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(54) Title: ENVIRONMENT FRIENDLY NON-TOXIC METHANOTROPH FORMULATION FOR MITIGATION OF METHANE EMISSION IN RICE

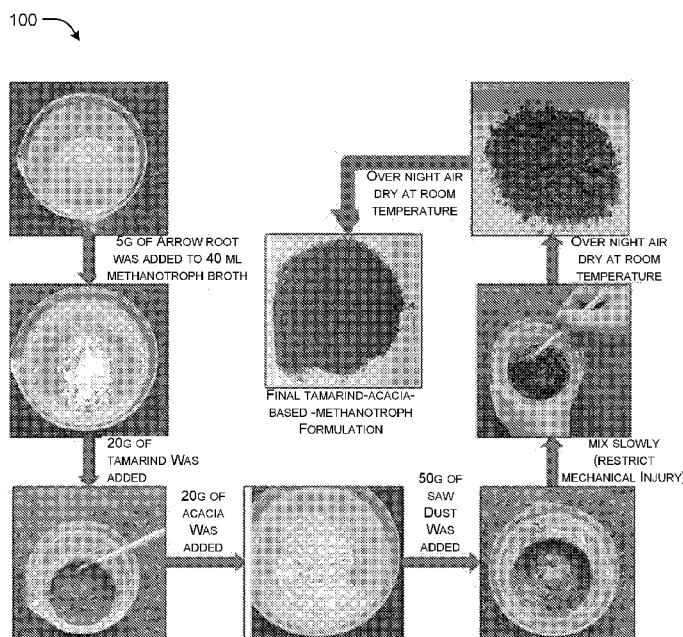


FIG. 1

(57) Abstract: The present disclosure pertains to reduction of greenhouse gas emission. In particular, the present disclosure provides an environment friendly, non-toxic (green) methanotroph formulation comprising *Methylobacterium* sp. (MT22_NRRI) for mitigation of methane emission from lowland rice; and a method of preparing the methanotroph formulation.

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ENVIRONMENT FRIENDLY NON-TOXIC METHANOTROPH FORMULATION FOR MITIGATION OF METHANE EMISSION IN RICE

FIELD OF THE INVENTION

5 [0001] The present disclosure pertains to reduction of greenhouse gas emission. In particular, the present disclosure provides an environment friendly, non-toxic (green) methanotroph formulation for mitigation of methane emission from lowland rice; and a method of preparing the methanotroph formulation.

BACKGROUND OF THE INVENTION

10 [0002] The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the present invention, or that any publication specifically or implicitly referenced is prior art.

15 [0003] Climate change and global warming is one of the most challenging issues faced by world community. The primary cause of climate change is the emissions of three major greenhouse gases (GHGs) i.e., methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O). Agricultural sector contributes about 20% of the total GHG emissions. Rice paddy contributes about 30% of global agricultural CH_4 emissions. Specifically, the lowland flooded rice fields are more favourable for the activity of methanogens (methane producing 20 archaea) resulting higher CH_4 production as compared to the aerobic conditions.

25 [0004] Methanotrophs (methane oxidizing bacteria) and methanogenesis (methane producing archaea) are the two important groups of microbes which play an important role in CH_4 emission from soil to the atmosphere in rice field. Thus, to reduce the methane emission in the agricultural system, reduction of methanogenic activities and enriching the methanotrophs in soil is a better ecofriendly option.

[0005] The present invention discloses the use of a novel methanotroph composition for reducing methane emission from lowland ecosystem.

OBJECTS OF THE INVENTION

[0006] Objects of the present invention is to reduce methane emission from lowland rice.

30 [0007] An object of the present invention is to provide an environment friendly, non-toxic formulation for reducing methane emission from lowland rice.

[0008] An object of the present invention is to provide an environment friendly, non-toxic methanotroph formulation for reducing methane emission from lowland rice.

[0009] Another object of the present invention is to provide an environment friendly, non-toxic methanotroph formulation comprising *Methylobacterium* sp. (MT22_NRRI) for 5 reducing methane emission from lowland rice.

[00010] Yet another object of the present invention is to provide a method for reducing methane emission from lowland ecosystem.

SUMMARY OF THE INVENTION

[00011] This summary is provided to introduce a selection of concepts in a simplified 10 form that are further described below in Detailed Description section. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[00012] Aspects of the present disclosure pertains to reduction of greenhouse gas 15 emission. In particular, the present disclosure provides an environment friendly, non-toxic (green) methanotroph formulation for mitigation of methane emission from lowland rice; and a method of preparing the methanotroph formulation.

