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**WO 03/049538 A3**

(54) Title: METHODS FOR THE COST-EFFECTIVE SACCHARIFICATION OF LIGNOCELLULOSIC BIOMASS

(57) Abstract: The invention provides methods for the cost-effective saccharification of polysaccharides in lignocellulosic biomass, particularly in crop residues. In one embodiment of the invention polysaccharide-degrading enzymes are expressed in the seeds of plants, preferably in germ (embryo) tissue of seed. Corn crop plants are used in one embodiment of the invention. The corn seeds and the corn stover are harvested concurrently in a single pass harvesting operation to lower costs. The corn seeds are fractionated which allows for additional uses for separated tissue. The endosperm can be used as a source of starch for existing industries to produce by-product credits. In an embodiment, the starch is used to produce ethanol in currently existing facilities and the tissue, preferably germ, containing the polysaccharide-degrading enzymes can be used as the enzyme source. The appropriate tissues that express polysaccharide-degrading enzymes, or extracts thereof, are combined with the corn stover and the combination is exposed to conditions favorable for the conversion of the cell wall polysaccharides in the corn stover into fermentable sugars. The fermentable sugars can then be utilized by microorganisms to produce ethanol or other desired fermentative products.

INTERNATIONAL SEARCH REPORT

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<p><b>A. CLASSIFICATION OF SUBJECT MATTER</b></p> <p>IPC(7) :C12N 15/56, 15/82; C12P 7/10, 19/00, 19/02, 19/12, 21/02          US CL :800/278, 284, 287, 320.1; 435/69.1, 96, 100, 105, 161, 165, 468          According to International Patent Classification (IPC) or to both national classification and IPC</p>																				
<p><b>B. FIELDS SEARCHED</b></p> <p>Minimum documentation searched (classification system followed by classification symbols)          U.S. : 800/278, 284, 287, 320.1; 435/69.1, 96, 100, 105, 161, 165, 468</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)</p>																				
<p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>Y</td> <td>US 5,543,576 A (VAN OOIJEN et al) 06 August 1996, col. 3, lines 37-54; col. 5, lines 7-21 and 50-63; col. 6, lines 9-13; col. 7, lines 28-34 and 51-60; col. 9, lines 36-42; col. 10, lines 13-30 and 46-51; col. 15, lines 5-60; col. 17, lines 35-54; col. 20; col. 21, line 41 through col. 22, line 18; claims 1-7, 13 and 14.</td> <td>1-20</td> </tr> <tr> <td>Y</td> <td>US 5,981,835 A (AUSTIN-PHILLIPS et al) 09 November 1999, see col. 1, lines 30-67; col. 2, lines 1-6, 15-18 and 47-57; col. 3, lines 21-34 and 59-67; col. 4, lines 1-9; col. 7, lines 47-60; col. 10, lines 32-43; col. 16, line 38 through col. 17, line 7; claims 1, 4-5, 12 and 14-17.</td> <td>7, 9-10 and 13-20</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	Y	US 5,543,576 A (VAN OOIJEN et al) 06 August 1996, col. 3, lines 37-54; col. 5, lines 7-21 and 50-63; col. 6, lines 9-13; col. 7, lines 28-34 and 51-60; col. 9, lines 36-42; col. 10, lines 13-30 and 46-51; col. 15, lines 5-60; col. 17, lines 35-54; col. 20; col. 21, line 41 through col. 22, line 18; claims 1-7, 13 and 14.	1-20	Y	US 5,981,835 A (AUSTIN-PHILLIPS et al) 09 November 1999, see col. 1, lines 30-67; col. 2, lines 1-6, 15-18 and 47-57; col. 3, lines 21-34 and 59-67; col. 4, lines 1-9; col. 7, lines 47-60; col. 10, lines 32-43; col. 16, line 38 through col. 17, line 7; claims 1, 4-5, 12 and 14-17.	7, 9-10 and 13-20									
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<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document published on or after the international filing date</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&amp;"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>"F" document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means			"F" document published prior to the international filing date but later than the priority date claimed		
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<p>Date of the actual completion of the international search</p> <p>15 APRIL 2003</p>		<p>Date of mailing of the international search report</p> <p>10 JUN 2003</p>																		
<p>Name and mailing address of the ISA/US Commissioner of Patents and Trademarks          Box PCT          Washington, D.C. 20231          Facsimile No. (703) 305-3230</p>		<p>Authorized officer</p> <p>DAVID T. FOX <i>Della Collins for</i></p> <p>Telephone No. (703) 308-0196</p>																		

## INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/39461 A1 (HOWARD, J. A.) 11 September 1998, see page 4, line 3 through page 5, line 3; page 7, line 13 through page 9, line 15; page 11, lines 3-15; page 14, line 25 through page 15, line 17; page 15, line 31 through page 16, line 8; page 18, lines 16-31; page 19, lines 21-31; page 21, lines 15-26.	5-7 and 13-20
Y	WO 99/16890 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 08 April 1999, see page 30, line 10 through page 32, bottom line.	5-6 and 14-15