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[Continued on next page]

(54) Title: FERMENTATIVE PRODUCTION OF ALCOHOLS

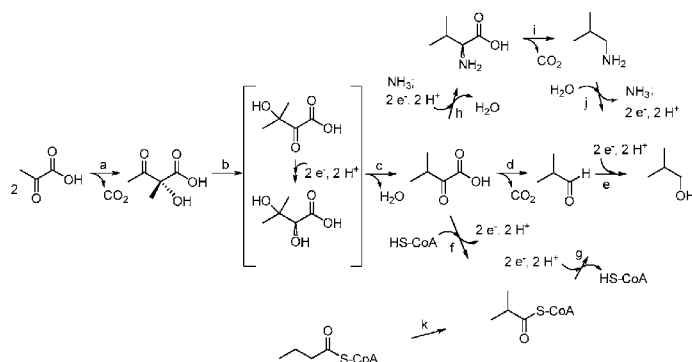


FIG. 3

(57) Abstract: The invention relates to the fields of industrial microbiology and alcohol production. The invention also relates to the development of a microorganism capable of producing fermentation products via an engineered pathway, and uses of the microorganism. A process for producing butanol comprising the following steps: (a) providing a butanologen, (b) contacting the butanologen with one or more carbon substrates under conditions wherein butanol is produced at an effective yield; (c) collecting the butanologen; (d) recovering butanol at a concentration of at least about 6 g/L; (e) contacting the collected butanologen of (c) with one or more carbon substrates under conditions wherein butanol is produced at an effective yield and wherein the effective yield is at least about 90% of the effective yield of (b); (f) repeating steps (c)-(e); and, optionally exposing the collected butanologen of (c) to conditions of pH less than or equal to about 2.0 for at least about 1 hour in the presence of at least about 0.3% butanol. The invention also relates to the methods to improve cell viability and productivity and the use of recycling and acid washing to increase the yield of fermentation

products.



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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24 October 2013

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/072079

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P7/16

ADD.

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)

C12P

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)

EP0-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/159962 A1 (BUTAMAX TM ADVANCED BIOFUELS [US]; BURLEW KEITH H [US]; DICOSIMO ROBER) 22 December 2011 (2011-12-22)	1-3,5,6, 9-11,13, 18-25
Y	[00130]-[00133]; page 33, line 24 - line 29; figure 3 page 45, line 21 - line 25 -----	4,7,8,26
X	WO 2011/097711 A1 (IOGEN ENERGY CORP [CA]; TOLAN JEFFREY S [CA]; FOODY BRIAN [CA]) 18 August 2011 (2011-08-18) [0072]-[0074],[0078]-[0080],[0082] ----- -/-	1,3, 9-11, 18-25

☒ 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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

8 May 2013

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03/09/2013

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/072079

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	XIAOPING YANG ET AL: "Enhanced acetone-butanol fermentation using repeated fed-batch operation coupled with cell recycle by membrane and simultaneous removal of inhibitory products by adsorption", BIOTECHNOLOGY AND BIOENGINEERING, WILEY & SONS, HOBOKEN, NJ, US, vol. 47, no. 4, 20 August 1995 (1995-08-20), pages 444-450, XP002505045, ISSN: 0006-3592, DOI: 10.1002/BIT.260470405 page 444, column 1, paragraph 4 - column 2, paragraph 1; table 1 -----	4
Y	STEEN ERIC J ET AL: "Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol", MICROBIAL CELL FACTORIES, BIOMED CENTRAL, LONDON, NL, vol. 7, no. 1, 3 December 2008 (2008-12-03), page 36, XP021049875, ISSN: 1475-2859, DOI: 10.1186/1475-2859-7-36 page 2 of 8, column 2, paragraph 2 - paragraph 3; figure 1 -----	7,8
Y	CHEN XIAO ET AL: "Increased isobutanol production in Saccharomyces cerevisiae by overexpression of genes in valine metabolism.", BIOTECHNOLOGY FOR BIOFUELS 2011, vol. 4, 21, 28 July 2011 (2011-07-28), page 12PP, XP002696693, ISSN: 1754-6834, DOI: 10.1186/1754-6834-4-21 page 3 of 12, column 1, paragraph 3 - column 2, paragraph 3; figures 2,4 -----	26
A	EZEJI T C ET AL: "Acetone butanol ethanol (ABE) production from concentrated substrate: Reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 63, no. 6, 1 February 2004 (2004-02-01), pages 653-658, XP002512560, ISSN: 0175-7598, DOI: 10.1007/S00253-003-1400-X [retrieved on 2003-08-09] the whole document ----- -/--	1-27

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/072079

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ATSUMI S ET AL: "Metabolic engineering of Escherichia coli for 1-butanol production", METABOLIC ENGINEERING, ACADEMIC PRESS, US, vol. 10, no. 6, 1 November 2008 (2008-11-01), pages 305-311, XP025800632, ISSN: 1096-7176, DOI: 10.1016/J.YMBEN.2007.08.003 [retrieved on 2008-11-01] the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/072079

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims 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. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional 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 fees, this Authority did not invite payment of additional fees.
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.:

1-27

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27

A process for producing butanol comprising:

- (a) providing a butanologen,
- (b) contacting the butanologen with one or more carbon substrates under conditions wherein butanol is produced at an effective yield;
- (c) collecting the butanologen;
- (d) recovering butanol at a concentration of at least about 6 g/L;
- (e) contacting the collected butanologen of (c) with one or more carbon substrates under conditions wherein butanol is produced at an effective yield and wherein the effective yield is at least about 90% of the effective yield of (b);
- (f) repeating steps (c)-(e); and, optionally exposing the collected butanologen of (c) to conditions of pH less than or equal to about 2.0 for at least about 1 hour in the presence of at least about 0.3% butanol.

2. claims: 28-59

A composition having pH of less than about 2 and comprising a sucrose utilizing isobutanologen comprising an engineered isobutanol pathway and a microorganism comprising reduced or eliminated phosphodiesterase and/or phosphodiesterase activity, wherein the microorganism produces butanol.

3. claims: 60-71

Methods for improving cell viability and productivity, processes for producing an alcohol and a composition comprising a nutrient rich medium.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/072079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011159962 A1	22-12-2011	AR 083153 A1	06-02-2013
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