Publication 2011/0183396, *Production of yeast having increased cellulose hydrolysis ability* (inventors: Hideo Noda, Shohei Kaneko, Akihiko Kondo; assignees: Kansai Chemical Engineering Co., Ltd., and Bio-Energy Corporation; 28 July 2011) teaches a specially tailored yeast incorporating into a noncellulolytic yeast both a gene for an enzyme capable of hydrolyzing crystalline cellulose and a gene for an enzyme capable of hydrolyzing noncrystalline cellulose. As the publication notes, cellulosic biomass has a crystalline portion and a noncrystalline portion. The enzymatic hydrolic reaction works well on the noncrystalline portion but is not nearly as effective on the crystalline portion of the cellulosic biomass. A transformed yeast and process that can target both aspects of the cellulose biomass can thus more efficiently convert cellulose biomass into biofuels products.

US Patent Publication 2011/0183382, *Methods and compositions for producing chemical products from* C. phytofermentans (inventors: Matthias Schmalisch, Chelsea Ju, Francis H. Verhoff, Gregory S. Coli; assignee: Qteros, Inc.; 28 July 2011) teaches the use of *C. phytofermentans* genetically engineered for hydrolysis and fermentation to produce commercial biofuels. The inventors aimed to address two identified problems. First, they noted that microbial fermentation requires adaptation of strains to particular feedstocks and fermentation media; given that certain species are particular to the products they synthesize, different microbes must be adapted to any process to make more than one product. Second, while Clostridia is known as a "natural synthesizer of chemical products," many species can only ferment biomass to a few specific products and, generally, in low amounts. The publication describes a composition for production of fermentation end-products comprising carbonaceous biomass and an organism capable of direct hydrolysis and fermentation of the

AUTHOR'S NOTE: In general, when patents and patent applications are reviewed, it is instructive to keep a few points in mind. There are two types of published patent applications—those that have issued and those that have published but are still pending and under examination. This article will primarily focus on patent publications, since those documents are indicative of more recent research and development of biofuels. In fact, the patent applications being published in June and July of 2011 were first filed between December 2009 and February 2010.

An issued patent has a fixed set of claims at the end of that patent that define the invention regardless of what is included in the detailed description section. Anyone reviewing issued patents for an indication of the actual invention should turn to the claims section first. The detailed description section should primarily be used to provide context of the claims and/or definitions of some of the terms/phrases in the claims. Issued patents are in force 20 years from the filing date, and they give

biomass, wherein Clostridia strains are modified to produce higher product yields than possible with wild-type organisms.

US Patent Publication 2011/0183381, *Thermocellulases for lignocellulosic degradation* (inventors: Rolf Prade, Hongliang Wang; assignee: The Board of Regents for Oklahoma State University; 28 July 2011) teaches a thermostable cellulase enzyme cocktail of different enzymes that work to produce biofuels more efficiently and with better yield. In this case, the inventors use at least one of each of a thermostable endoglucanase, an exo-processive-endoglucanase, and a β-glucosidase, and claim that this mixture carries "out the complete, coordinated hydrolysis of a crystalline cellulose to monomeric glucose." The approach tackles the recalcitrance of the cellulosic polymer to degradation due to the extensive intermolecular hydrogen bonding between cellulose polymer chains.

US Patent Publication 2011/0183393, *Methods of increasing dihydroxy acid dehydratase activity to improve production of fuels, chemicals, and amino acids* (inventors: Catherine Asleson Dundon, Aristos Aristidou, Andrew Hawkins, Doug Lies, Lynne H. Albert; assignee: Gevo, Inc.; 28 July 2011) teaches a method of production of recombinant microorganisms that comprise a dihydroxyacid dehydratase-/(DHAD)-requiring biosynthetic pathway. The inventors note that DHAD catalyzes the conversion of 2,3-dihydrooxyisovalerate to α-ketoisovalerate as part of isobutanol biosynthetic pathways but cite the challenge that DHAD-activity must be at levels significantly higher than in non-engineered microorganisms in order to produce the yields needed for commercially viable productions. The claimed recombinant microorganisms are engineered to improve DHAD activity.

US Patent Publication 2011/0165635, *Methods and materials for processing a feedstock* (inventors: Gregory P. Copenhaver, Daphne Preuss, Jennifer Mach; assignee: Chromatin, Inc.; 7 July 2011) teaches a method of processing feedstocks for biofuel production employing transgenic organisms (plants, fungi, and algae) modified to incorporate enzymatic activity with potential to break down down cellulose and lignocellulose; the enzymes include glycoside hydrolases (families 1 through 32). Modified organisms are mixed with the feedstock. Embodiments accommodate feedstocks including lignocellulosic material, recycled materials, forestry waste, industrial waste materials, livestock waste, and municipal wastes, oilseeds, algae, animal waste, and vegetable oil; other embodiments further comprise thermochemical, chemical, and/or biochemical treatments.

the patent owner the right to *exclude* others from making, using and/or selling the claimed invention in the country where the patent issued.

