

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
14 August 2003 (14.08.2003)

PCT

(10) International Publication Number
WO 2003/066826 A3

- (51) International Patent Classification⁷: **C12P 07/06**, 07/16, C12N 1/20, 15/00
- (21) International Application Number: PCT/US2003/003670
- (22) International Filing Date: 6 February 2003 (06.02.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/355,180 8 February 2002 (08.02.2002) US
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- (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, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, 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, 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: 3 March 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.*



WO 2003/066826 A3

(54) Title: METHODS FOR PRODUCING ETHANOL FROM CARBON SUBSTRATES

(57) Abstract: The present invention provides means for the production of desired end-products of in vitro and/or in vivo bioconversion of biomass-based feed stock substrates, including but not limited to such materials as starch and cellulose. In particularly preferred embodiments, the methods of the present invention do not require gelatinization and/or liquefaction of the substrate. In particularly preferred embodiments, the present invention provides means for the production of ethanol.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/03670

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12P 07/06, 07/16; C12N 1/20, 15/00
 US CL : 435/160, 161, 183, 252.3, 320.1

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. : 435/160, 161, 183, 252.3, 320.1

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)
 CAS STN, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INGRAM et al. Genetic engineering of ethanol production in Escherichia coli. Appl Environ Microbiol. October 1987. Vol. 53, No. 10, pgs. :2420-5.	1, 2, 4, 6, 8-11, 15-20
X	OHTA et al. Genetic improvement of Escherichia coli for ethanol production: chromosomal integration of Zymomonas mobilis genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. Appl Environ Microbiol. April 1991. Vol. 57, No.4, pgs.:893-900.	1, 2, 4, 6, 8-11, 15-20
X	ARFMAN et al. Use of the tac promoter and lacIq for the controlled expression of Zymomonas mobilis fermentative genes in Escherichia coli and Zymomonas mobilis. J Bacteriol. November 1992, Vol. 174, No.22. pgs.7370-7378.	1, 2, 4, 6, 8-11, 15-20
A	CONWAY et al. Promoter and nucleotide sequences of the Zymomonas mobilis pyruvate decarboxylase. J Bacteriol. March 1987 Vol.169, NO.3, pgs.949-54.	1-21
A	OHTA et al. Metabolic engineering of Klebsiella oxytoca M5A1 for ethanol production from xylose and glucose. Appl Environ Microbiol. October 1991., Vol. 10, pgs. 2810-5.	1-21

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"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: 13 November 2004 (13.11.2004)
 Date of mailing of the international search report: 22 DEC 2004

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/03670

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.: 1-21

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.

- I. Claims 1-21, drawn to a method for producing an alcohol by contacting a carbon substrate with at least one substrate-converting enzyme to produce an intermediate and contacting the intermediate with at least one intermediate-converting enzyme, wherein said intermediate is substantially all converted by said intermediate enzyme to said alcohol.
- II. Claims 22-37, drawn to a method for producing an alcohol by contacting a carbon substrate with at least one substrate-converting enzyme to produce an intermediate and contacting the intermediate with at least one intermediate-converting enzyme, wherein said intermediate is substantially all converted by said intermediate enzyme to said alcohol end-product, and wherein the presence of said alcohol end-product does not further inhibit production of said alcohol end-product.

The inventions listed as Groups I and II 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:

The technical feature shared by inventions of Groups I and II is the production of alcohol. However, this technical feature has already been taught in the prior art by Ingram et al. (Genetic engineering of ethanol production in *Escherichia coli*. Appl Environ Microbiol. 1987 Oct; 53(10):2420-5).

Ingram et al. teach genetically engineered *Escherichia coli* transformed with the *Zymomonas mobilis* gene encoding pyruvate decarboxylase, which catalyzes the decarboxylation of pyruvate to acetaldehyde and carbon dioxide, and the gene encoding alcohol dehydrogenase II, which catalyzes the reduction of acetaldehyde to ethanol (see p. 2420, left column, 3rd paragraph); and a method for producing ethanol using said genetically engineered *Escherichia coli* (see pp. 2421-2423). Thus, the Ingram et al. reference anticipate claim 1 and teach the technical feature shared by inventions of Groups I and II.

Thus, the technical feature is not special since it was known in the prior art and therefore cannot make a contribution over the prior art.