[00013] In an aspect, the present disclosure relates to an environment friendly, non-toxic methanotroph formulation comprising *Methylobacterium* sp. (MT22_NRRI) for reducing methane emission from lowland rice.

[00014] In an aspect, the present disclosure relates to an environment friendly, non-toxic methanotroph formulation for reducing methane emission from lowland rice, said 20 formulation comprises:

- a) a methanotroph broth in a volume of about 38% - 42% v/w of the formulation;
- b) tamarind seed coat powder in an amount of 18%-22% wt. of the formulation;
- c) gum acacia in an amount of 18%-22% wt. of the formulation;
- d) arrowroot powder in an amount of 4%-5% wt. of the formulation; and
- e) saw dust in an amount of 45%-55% wt. of the formulation.

[00015] In another aspect, the present disclosure relates to a method for producing an 25 environment friendly, non-toxic methanotroph formulation, said method comprises the steps of:

- a) providing a methanotroph broth;
- b) adding and mixing arrowroot to the methanotroph broth;

- c) adding and mixing gum acacia and tamarind seed coat powder to the arrowroot added methanotroph broth to obtain a pre-final formulation;
- d) adding saw dust to the pre-final formulation in plurality of parts, followed by slow mixing to obtain powdery form of the methanotroph formulation; and
- e) air-drying the methanotroph formulation, followed by freeze storing.

5 [00016] In yet another aspect, the present disclosure relates to a method for reducing methane emission from rice-based cropping system, said method comprises the step of: applying the methanotroph formulation as claimed in claim 1 at 7 and 45 days after transplanting of rice at a dose of 1.0-1.5 kg per acre of paddy field.

10 [00017] Various objects, features, aspects and advantages of the inventive subject matter will become more apparent from the following detailed description of preferred embodiments.

BRIEF DESCRIPTION OF DRAWINGS

15 [00018] Characteristics and advantages of the subject matter as disclosed in the present disclosure will become clearer from the detailed description of an embodiment thereof, with reference to the attached drawing, given purely by way of an example, in which:

[00019] **FIG. 1** represents the step-wise method of producing the Methanotroph Formulation of the present invention.

20 [00020] **FIG. 2** represents the bar graph of Methane reduction percentage by the application of the Methanotroph Formulation of the present invention as compared to Control recommended dose of fertilizers (RDF) in rice-based cropping system.

DETAILED DESCRIPTION OF THE INVENTION

25 [00021] The following is a detailed description of embodiments of the disclosure. The embodiments are in such detail as to clearly communicate the disclosure. However, the amount of detail offered is not intended to limit the anticipated variations of embodiments; on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure as defined by the appended claims.

30 [00022] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

[00023] Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases “in one embodiment” or “in an embodiment” in various places 5 throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[00024] In some embodiments, numbers have been used for quantifying weight percentages, angles, and so forth, to describe and claim certain embodiments of the invention 10 and are to be understood as being modified in some instances by the term “about.” Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits 15 and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found 20 in their respective testing measurements.

[00025] Various terms as used herein are shown below. To the extent a term used in a claim is not defined below, it should be given the broadest definition persons in the pertinent art have given that term as reflected in printed publications and issued patents at the time of filing.

[00026] As used in the description herein and throughout the claims that follow, the meaning of “a,” “an,” and “the” includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of “in” includes “in” and “on” 25 unless the context clearly dictates otherwise.

[00027] Unless the context requires otherwise, throughout the specification which follow, 30 the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense that is as “including, but not limited to.”

[00028] The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range.

Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein.

[00029] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and

5 all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

10 **[00030]** Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion 15 occurs, the specification is herein deemed to contain the group as modified.

[00031] The description that follows, and the embodiments described therein, is provided by way of illustration of an example, or examples, of particular embodiments of the principles and aspects of the present disclosure. These examples are provided for the purposes of explanation, and not of limitation, of those principles and of the disclosure.

20 **[00032]** The headings and abstract of the invention provided herein are for convenience only and do not interpret the scope or meaning of the embodiments.

