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<i>C12P 7/04</i> (2006.01)	<i>C12P 13/00</i> (2006.01)
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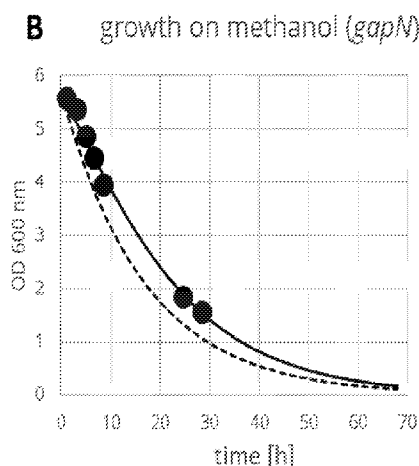
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: ENGINEERED MICROORGANISMS WITH G3P----> 3PG ENZYME AND/OR FRUCTOSE-1,6-BISPHOSPHATASE INCLUDING THOSE HAVING SYNTHETIC OR ENHANCED METHYLOTROPHY

Fig. 10



(57) Abstract: Described herein are engineered cells including ones having synthetic methylo-trophy which include an NADH-dependent enzyme capable of converting G3P to 3PG (e.g., *6. methanolicus gapN*) and/ or fructose-1, 6-bisphosphatase, along with hexulose-6-phosphate synthase, 6-phospho-3-hexuloisomerase, a phosphoketolase, or a combination thereof. Engineered cells of the disclosure beneficially maintain adequate pool sizes of phosphorylated C3 and/or C4 compounds, and/or provide increased levels of NADPH. As such, the modifications allow for the generation of C6 compounds from C1 (e.g. a methanol feedstock) and C5 compounds, the regeneration of C5 compounds from C6 compounds by carbon rearrangement, and an improved balance between regeneration of C5 compounds and lower glycolysis.



**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

**(88) Date of publication of the international search report:**  
06 February 2020 (06.02.2020)

**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/US2019/039207

A. CLASSIFICATION OF SUBJECT MATTER					
INV.	C12P5/00	C12P5/02	C12P7/04	C12P7/16	C12P7/18
	C12P7/24	C12P7/40	C12P7/42	C12P7/46	C12P7/64
	C12P13/00	C12N9/02	C12N9/16	C12N9/88	C12N9/90
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 C12N

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)  
EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, IBM-TDB, 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 2015/108777 A1 (PAPOUTSAKIS, E. ET AL.) 23 July 2015 (2015-07-23)  the whole document see especially: page 21, lines 8-19  -----	1-4, 6-23, 26-84
A	WO 2014/153036 A1 (REGENTS OF THE UNIVERSITY OF CALIFORNIA) 25 September 2014 (2014-09-25) page 2, paragraph 5 - page 8, paragraph 10 pages 77-86; claims 1-7, 11-15, 28, 38-46, 69  -----  -/--	1-4, 6-23, 26-84

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 but 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  16 October 2019	Date of mailing of the international search report  10/01/2020
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Fuchs, Ulrike
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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2019/039207

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>KOCHARIN, K. ET AL.: "Improved Polyhydroxybutyrate Production by Saccharomyces cerevisiae Through the Use of the Phosphoketolase Pathway", BIOTECHNOLOGY AND BIOENGINEERING, vol. 110, no. 8, 26 August 2013 (2013-08-26), pages 2216-2224, XP055101987, DOI: 10.1002/bit.24888 abstract page 2217, column 1, line 20 - column 2, line 24 page 2218; table 1 strain SCKK035 page 2221, column 1, line 1 - page 2222, column 2, line 6; figures 4, 5</p> <p style="text-align: center;">-----</p>	<p>1-4, 6-23, 26-84</p>
A	<p>DATABASE UniProt [Online]</p> <p>28 February 2018 (2018-02-28), IRLA, M. ET AL.: "Full=NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, EC=1.2.1.9", XP002794966, Database accession no. I3EBG2 cited in the application the whole document</p> <p style="text-align: center;">-----</p>	<p>1-4, 6-23, 26-84</p>

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/039207

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a.  forming part of the international application as filed:

in the form of an Annex C/ST.25 text file.

on paper or in the form of an image file.

b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c.  furnished subsequent to the international filing date for the purposes of international search only:

in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2019/039207

## 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.:

1-4, 6-23, 26-84(all partially)

### 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: 1-4, 6-23, 26-84(all partially)

engineered microorganism having synthetic or enhanced methylotrophy comprising:  
 (a) exogenous enzyme A that (ai) is capable of converting glyceraldehyde 3-phosphate (G3P) to 3-phosphoglycerate (3PG), or (aii) has at least 50% sequence identity to SEQ ID NO: 1 (B. methanolicus gapN), wherein (ai) or (aii) is capable of reducing NADP to NADPH, and  
 (b) an exogenous enzyme B which is (bi) a phosphoketolase, (bii) a hexulose-6-phosphate synthase, (biii) 6-phospho-3-hexuloisomerase, or any combination of (bi), (bii) and (biii)  
 method for producing a bioderived compound, comprising culturing engineered microorganism of any one of claims 68-80 under conditions and for a sufficient period of time to produce said bioderived compound  
 method for growing a non-natural microbial organism comprising culturing engineered microorganism of any one of the claims 1-80 in a medium comprising carbon-containing feedstock that comprises methanol

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2. claims: 24, 25(completely); 1-4, 6-23, 26-84(partially)

engineered microorganism having synthetic or enhanced methylotrophy comprising:  
 (a) exogenous enzyme A that (aiiui) is a fructose-1,6-bisphosphatase, and  
 (b) an exogenous enzyme B which is (bi) a phosphoketolase, (bii) a hexulose-6-phosphate synthase, (biii) 6-phospho-3-hexuloisomerase, or any combination of (bi), (bii) and (biii)  
 method for producing a bioderived compound, comprising culturing engineered microorganism of any one of claims 68-80 under conditions and for a sufficient period of time to produce said bioderived compound  
 method for growing a non-natural microbial organism comprising culturing engineered microorganism of any one of the claims 1-80 in a medium comprising carbon-containing feedstock that comprises methanol

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3. claims: 5(completely); 6-23, 26-81, 83, 84(partially)

engineered microorganism comprising an exogenous enzyme that (i) is capable of converting glyceraldehyde 3-phosphate (G3P) to 3-phosphoglycerate (3PG), or (ii) has at least 50% sequence identity to SEQ ID NO: 1 (B. methanolicus gapN), wherein enzyme A is capable of reducing NADP to NADPH  
 (b) an exogenous enzyme B which is (bi) a phosphoketolase, (bii) a hexulose-6-phosphate synthase, (biii)

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

6-phospho-3-hexuloisomerase, or any combination of (bi),  
(bii) and (biii)  
method for producing a bioderived compound, comprising  
culturing engineered microorganism of any one of claims  
68-80 under conditions and for a sufficient period of time  
to produce said bioderived compound

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/039207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015108777 A1	23-07-2015	US 2016333308 A1	17-11-2016
		US 2019048310 A1	14-02-2019
		WO 2015108777 A1	23-07-2015
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WO 2014153036 A1	25-09-2014	US 2016017339 A1	21-01-2016
		WO 2014153036 A1	25-09-2014
		WO 2014153207 A2	25-09-2014
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