



- (51) **International Patent Classification:**
C12P 5/00 (2006.01) *C07H 21/00* (2006.01)
- (21) **International Application Number:**
PCT/US2012/051925
- (22) **International Filing Date:**
22 August 2012 (22.08.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/526,178 22 August 2011 (22.08.2011) US
- (71) **Applicant (for all designated States except US):** **JOULE UNLIMITED TECHNOLOGIES, INC.** [US/US]; 18 Crosby Drive, Bedford, MA 01730 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** **REPPAS, Nikos, Basil** [US/US]; c/o Joule Unlimited Technologies, Inc., 18 Crosby Drive, Bedford, MA 01730 (US). **RIDLEY, Christian, Perry** [US/US]; c/o Joule Unlimited Technologies, Inc., 18 Crosby Drive, Bedford, MA 01730 (US). **DEARBORN, Amy** [US/US]; c/o Joule Unlimited Technologies, Inc., 18 Crosby Drive, Bedford, MA 01730 (US).
- (74) **Agents:** **HURT, Nicholas, S.** et al.; Fenwick & West LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (US).
- (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, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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.
- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title:** IMPROVED COMPOSITIONS AND METHODS FOR THE BIOSYNTHESIS OF 1-ALKENES IN ENGINEERED MICROORGANISMS

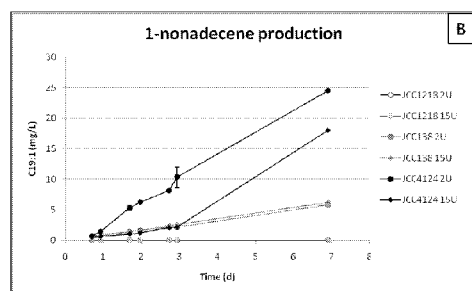
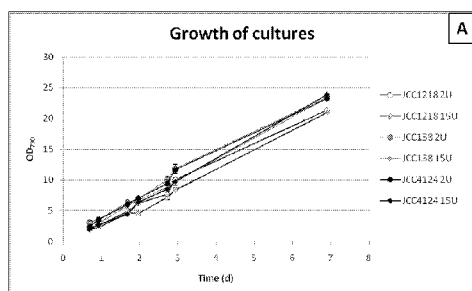


Figure 4

(57) **Abstract:** Various 1-alkenes, including 1-nonadecene and 1-octadecene, are synthesized by the engineered microorganisms and methods of the invention. In certain embodiments, the microorganisms comprise a recombinant alpha-olefin-associated enzyme. This enzyme may be expressed in combination with a recombinant alkene synthase pathway-related gene. The engineered microorganisms may be photosynthetic microorganisms such as cyanobacteria.



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:
18 April 2013

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/51925

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P 5/00; C07H 21/00 (2012.01)

USPC - 435/166; 536/23.2

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)

IPC(8): C12P 5/00; C07H 21/00 (2012.01)

USPC: 435/166; 536/23.2

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)

PubWEST (DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents, PatBase

Search Terms Used: hydrocarbon\$, alkene\$, cyanobacter\$, Synechococcus, 1-alkene synthase, nirA, urea, repress\$, 1,12-nonadecadiene, nonadecadiene, nonadecadienoic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0330642 A1 (RIDLEY et al.) 30 December 2010 (30.12.2010) esp; abstract paras [0004], [0007]-[0009], [0012]-[0016], [0022], [0028], [0029], [0110], [0224], [0225], SEQ ID NO: 2; Figs. 5, 6; Tables 2, 3; claims 1, 9.	1-5, 14-16, 19-23, 25-27 ----- 17, 18, 28 ----- 24
Y	US 2011/0111470 A1 (BERRY et al.) 12 May 2011 (12.05.2011) esp; abstract, para [0021], [0052], [0165], [0206], [0208], Table 13, SEQ ID NO: 16.	17, 18, 28
A	Mendez-Perez et al. "Modular Synthase-Encoding Gene Involved in a-Olefin Biosynthesis in Synechococcus sp. Strain PCC 7002" Appl. Environ. Microbiol.; 29 April 2011; Vol. 77, No. 12; pg. 4264-4267. esp: pg 4265 second column, last paragraph, Fig. 1, Table 2.	24
A	Li et al. NCBI reference sequence YP_001734428.1 polyketide synthase [Synechococcus sp. PCC 7002] 09 March 2011 (available at < http://www.ncbi.nlm.nih.gov/protein/170077790?sat=15&satkey=1709135 >); downloaded on 12 November 2012	1-5, 14-28
A	Qi et al. "Application of the Synechococcus nirA Promoter To Establish an Inducible Expression System for Engineering the Synechocystis Tocopherol Pathway"; Appl. Environ. Microbiol.; 2005; Vol. 71, No. 10; pg.5678-5684. entire article.	28
X	US 2011/0009674 A1 (Reppas et al.) 13 January 2011 (13.01.2011) entire document.	1-5
X, P	US 2012/0208253 A1 (Ridley et al.) 16 August 2012 (16.08.2012) entire document.	1-5, 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

