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(71) Applicant: ENVIRONMENTAL BIOORGANIC SCIENCES CORPORATION [US/US]; 1980 Festival Plaza Drive, Las Vegas, Nevada 89135 (US).

(72) Inventors: DICKERSON, James Rodney; c/o 1980 Festival Plaza Drive, Las Vegas, Nevada 89135 (US). BURBANK, Malcolm; c/o 1980 Festival Plaza Drive, Las Vegas, Nevada 89135 (US). ROGERS, Valerie; c/o 1980 Festival Plaza Drive, Las Vegas, Nevada 89135 (US).

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(54) Title: MICROBIAL COMPOSITIONS AND METHOD FOR PRODUCING THEREOF FOR USE IN TREATMENT OF CONTAMINATED SOIL, WATER, AND/OR SURFACES

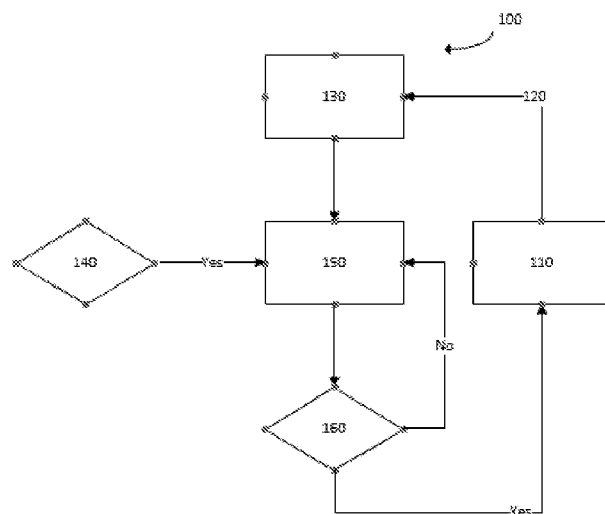


Figure 1

(57) Abstract: There is provided microbial compositions and methods for producing thereof and use of compositions thereof in treatment of contaminated soil, water, and/or surfaces. In one aspect, there is provided method for reducing microbial contamination of a microbial contaminated body, the method comprises: inactivating resident vegetative microbiology from an extract obtained from a contaminated of body to inactivate the resident vegetative microbiology in the extract, selecting one or more soil-based microbes suitable for growth in the contaminated body, growing the one or more soil-based microbes with the inactivated extract to allow the one or more soil-based microbes to adapt to the inactivated extract, releasing the one or more soil-based microbes into the contaminated body where the one or more soil-based microbes dominate and reduce microbial contamination of the microbial contaminated body.



SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER

INV. **C12N1/20 C12N1/36 B09C1/10 B09B3/00 C02F3/34**
C05F11/08 A61K35/74 A62D3/02

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)

C12N B09C C02F C05G C05F A61K A62D B09B

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG R. ET AL: "Enhanced removal of Microcystis bloom and microcystin-LR using microcosm constructed wetlands with bioaugmentation of degrading bacteria", CHEMOSPHERE, vol. 210, 26 June 2018 (2018-06-26), pages 29-37, XP085468373, ISSN: 0045-6535, DOI: 10.1016/J.CHEMOSPHERE.2018.06.140 the whole document ----- -----	1-6

☒ 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

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13/01/2022

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

van de Kamp, Mart

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2021/056313

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUANG J. ET AL: "Suppression of Fusarium wilt of banana by combining acid soil ameliorant with biofertilizer made from <i>Bacillus velezensis</i> H-6", EUROPEAN JOURNAL OF PLANT PATHOLOGY, vol. 154, 8 February 2019 (2019-02-08), pages 585-596, XP036829517, ISSN: 0929-1873, DOI: 10.1007/S10658-019-01683-5 [retrieved on 2019-02-08] the whole document -----	1
A	WO 2018/081441 A1 (NUTECH VENTURES [US]) 3 May 2018 (2018-05-03) the whole document -----	1
A	ISLAM F. ET AL: "Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation", ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH, vol. 23, no. 1, 21 September 2015 (2015-09-21), pages 220-233, XP035937306, ISSN: 0944-1344, DOI: 10.1007/S11356-015-5354-1 [retrieved on 2015-09-21] the whole document -----	1
A	LÜRLING M. ET AL: "Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation", WATER RESEARCH, vol. 47, 30 August 2013 (2013-08-30), pages 6527-6537, XP028744817, ISSN: 0043-1354, DOI: 10.1016/J.WATRES.2013.08.019 the whole document -----	1-6
A	EP 3 628 651 A1 (BIORIZON BIOTECH S L [ES]) 1 April 2020 (2020-04-01) the whole document -----	1-6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/056313

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.:

1-6

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: 1-6

A method for reducing cyanobacteria in a cyanobacteria-contaminated body of water, the method comprises: inactivating resident vegetative microbiology from an extract obtained from a cyanobacteria-contaminated body of water to inactivate the resident vegetative microbiology in the extract, selecting one or more soil-based microbes suitable for growth in the cyanobacteria-contaminated body of water, growing the one or more soil-based microbes with the inactivated extract to allow the one or more soil-based microbes to adapt to the inactivated extract, releasing the one or more soil-based microbes into the cyanobacteria-contaminated body of water where the one or more soil-based microbes dominate and reduce cyanobacteria of the cyanobacteria-contaminated body of water.