[00033] The following discussion provides many example embodiments of the inventive subject matter. Although each embodiment represents a single combination of inventive elements, the inventive subject matter is considered to include all possible combinations of 25 the disclosed elements. Thus if one embodiment comprises elements A, B, and C, and a second embodiment comprises elements B and D, then the inventive subject matter is also considered to include other remaining combinations of A, B, C, or D, even if not explicitly disclosed.

[00034] Various terms are used herein. To the extent a term used in a claim is not defined 30 below, it should be given the broadest definition persons in the pertinent art have given that term as reflected in printed publications and issued patents at the time of filing.

[00035] While a particular form of the invention has been illustrated and described, it will be apparent that various modifications can be made without departing from the spirit and scope of the invention.

5 **[00036]** The present disclosure pertains to reduction of greenhouse gas emission. In particular, the present disclosure provides an environment friendly, non-toxic methanotroph formulation for mitigation of methane emission from lowland ecosystem; and a method of preparing the methanotroph formulation.

10 **[00037]** In an embodiment, the present invention provides an environment friendly, non-toxic methanotroph formulation reduces methane emission by 1-25% on the overall emission. For example, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 18%, 20%, 22%, 25%., Most preferably 4% to 15%.

15 **[00038]** In an embodiment, the present invention provides an environment friendly, non-toxic methanotroph formulation comprising methanotrophic bacterium for mitigation of methane emission from lowland ecosystem, wherein the bacterium is *Methylobacterium* sp. (MT22_NRRI) with National Center for Biotechnology Information (NCBI) accession number: MZ683316.

20 **[00039]** In an embodiment of the present invention, the methanotrophic bacterium is incubated in Nitrate Mineral Salts medium, and the resulting broth after 5 days of incubation having 1^8 CFU/ml to 15^8 CFU/ml is used in the methanotroph formulation. For example 1^8 CFU/ml, 2^8 CFU/ml, 3^8 CFU/ml, 4^8 CFU/ml, 5^8 CFU/ml, 6^8 CFU/ml, 7^8 CFU/ml, 8^8 CFU/ml, 9^8 CFU/ml, 10^8 CFU/ml, 11^8 CFU/ml, 12^8 CFU/ml, 13^8 CFU/ml, 14^8 CFU/ml, or 15^8 CFU/ml. Most preferably 10^8 CFU/ml.

25 **[00040]** In an embodiment of the present invention, the methanotrophic broth is used based on the total weight of the formulation in a volume of about at least 29.6%, arrowroot powder 3.7%, tamarind seed coat powder 14.8%, gum acacia 14.8%, and saw dust 37% of the formulation to preparation of methanotroph formulation.

30 **[00041]** In an embodiment of the present invention, the methanotroph formulation further comprises tamarind seed coat powder (inhibit methanogenic activities), gum acacia (used as adhesive materials and also as substrate for bacteria), arrowroot powder (as binding agent), and saw dust (used as a filler material).

[00042] In an embodiment of the present invention, 135 g of formulation contains 5 gm of arrowroot powder, 20g of tamarind seed coat powder, 20g of gum acacia, and 50g of saw dust.

[00043] In an embodiment, the present invention provides a non-toxic methanotroph formulation for reducing methane emission from lowland rice, said formulation comprises:

- 5 a) a methanotroph broth in a volume of about 29.6% v/w of the formulation;
- b) tamarind seed coat powder in an amount of 14.8% wt. of the formulation;
- c) gum acacia in an amount of 14.8% wt. of the formulation;
- d) arrow root powder in an amount of 3.7% wt. of the formulation; and
- e) saw dust in an amount of 37% wt. of the formulation.

[00044] In an embodiment of the present invention, the methanotroph formulation is granular in size ranging about 0.5 mm-2.5 mm. For example 0.5 mm, 0.6 mm, 0.77 mm, 0.88 mm, 0.9 mm, 1.0 mm, 1.1 mm, 1.2 mm, 1.3 mm, 1.4 mm, 1.5 mm, 1.6 mm, 1.7 mm, 1.8 mm, 1.9 mm, 2.0 mm, 2.1 mm, 2.2 mm, 2.3 mm, 2.4 mm, or 2.5 mm.

[00045] In an embodiment of the present invention, the rice-based cropping system is lowland ecosystem.