"&" document member of the same patent family

Date of the actual completion of the international search

22 January 2013 (22.01.2013)

Date of mailing of the international search report

08 FEB 2013

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/51925

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Smith et al. Structure and composition of hydrocarbons and fatty acids from a marine blue-green alga, <i>Synechococcus</i> sp. BBA Lipids and Lipid Metab. 1982 vol 710 iss. 3, pgs 485-492. abstract only.	24
A	US 2010/0105955 A1 (ALIBHAI et al.) 29 April 2010 (29.04.2010) entire document	1-5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/51925

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:

This application contains the following inventions or groups of inventions which are not so linked 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 I+ claims 1-28 directed to a method for the biosynthetic production of 1-alkenes. The first invention is restricted to the method wherein said recombinant 1-alkene synthase is at least 90% identical to YP_001734428 from *Synechococcus* sp. PCC 7002. (Applicant may elect other recombinant 1-alkane-synthase(s) to be searched by paying an additional fee for each election. For example, applicant may elect the method wherein said recombinant alpha-olefin enzyme is at least 90% identical to SEQ ID NO: 11. Further, if applicable, applicant may designate one nucleic acid to be searched which corresponds to the polypeptide elected. For example, if applicant elects polypeptide SEQ ID NO: 13, applicant may also elect the corresponding nucleic acid which codes for the elected polypeptide (presumably polynucleotide SEQ ID NO: 12, for example) for no additional fee).

- Please see extra sheet for continuation -

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 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.: 1-5 and 14-28; limited to a sequence at least 90% identical to YP_001734428

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/51925

Continuation of Box III: Lack of Unity of Invention

Group II+ claims 29-47 directed to an isolated or recombinant polynucleotide; wherein the first invention is limited to SEQ ID NO: 6 or a degenerate variant thereof. (Applicant may elect any isolated polynucleotide to be searched by paying an additional fee for each election from primary claim 29. For example, applicant may elect a nucleic acid sequence that is at least 85% identical to SEQ ID NO: 12. Further, applicant must designate, for dependent claims 33, 34, and 37, the specific vector sequence to be elected. Each nucleic acid sequence/vector combination will be considered a separate, electable invention).

Group III: claim 48, directed to a method for identifying a modified gene that improves 1-alkene synthesis.

The inventions listed as Groups I+ to III do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated as above. The shared technical feature of all the Groups is a polynucleotide involved in 1-alkene biosynthesis. However, this common technical element does not represent an improvement over the prior art of US 2010/0330642 A1 to Ridley et al. (hereinafter Ridley), which teaches a polynucleotide involved in 1-alkene biosynthesis (abstract, paras [0004], [00907], [0010], [0011]). Further to Group I+, Ridley teaches a method for the biosynthetic production of 1-alkenes (abstract, para [0004], [0007]), comprising culturing an engineered microorganism in a culture medium (para [0014], claim 1), wherein said engineered microorganism comprises a recombinant alpha-olefin-associated enzyme (para [0007], [0008]), wherein said engineered microorganism produces 1-alkenes (para [0004]), and wherein the amount of said 1-alkenes produced by said engineered microorganism is greater than the amount that would be produced by an otherwise identical microorganism, cultured under identical conditions, but lacking said recombinant alpha-olefin-associated enzyme (para [0014]) wherein said engineered microorganism is a cyanobacterium and said cyanobacterium is a *Synechococcus* species (para [0022]) wherein said engineered microorganism comprises a recombinant 1-alkene synthase (para [0004], [0012], [0013]) wherein said recombinant 1-alkene synthase is at least 90% identical to YP_001734428 from *Synechococcus* sp. PCC 7002 (SEQ ID NO: 2 at 100% homology. Note that claimed enzyme YP_001734428 is identical to claimed enzyme SEQ ID NO: 5. See NCBI Reference Sequence: YP_001734428.1 available at <<http://www.ncbi.nlm.nih.gov/protein/170077790?sat=15&satkey=1709135>>). The Groups are different because the different polypeptide sequences represented by YP_001734428, YP_0001735499, YP_003887108.1, YP_002377175, ZP_08425909.1, ZP_08432358, YP_003265309, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 19 are different structures that are not common to one another but are different because they are composed of unique amino acid sequences. Likewise, the different polynucleotide sequences represented by SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18 are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences. Group II+ requires an isolated or recombinant polynucleotide which is not required by the other Groups. The Groups are different because the different polynucleotide sequences represented by SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18 are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences. Group III requires identifying a polynucleotide sequence expressing an enzyme involved in 1-alkene biosynthesis, expressing said enzyme from a recombinant form of the polynucleotide sequence in a host cell and screening the host cell for increased activity of said enzyme or increased production of 1-alkene, none of which is required by any of the other Groups.

Therefore the inventions listed as Groups I+ to III do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-28 restricted to the method wherein said recombinant 1-alkene synthase is at least 90% identical to YP_001734428 from *Synechococcus* sp. PCC 7002 (specifically claims 1-5 and 14-28) without additional fees. (If it is the case that the polypeptide of YP_001734428 is encoded by any of the claimed nucleic acid sequences, applicant must so indicate and said nucleic acid sequence with specificity and said nucleic acid sequence will be searched as appropriate with the first invention for no additional fee). In order for other inventions to be examined, the appropriate examination fees must be paid.