Published patent applications are useful to provide notice to the general public and/or competitors as to potential future patents and their scope. Between the filing date or priority date of the application and the publication date, the patent application is not published, and therefore, not readily available to the public for review.

Inventors use pending patent publications to put likely competitors on notice that they may have enforceable patent rights at some point in the future. Competitors use patent publications to scope out the technology landscape. It is important to note, however, that the existence of a patent publication does not necessarily mean that a patent will issue from that application or that the final issued set of claims will be the same or resemble the claims in the publication. These publications should primarily be used for notice and information purposes.

Process efficiency

US Patent Publication 2011/0165639, *Refinery process to produce biofuels and bioenergy products from home and municipal solid waste* (inventors: Miguel Ascon, Dolores Ascon; assignee: BriJen Biotech, LLC; 7 July 2011) teaches a process for using home and municipal solid waste as a carbon source to support the metabolism of synthetic microorganisms that are then used to produce biofuels and bioenergy products. Contemplated systems described in this publication contain tubular bioreactors that convert the pretreated biomass to fuels through biosynthesis. Bioreactors contain naturally or genetically engineered microorganisms and may also be anaerobic multiphase bioreactors having fermentative and/or methanogenic microbes forming biofilms on solid surfaces. This system is designed to have several points of conversion of waste into fuels, energy, and other useful by-products/products.

US Patent Publication 2011/0136210, *Use of methylsulfonylmethane (MSM) to modulate microbial activity* (inventors: Rodney L. Benjamin, Jeffrey Varelman, Anthony L. Keller; assignee: Biogenic Innovations, LLC; 9 June 2011) teaches enhancement or inhibition of microbial activity, including increased or more efficient fermentation of a starter material, through the use of MSM. This application is a bit broader than just an application to biofuels and biodiesel production. The inventors disclose that using about 0.5% to about 5% (by weight of the moisture content of the medium) MSM is used to enhance fermentation efficiency. MSM is used in larger amounts, such as above about 10%, to inhibit detrimental microbial activity, such as H1N1 influenza contamination. The inventors suggest that "enhancing fermentation efficiency comprises an at least 50% increase in carbon dioxide production in the presence of MSM by the microorganism" as compared to the same microorganism with no MSM present.

US Patent Publication 2011/0177564, *Bioprocess and microbe engineering for total carbon utilization in biofuel production* (inventor: Gregory Stephanopolous; assignee: Massachusetts Institute of Technology; 21 July 2011) discloses the utilization of an aerobic and anaerobic pathway to produce biofuels or biomass. The inventors use two different bioreactors to separate out the aerobic production of triacylglycerol (TAG) and the anaerobic reduction of CO_2 in the presence of hydrogen or an electric current. These two different reactors, according to the inventors, allow for each reactor to be specifically tailored to provide maximum efficiency and product recovery. For the TAG production, an oleaginous microbe is optimized. Bacteria is utilized for the anaerobic CO_2 fixation, and in some embodiments,

the bacteria is genetically modified or "pathway-engineered," such as by increasing the activity of the reductive acetyl CoA pathway in the organism. The inventors contemplate *Clostridia* as is the primary bacterium used.

Microorganism biofilms or membranes

US Patent Publication 2011/0183390, *Method of conversion of syngas using microorganism on hydrophobic membrane* (inventors: Robert Hickey, Rahul Basu, Rathin Datta, Shih-Perng Tsai; 28 July 2011) teaches the creation of and use of a microorganism biofilm on a membrane wherein a fermentation liquid interacts with the microorganisms in order to produce liquid fuel from syngas components (gases containing CO and/or a mixture of CO_2 and H_2) on the other side of the membrane. The syngas permeates the membrane and supports the growth and metabolism of the bacteria on the biofilm. The inventors state that one of the important developments in this application is that the biofilm is retained on the surface of the membrane and that there is no substantial penetration of the membrane by the biofilm. Contemplated microorganisms include *Clostridium ragsdalei, Butyribacterium methylotrophicum, Clostridium ljungdahlii, Clostridium autoethanogenum, Clostridium woodii,* and *Clostridium carboxydivorans.*

A review of the patent publications from late 2010 and the better part of 2011 indicate that fermentation of feedstocks remains a key focus in industrial biotechnology R&D. Much research is directed to modifying or blending conventional enzymes in order to boost production efficiency and yield.

Part 2 of this article (forthcoming in December 2011 issue of *Industrial Biotechnology*) will examine the growth of this area of research over the last 3-5 years, discuss trends, and bring the reader up to the current time period with respect to subject matter considered material for patent applications.

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