[00046] In an embodiment, the present invention provides a method for producing an environment friendly, non-toxic methanotroph formulation, said method comprises the steps of:

- 20 a) providing a methanotroph broth;
- b) adding and mixing arrowroot to the methanotroph broth;
- c) adding and mixing gum acacia and tamarind seed coat powder to the arrowroot added methanotroph broth to obtain a pre-final formulation;
- d) adding saw dust to the pre-final formulation in plurality of parts, followed by slow mixing to obtain powdery form of the methanotroph formulation; and
- e) air-drying the methanotroph formulation, followed by freeze storing.

[00047] In a further embodiment, the present invention provides a method for reducing methane emission from rice-based cropping system, said method comprises the step of: applying the methanotroph formulation at 7 and 45 days after transplanting of rice at a dose of 1-1.5 kg per acre of paddy field.

[00048] While the foregoing description discloses various embodiments of the disclosure, other and further embodiments of the invention may be devised without departing from the basic scope of the disclosure. The invention is not limited to the described embodiments, versions or examples, which are included to enable a person having ordinary skill in the art to make and use the invention when combined with information and knowledge available to the person having ordinary skill in the art.

EXAMPLES

[00049] The present disclosure is further explained in the form of following examples. However, it is to be understood that the foregoing examples are merely illustrative and are not to be taken as limitations upon the scope of the invention. Various changes and 5 modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications may be made without departing from the scope of the invention.

[00050] Example 1: Preparation of Tamarind-Acacia-based Methanotroph Formulation

FIG. 1 provides the preparation in a step-wise manner. 40 mL of methanotroph broth (after 5 10 days of incubation in nitrate minimal salts (NMS) media) (10^8 cfu mL⁻¹) was taken in a 500 mL of beaker. 5 g of arrowroot was added to the broth and mixed properly by using glass rod. After that gum-acacia and tamarind seed coat powder (20 g each) were added and mixed 15 gently. Then 50 g of saw dust (10g saw dust added at a time to the broth and repeated it for 5 times) was added and mixed slowly (restrict mechanical injury), followed by air drying the same overnight in room temperature (25°C). The final product was stored in freeze condition (at 4°C).

[00051] Example 2: Reduction of methane emission by the application of 'Tamarind-Acacia-based Methanotroph Formulation' in rice

'Tamarind-Acacia-based Methanotroph Formulation' was applied in the rice-based cropping 20 system (rice-rice and rice-green gram) in lowland for both the *kharif* and *rabi* seasons. The formulated products applied at two splits (at 7 and 45 days after transplanting) in rice, showed more effective than the single dose of application (only after 7 days). The percentage 25 of methane emission reduction due to the application of tamarind-acacia-based methanotroph formulation was ranged from 4.6 to 12.1% as compared to the recommended doses of fertilizers (used as control) treatment (FIG. 2).

[00052] Various modification and variation of the described assays, techniques and various means disclosed herein to implement the assays/methods in accordance with the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with 30 specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

We Claim:

1. A non-toxic methanotroph formulation for reducing methane emission from lowland ecosystem, said formulation comprises:

5 a) a methanotroph broth in a volume of about 29.6% wt. of the formulation;
 b) Tamarind seed coat powder in an amount of 14.8% wt. of the formulation;
 c) gum acacia in an amount of 14.8% wt. of the formulation;
 d) arrow root powder in an amount of 3.7% wt. of the formulation; and
 e) saw dust in an amount of 37% wt. of the formulation.

10

2. The formulation as claimed in claim 1, wherein the methanotroph is *Methylobacterium* sp. (MT22_NRRI) with NCBI accession number: MZ683316.

15 **3.** The formulation as claimed in claim 1-2, wherein the methanotroph broth comprises the methanotroph after 5 days of incubation in nitrate minimal salts (NMS) media.

4. The formulation as claimed in claim 1-3, wherein the volume of the methanotroph broth comprises 10^8 CFU/ml of methanotroph.

20 **5.** The formulation as claimed in claim 1, wherein the formulation is granular formulation having a size of 0.5 mm to 2.5 mm.

6. The formulation as claimed in claim 1, wherein the formulation reduces methane emission by 4.6% to 12.1%.

25

7. The formulation as claimed in claim 1, wherein the lowland ecosystem is rice based cropping system.

30 **8.** A method for producing non-toxic methanotroph formulation as claimed in claim 1, said method comprises the steps of:

 a) providing a methanotroph broth as claimed in claim 1;
 b) adding and mixing arrowroot to the methanotroph broth;
 c) adding and mixing gum acacia and tamarind seed coat powder to the arrowroot added methanotroph broth to obtain a pre-final formulation;

- d) adding saw dust to the pre-final formulation in plurality of parts, followed by slow mixing to obtain powdery form of the methanotroph formulation; and
- e) air-drying the methanotroph formulation, followed by freeze storing.

