

(51) International Patent Classification:
C12P 7/64 (2006.01)(21) International Application Number:
PCT/US2012/024358(22) International Filing Date:
8 February 2012 (08.02.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/440,686 8 February 2011 (08.02.2011) US
61/442,261 13 February 2011 (13.02.2011) US
61/532,745 9 September 2011 (09.09.2011) US(71) Applicant (for all designated States except US): **PHYCAL INC.** [US/US]; 51 Alpha Park Drive, Highland Heights, Ohio 44143 (US).

(72) Inventor; and

(71) Applicant : **ABAD, Mark, Scott** [US/US]; 118 Glen Road, Webster Groves, Missouri 63119-2431 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **POSTIER, Bradley, Lynn** [US/US]; 118 Glen Road, Webster Groves, Missouri 63119-2431 (US). **SWANSON, Andrew, Keith** [CA/US]; 2764 Berkshire Rd, Cleveland Heights, Ohio 44106 (US). **LAUDERDALE, George, William** [US/US]; 3822 Braidwood Dr., Fort Collins, Colorado 80524 (US). **KESSLER, Ben, Ami** [US/US]; 2435 Overlook Road, Apt. 30, Cleve-land Heights, Ohio 44106 (US). **ALLNUTT, F C Thomas** [US/US]; 14692 Mustang Path, Glenwood, Maryland 21738 (US). **PARK, Jonathan, D.** [US/US]; 201 North Street, Chardon, Ohio 44024 (US). **SPOO-CHUPKA, Margaret** [US/US]; 253 High Street, Chagrin Falls, Ohio 44022 (US). **SLOMCZYNSKI, David, Joseph** [US/US]; 5105 Dogwood Trail, Lyndhurst, Ohio 44124 (US). **KWI-ATKOWSKI, Jason, Richard** [US/US]; 3356 E Fairfax Rd, Cleveland Heights, Ohio 44118 (US).(74) Agent: **MIZER, Susan, L.**; Tucker Ellis & West LLP, 925 Euclid Avenue, 1150 Huntington Building, Cleveland, Ohio 44115 (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, 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, MD, 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,

[Continued on next page]

(54) Title: METHODS FOR IMPROVED MIXED TROPHIC ALGAL CULTURE

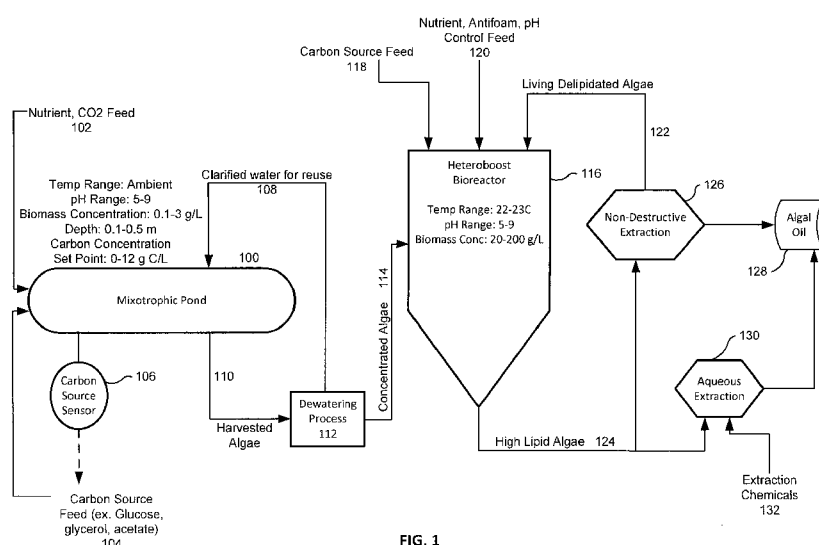


FIG. 1

(57) Abstract: The subject application is directed to methods, designs, and tools for improving the production of bioproducts from photosynthetic organisms using multiple trophic states in combination to achieve better production of a bioproduct or series of bioproducts. The subject application provides cost improvements with production of these bioproducts in a heterotrophic boost step in an enclosed bioreactor fed by a fixed carbon source as an intermediate within the production process.



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).