2. claims: 7-13

A method for decontaminating irrigation water and restoring soil contaminated with contaminated irrigation water, the method comprises: inactivating contaminated irrigation water to inactivate vegetative microbiology to produce inactivated irrigation water, selecting one or more microbes suitable for outcompeting microbiology in the soil contaminated with the inactivated irrigation water, growing, under aerobic conditions, the one or more microbes with the inactivated irrigation water to allow the one or more microbes to adapt to the inactivated irrigation water and to inactivate any obligate anaerobic bacteria endospores that may have survived inactivation, releasing the one or more microbes and the inactivated irrigation water into the soil contaminated with contaminated irrigation water where the one or more soil-based microbes dominate and reduce contamination and restore the soil.

3. claims: 14-27

A biofertilizer composition for soil inoculation and/or foliar application, the composition produced according to a method comprising: macerating an extract of agricultural by-waste, inactivating the extract to inactivate resident vegetative microbiology in the extract, flowing inactivated extract into a holding reservoir and a portion of the inactivated extract into a culture reservoir, growing in the culture reservoir one or more soil-based microbes with the portion of the inactivated extract to allow the one or more soil-based microbes to adapt to the inactivated extract, flowing a portion of the one or more adapted

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

soil-based microbes when the one or more adapted microbes are in a vegetative or active form and capable of assimilating nutrients or chemicals into the holding reservoir until the concentration of the one or more adapted soil-based microbes in the holding reservoir is from about 10^6 and 10^9 colony forming units (cfu)/ml. Use of said composition to inoculate soil and/or apply to foliage to restore an agricultural microbiome.

4. claims: 28-40

A method for treating foot rot afflicted livestock, the method comprising: contacting a lower extremity of foot rot afflicted livestock with a microbial composition, the composition comprises at least one microbe adapted to degrade livestock manure and/or produce a bacteriostatic antibiotic. A microbial composition for treating foot rot afflicted livestock, the composition comprises: at least one microbe adapted to degrade livestock manure and/or produce a bacteriostatic antibiotic.

5. claims: 41-52

A method of producing copper tolerant microbes adapted to degrade copper contaminated organic waste, the method comprises: culturing copper intolerant microbes in a solid growth medium containing a base copper level at about 35 degrees for an incubation time of about 6 to about 24 hours, selecting the base copper level tolerant microbes and growing the base copper level tolerant microbes in a liquid nutritional medium, harvesting at least a portion of the base copper level tolerant microbes and growing the at least a portion of the base copper level tolerant microbes in a solid growth medium containing an elevated copper level at about 35 degrees for an incubation time of about 6 to about 24 hours, obtaining copper tolerant microbes by selecting the elevated copper tolerant microbes. Copper tolerant microbes adapted to degrade copper contaminated organic waste produced according to a method that comprises: culturing copper intolerant microbes in a growth medium containing a base copper level at about 35 degrees for an incubation time of about 6 to about 12 hours, selecting the base copper level tolerant microbes and growing the base copper level tolerant microbes in a liquid nutritional medium, obtaining at least a portion of the base copper level tolerant microbes and growing then at least a portion of the base copper level tolerant microbes in a growth medium containing an elevated copper level at about 35 degrees for an incubation time of about 6 to about 12 hours, obtaining copper tolerant microbes by selecting the elevated copper level tolerant microbes, and use of said copper tolerant microbes for degrading copper contaminated organic waste.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. claims: 53-67

A method for remediating metal-impacted water and/or remediating organic contaminated water, the method comprises: flowing metal-impacted water and/or organic contaminated water to an aerated fluidized bed reactor comprising sand and soil-based bacteria to reduce susceptible metals in the water and/or hydrocarbon degrading bacteria to reduce organic contaminants in the water, retaining for a first period sufficient to allow the soil-based bacteria to reduce susceptible metals in the water and/or the hydrocarbon degrading bacteria to remove organic contaminants in the water, and form treated water. flowing clean water, when the treatment time has lapsed, to the first fluidized bed to displace the treated water, flowing the displaced treated water containing any susceptible metals not reduced in the aerated fluidized bed reactor and/or organic contaminants not removed in the aerated fluidized bed reactor to an anaerobic fluidized bed reactor comprising sand and anaerobic or facultative bacteria, retaining for a second period sufficient to allow the anaerobic or facultative bacteria to reduce any susceptible metals not reduced in the aerated fluidized bed reactor and/or remove organic contaminants not removed in the aerated fluidized bed reactor, and form remediated metal-impacted water and/or remediated organic contaminated water.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2021/056313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018081441 A1	03-05-2018	US 2019269740 A1 WO 2018081441 A1	05-09-2019 03-05-2018
EP 3628651 A1	01-04-2020	CN 110937936 A EP 3628651 A1 ES 2693793 A1 US 2020095176 A1	31-03-2020 01-04-2020 13-12-2018 26-03-2020