

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
8 May 2008 (08.05.2008)

PCT

(10) International Publication Number  
WO 2008/055138 A3

- (51) **International Patent Classification:**  
C12N 9/24 (2006.01) C12N 9/00 (2006.01)
- (21) **International Application Number:**  
PCT/US2007/082968
- (22) **International Filing Date:** 30 October 2007 (30.10.2007)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
60/855,671 31 October 2006 (31.10.2006) US  
11/619,314 3 January 2007 (03.01.2007) US
- (71) **Applicant (for all designated States except US):**  
MANTROSE-HAEUSER CO., INC. [US/US]; 113  
Olive Street, Attleboro, MA 02703 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** UPPALANCHI,  
Arun, Kumar [IN/US]; 180 North Street, Foxboro,  
MA 02035 (US). GAN, Xiangdong [CN/US]; 65 East  
Washington Street, Apt #7205, North Attleboro, MA  
02760 (US). ZHONG, Bin [CN/US]; 70 Waterman Street,  
Cumberland, RI 02864 (US).
- (74) **Agent:** MOORHEAD, Sean, T.; Calfee, Halter & Gris-  
wold LLP, 1400 Keybank Center, 800 Superior Avenue,  
Cleveland, OH 44114 (US).
- (81) **Designated States (unless otherwise indicated, for every  
kind of national protection available):** AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH,  
CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG,  
ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL,  
IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,  
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,  
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL,  
PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY,  
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,  
ZM, ZW.
- (84) **Designated States (unless otherwise indicated, for every  
kind of regional protection available):** ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,  
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— with international search report
- (88) **Date of publication of the international search report:**  
14 August 2008

(54) **Title:** ENZYMATIC HYDROLYSIS OF STARCH

(57) **Abstract:** Provided herein are methods of increasing the enzymatic rate of hydrolysis of starch substrates. In certain embodiments the method comprises contacting a starch substrate with one or more amylase family enzymes in the presence of greater than 0.001 mM manganese ions. In certain embodiments, the method comprises contacting a starch substrate with one or more glucoamylase and/or  $\beta$ -amylase enzymes in the presence of greater than 0.001 mM calcium ions ( $\text{Ca}^{++}$ ). In certain embodiments, the method comprises contacting the starch substrate with a  $\beta$ -amylase in the presence of greater than 0.001 mM manganese ion, calcium ion, magnesium ion ( $\text{Mg}^{++}$ ), strontium ion ( $\text{Sr}^{++}$ ), barium ion ( $\text{Ba}^{++}$ ) or any combination of said metal ions. In certain embodiments the method comprises contacting the starch substrate with a glucoamylase in the presence of greater than 0.001 mM manganese ion, calcium ion, lithium ion ( $\text{Li}^{+}$ ), potassium ion ( $\text{K}^{+}$ ) or any combination of said metal ions. Also provided herein are compositions and kits for hydrolyzing starch.



WO 2008/055138 A3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 07/82968

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 9/24; C12N 9/00 (2008.01)

USPC - 435/183; 435/ 200

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC- 435/183, 200

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
USPC - 435/201, 202, 203, 204, 205 (text search, see terms below)

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

PubWEST(PGPB,USPT,EPAB,JPAB); Google/Scholar; PubMed (text search, see terms below)

Search Terms: alpha-amylase, beta-amylase, glucoamylase, gamma-amylase, amyloglucosidase, starch, alpha-amylase, amylopectin, hydrolysis, hydrolyze, liquefy, liquefying, manganese, calcium.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,113,509 A (LEACH et al.) 12 September 1978 (12.09.1978), particularly (col 2, ln 29-30, 45-61), (col 3, ln 4-8, 42), (col 4, ln 38-55), (col 5, ln 10-13, 59-63), (col 6, ln 59-66), (col 8, ln 60-67), (col 9, ln 36-41), (col 11, ln 40).	25, 35, 46-49
Y		1-24, 26-34, 36-45, 50-53
Y	Selvakumar et al. Purification and characterization of glucoamylase produced by <i>Aspergillus niger</i> in solid state fermentation. Letters in Applied Microbiology, 1996, vol 23, pp 403-406, particularly Abstract, Table 2, (page 403, para 1), (page 405, para 1).	1-24, 26-34, 36-45, 50-53
Y	Zhao et al. Molecular cloning, characterization, and differential expression of a glucoamylase gene from the Basidiomycetous fungus <i>Lentinula edodes</i> . Applied and Environmental Microbiology, June 2000, vol 66, pp 2531-2535, particularly Abstract.	24

 Further documents are listed in the continuation of Box C.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

07 March 2008 (07.03.2008)

Date of mailing of the international search report

09 MAY 2008

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450  
Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774