— *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))*

Published:

— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:

8 November 2012

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24358

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P 7/64 (2012.01)

USPC - 435/134; 435/289.1

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)

IPC(8): C12P 7/64 (2012.01)

USPC: 435/134; 435/289.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 435/257.1; 435/257.2; 435/252.1; 435/254.1; 554/124

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

PubWEST (PGPB,USPT,EPAB,JPAB); Google Patents; Google Scholar; esp@cenet: algae, bioreactor, microalgae, chlorella, vessel, hydrolysate, syrup, crude, raw, triacylglyceride, energy, glucose, light, air, sunlight, lipid, density, harvest, inhibitor, low density, high density, mass culture, inhibitor, Phycal, Bradley Postier, Thomas Allnutt

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0298159 A1 (WU et al.) 3 December 2009 (03.12.2009), abstract; para [0015], [0017], [0021], [0022], [0032], [0034], [0049], [0050], [0051], [0058], [0066], [0067], [0068], [0096], [0097].	1-14
Y	US 2010/0184197 A1 (DONG et al.) 22 July 2010 (22.07.2010), para [0003], [0006], [0057].	1-14
Y	WO 2010/009284 A2 (MUCH) 21 January 2010 (21.01.2010), abstract; pg 11, ln 5-8; pg 11, ln 28-36; Fig 2.	8, 9
Y	US 6,607,900 B2 (BAILEY et al.) 19 August 2003 (19.08.2003), abstract; col 3, ln 42-44; col 5, ln 19-21.	12
Y	WO 2010/132413 A1 (SWANSON et al.) 18 November 2010 (18.11.2010), para [0007], [0024].	14



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

25 July 2012 (25.07.2012)

Date of mailing of the international search report

10 SEP 2012

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 12/24358

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 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.

Group I: Claims 1-14, drawn to a method for the production of a bioproduct by growing a biomass organism.

Group II: Claims 15-21, drawn to a method for the production of a bioproduct by growing a recombinant organism.

Group III: Claims 22-28 and 36, drawn to a method for the production of a bioproduct wherein the method comprises introducing a crude biomass hydrolysate containing growth inhibitors into a bioreactor

---please see continuation in extra 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 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.:
1-14

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.

Continuation of Box No. III Observations where unity of invention is lacking

Group IV: Claims 29-35, drawn to a method for the production of a bioproduct wherein the method comprises concentrating the biomass by filtration or microfiltration

Group V: Claims 37-52, drawn to methods for production of an algal biomass

The inventions listed as Groups I-V 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 shared technical feature of the inventions listed as Groups I-V is the production of a bioproduct by growing a biomass organism. This shared technical feature fails to provide a contribution over the prior art, as evidenced by US 2009/0298159 A1 to Wu et al. (published 3 December 2009; hereinafter 'Wu'). Wu discloses a method for the production of a bioproduct (abstract), wherein the method comprises: growing a biomass organism in a mass culture system at a low density (para [0017]); harvesting the biomass organism (para [0049]); introducing the harvested biomass organism into a bioreactor at a high density (para [0050], [0068]); introducing a fixed carbon source into the bioreactor (para [0051]); and generating a bioproduct in the bioreactor (para [0066], [0067]). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

The shared technical feature of the inventions listed as Groups I and III is harvesting of a biomass organism, wherein the harvesting is performed so as not to damage or to minimize damage to the biomass organism. This shared technical feature fails to provide a contribution over the prior art, as evidenced by US 2010/0184197 A1 to Dong et al. (published 22 July 2010; hereinafter 'Dong'). Dong discloses harvesting of a biomass organism (para [0006] - "methods for harvesting biological materials, such as, for example microalgal cells"; para [0003] - "as global energy demands grow, the need for renewable and sustainable biofuels has become urgent, and thus simple and effective methods for harvesting biological materials are desired"), wherein the harvesting is performed so as not to damage or to minimize damage to the biomass organism (para [0057] - "Therefore, the harvesting method disclosed herein did no harm to the microalgae cells"). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

Further, the special technical feature of the inventions listed as Group II is a recombinant organism. This special technical feature is not shared by the inventions of Groups I, III-V. The special technical feature of the inventions listed as Group III is a crude biomass hydrolysate containing growth inhibitors. This special technical feature is not shared by the inventions of Groups I-II and IV-V. The special technical feature of the inventions listed as Group IV is the concentration of biomass by filtration or microfiltration. This special technical feature is not shared by the inventions of Groups I-III and V. The special technical feature of the inventions listed as Group V is the production of an algal biomass. This special technical feature is not shared by the inventions of Groups I-IV.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. Without a shared special technical feature, the inventions of Groups I-V lack unity with one another under PCT Rule 13.