5 **9.** A method for reducing methane emission from lowland rice, said method comprises the step of: applying the methanotroph formulation as claimed in claim 1, at 7 and 45 days after transplanting of rice at a dose of 1.0-1.5 kg per acre of rice field.

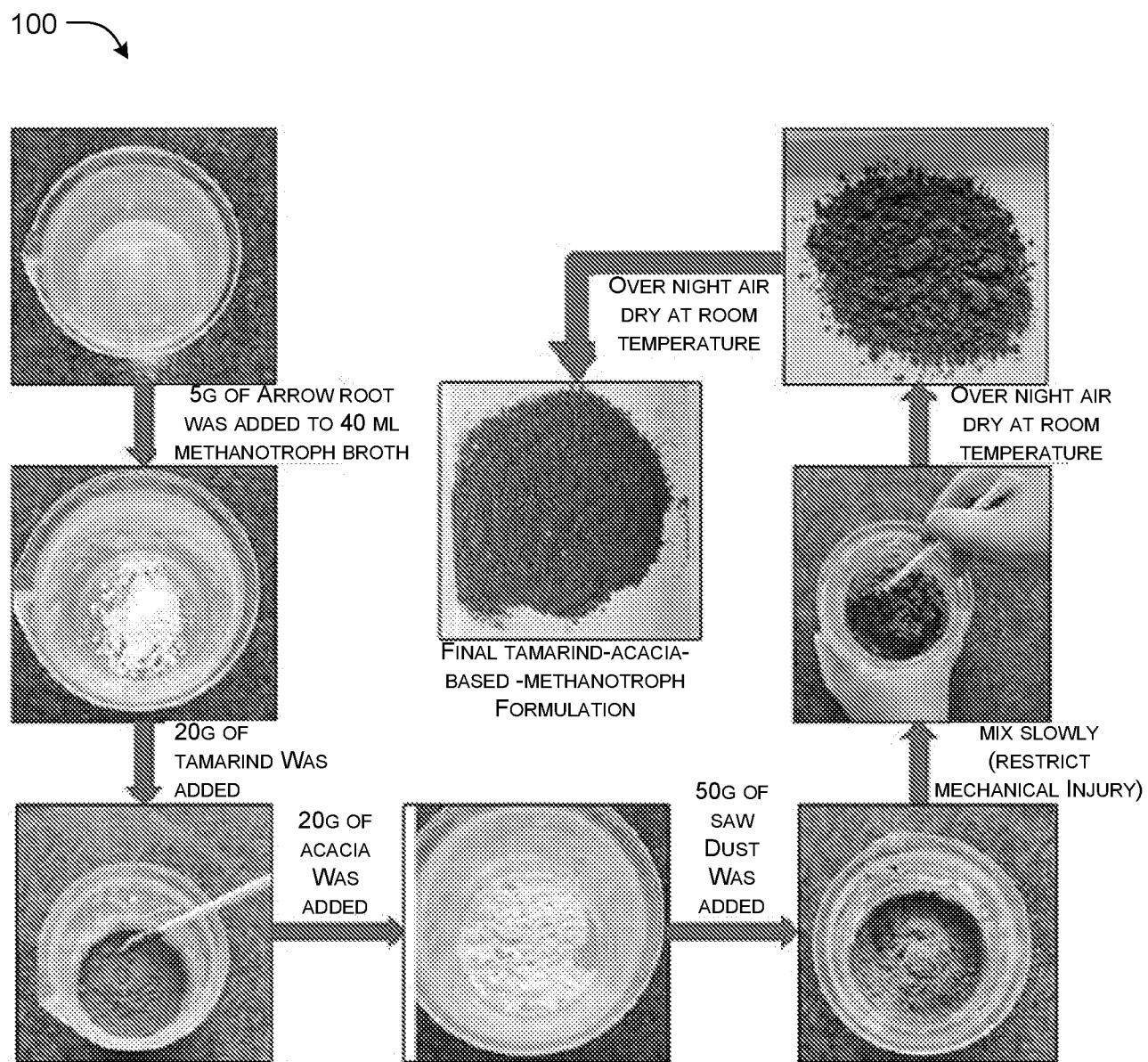


FIG. 1

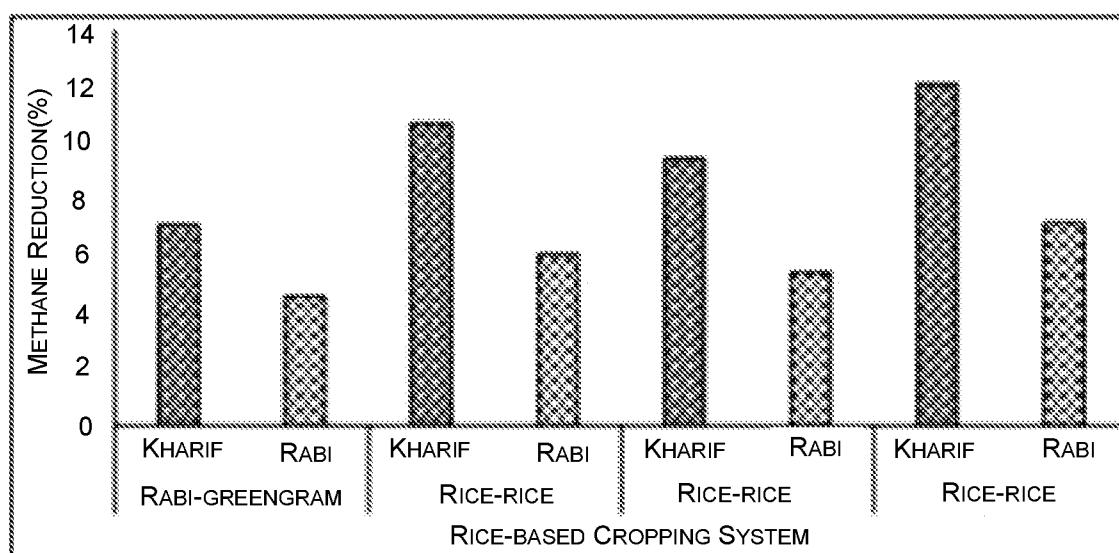


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/060450

A. CLASSIFICATION OF SUBJECT MATTER

C12N1/20, A01N63/20, C12R1/26, A01G7/06 Version=2024.01

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, A01N, C12R, A01G

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

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

PatSeer, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Davamani et al. Mitigation of methane gas emissions in flooded paddy soil through the utilization of methanotrophs, Science of the total environment 726 (2020) 138570. Whole document especially abstract, materials and methods and page 6	1-9
Y	US10757946B2 (NEWLEAF SYMBIOTICS INC) 01 SEPTEMBER 2020 (01-09-2020) Abstract, claims 1-21, col. 25	1-9
Y	IN202041022403A (STRING BIO PVT LTD) 02 JULY 2021 (02-07-2021) Whole document especially abstract, claims 1-9	1-9
Y	GenBank: MZ683316.1, Methylobacterium sp. strain MT22_NRRI 16S ribosomal RNA gene, partial sequence 09 AUGUST 2021 (09-08-2021) Whole document	2
Y	Chandini S Kumar and Sila Bhattacharya: Tamarind Seed: Properties, Processing and Utilization,	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"D" document cited by the applicant in the international application

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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

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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

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document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

15-02-2024

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/060450

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Critical Reviews in Food Science and Nutrition, 48:1, 1-20 (2008). Table 6 (page 12) ----- Y CN112876793B (BEIJING FORESTRY UNIVERSITY) 12 AUGUST 2022 (12-08-2022) Abstract ----- Y Malik et al. Effect of tamarind seed husk supplementation on ruminal methanogenesis, methanogen diversity and fermentation characteristics, 2017, Carbon Management, 8:4, 319-329. Abstract ----- Y Singh et al.: Effect of different additives on the persistence and insecticidal activity of native strains of <i>Bacillus thuringiensis</i> , Indian Journal of Microbiology (March 2007) 47:42-45. Abstract	1-9 1-9 1-9 1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IB2023/060450

Citation	Pub.Date	Family	Pub.Date
<hr/>			
IN 202041022403 A	02-07-2021	US 2023217929 A1	13-07-2023
		JP 2023527870 A	30-06-2023
		EP 4156941 A1	05-04-2023
		AU 2021279438 A1	19-01-2023
		WO 2021240472 A1	02-12-2021