

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018645 A3

(51) International Patent Classification⁷: **C12P 7/20**,
C12N 9/00, 9/24, C07H 21/04

(21) International Application Number:
PCT/US2003/026760

(22) International Filing Date: 25 August 2003 (25.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/405,896 23 August 2002 (23.08.2002) US

(71) Applicant: **E.I. DU PONT DE NEMOURS AND COMPANY** [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventor: **CAIMI, Perry, G.**; 7 Holly Drive, Kennett Square, PA 19348 (US).

(74) Agent: **FLOYD, Linda, Axiame**thy; E.I. DUPONT DE NEMOURS AND COMPANY, LEGAL PATENT RECORDS CENTER, 4417 Lancaster Pike, Wilmington, DE 19805 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
18 August 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: UTILIZATION OF STARCH PRODUCTS FOR BIOLOGICAL PRODUCTION BY FERMENTATION

(57) Abstract: This invention relates to a method for utilizing less purified starch in fermentation processes. One example is a recombinant *E. coli* containing a exogenous extracellular isoamylase activity that is capable of utilizing small oligomers containing (1, 6) linkages (including but not limited to isomaltose and panose) in fermentations to produce useful products. The invention is useful in large-scale industrial biofermentations by reducing the cost of the substrate carbohydrate.



WO 2004/018645 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/26760

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12P 7/20; C12N 9/00, 9/24; C07H 21/04

US CL : 435/69.1, 155, 159, 183, 200, 252.3, 320.1; 530/350; 536/23.2, 23.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 155, 159, 183, 200, 252.3, 320.1; 530/350; 536/23.2, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP1227152-A1 (NEST) 31 July 2002 (31.07.2002), SEQ ID NO:1101 encoding SEQ ID NO:318 in reference is 86% identical to SEQ ID NO:1 and can hybridize with SEQ ID NO:3,686,276 (LAFFEND et al.) 11 November 1997 (11.11.1997), see entire document.	1, 3
Y	EP0511393A1 (NIPPON MINING CO. LTD) 04 November 1992 (11.04.1992) see entire document specifically claim 5.	24-26, 28
Y	KRASIKOV et al. Alpha-glucosidases, Biochemistry (Moscow), March 2001, Vol. 66, No.3, pages 267-281.	24-26, 28
Y	YAMAMOTO et al. Purification and properties of an oligo-1,6-D-glucosidase from an alkalophilic Bacillus species. Carbohydrate Res., March 1990, Vol.197, pages 227-235.	24-26, 28



Further documents are listed in the continuation of Box C.



See patent family annex.

* 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 application or patent 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		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

27 May 2005 (27.05.2005)

Date of mailing of the international search report

22 JUN 2005

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner of Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Manjunath N. Rao, Ph.D.

Telephone No. 571-272-1600

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/26760

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-4, 24-26, 28, 32 SEQ ID NO:1 and 2 only

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claims 1-4, 24-26, 28, 32 drawn to an isolated polynucleotide with SEQ ID NO:1, encoding a non-chimeric polypeptide with SEQ ID NO:2 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule such as glycerol using the recombinant cell producing the polypeptide.

Group 2, claims 1-4, 24-26, 28 and 32 drawn to an isolated polynucleotide with SEQ ID NO:3, encoding a non-chimeric polypeptide with SEQ ID NO:4 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the polypeptide.

Group 3, claims 1-4, 24-26, 28 and 32 drawn to an isolated polynucleotide with SEQ ID NO:5, encoding a non-chimeric polypeptide with SEQ ID NO:6 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the polypeptide.

Group 4, claims 1-4, 24-26, 28 and 32 drawn to an isolated polynucleotide with SEQ ID NO:16, encoding a non-chimeric polypeptide with SEQ ID NO:17 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the polypeptide.

Group 5, claims 1-4, 24-26, 28 and 32 drawn to an isolated polynucleotide with SEQ ID NO:30, encoding a non-chimeric polypeptide with SEQ ID NO:31 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the polypeptide.

Group 6, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:2 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule such as glycerol using the recombinant cell producing the chimeric polypeptide.

Group 7, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:4 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 8, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:6 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 9, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:17 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 10, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:29 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 11, claims 5-23, 27, 29-31, 32, drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:31 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 12, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:33 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

INTERNATIONAL SEARCH REPORT

PCT/US03/26760

Group 13, claims 5-23, 27, 29-31, 32,33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:35 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 14, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:37 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 15, claims 5-23, 27, 29-31, 32,33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:39 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 16, claims 5-23, 27, 29-31, 32,33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:41 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 17, claims 5-23, 27, 29-31, 32, 33 drawn to an isolated polynucleotide encoding a chimeric polypeptide comprising a signal peptide with SEQ ID NO:24, 25, 26 or 27 operably linked to with SEQ ID NO:43 having α (1,6)linked glucose oligosaccharide hydrolyzing polypeptide and a method of making a target molecule using the recombinant cell producing the chimeric polypeptide.

Group 18, claim 34, drawn to a polypeptide that is 69% identical in amino acid sequence with that of SEQ ID NO:17.

The inventions listed as Groups 1-19 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, polynucleotides with SEQ ID NO:1, encoding a polypeptide with SEQ ID NO: 2, having α 1,6, glucose oligosaccharide hydrolyzing activity and methods of using said polynucleotide and polypeptide. Further pursuant to 37 C.F.R. 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

Continuation of B. FIELDS SEARCHED Item 3:

File BIOSIS, CAPLUS, EMBASE, MEDLINE, SCISEARCH, USPTO WEST, BIOTECHNO, BIOTECHABS, CANCERLIT, GENBANK,