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(54) Title: SYNTHETIC BIOFILM AND BIOREACTOR

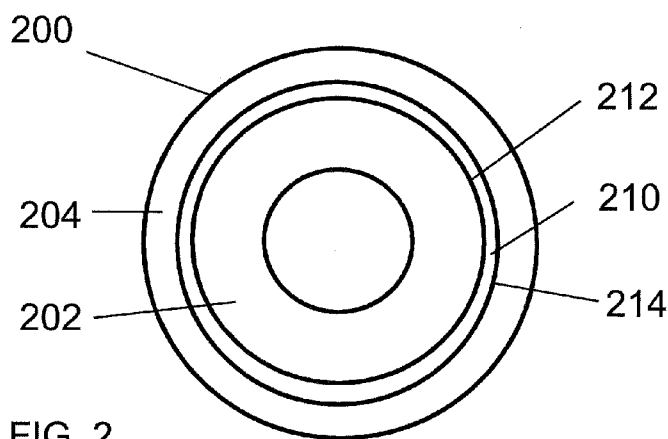


FIG. 2

(57) Abstract: A synthetic biofilm is described. Microorganisms are immobilized or embedded within a matrix that is gas-permeable and water-permeable. The synthetic biofilm can be coated on or embedded as a layer within a supporting substrate.



SYNTHETIC BIOFILM AND BIOREACTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/679,310, filed August 3, 2012. The contents of that application are hereby fully incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Award No. DE-AR0000095, Subaward 60026346/RF01209604 awarded by the Department of Energy ARPA-E. The government has certain rights in the invention.

BACKGROUND

[0003] The present disclosure relates to a bioreactor in which microorganisms are maintained to produce desirable products. The microorganisms are immobilized in an artificial or synthetic biofilm.

BRIEF DESCRIPTION

[0004] Described in various embodiments are different synthetic biofilms, as well as bioreactors that use such biofilms. The bioreactor designs of the present disclosure are based on artificially immobilizing microorganisms in various configurations and shapes. The unique shape and structure of each synthetic biofilm can be tailored to the specific reactor design. The bioreactors can be used to produce desirable or valuable products from microorganisms using specified inputs.

[0005] Disclosed in embodiments is a synthetic biofilm, comprising microorganisms immobilized within a matrix, wherein the matrix is water permeable and gas permeable.

[0006] The matrix may be made from or contain alginate, sol-gel silica, carrageenan, latex, polyvinyl alcohol, polystyrene sulfonate, or mixtures thereof.

[0007] The biofilm can further comprise a strengthening base dispersed throughout the biofilm. Such strengthening base may be cloth, metal or a foam.

[0008] The biofilm may be in the shape of a sphere, a sheet, or a tube.

[0009] The biofilm can be coated on or embedded within a supporting substrate. The supporting substrate can be in the shape of a tube or a sheet. The supporting substrate can be a porous metal or ceramic tube. In particular embodiments, the supporting substrate is a porous foam in which the biofilm is embedded as a distinct layer.

[0010] The microorganisms in the biofilm can be bacteria.

[0011] Also disclosed in embodiments is a bioreactor, comprising an synthetic biofilm and a supporting substrate. The synthetic biofilm comprises bacteria immobilized in an alginate matrix and a porous foam strengthening base.

[0012] The synthetic biofilm may be in the shape of a tube. A membrane may be located on a surface of the synthetic biofilm. In embodiments, a liquid flow is provided on one side of the synthetic biofilm, and a gas flow is provided on an opposite side of the synthetic biofilm.

[0013] These and other non-limiting aspects and/or objects of the disclosure are more particularly described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0015] **FIG. 1** is a diagram illustrating a synthetic biofilm coated on a supporting substrate.

[0016] **FIG. 2** is a diagram illustrating a synthetic biofilm embedded in a supporting substrate.

[0017] **FIG. 3** is a picture of a porous metal tube, which has been coated with a synthetic biofilm, mounted in a bioreactor.

[0018] **FIG. 4** is a picture of a synthetic biofilm that has a porous foam strengthening base, in the shape of a tube.

[0019] FIG. 5 is a picture showing the foam reinforced alginate tube of FIG. 4 with water flowing through the interior.

[0020] FIG. 6 is a picture of other synthetic biofilm tubes where the synthetic biofilm includes a foam strengthening base and surrounds a metal screen tube.

[0021] FIG. 7 is a picture of a synthetic biofilm that has a cloth strengthening base.

[0022] FIG. 8 is a picture showing the synthetic biofilm in the form of spheres. The spheres are made from microorganisms and alginate.

[0023] FIG. 9 is a picture showing the alginate spheres of FIG. 8 being used in a fluidized bed reactor.

[0024] FIG. 10 is a diagram illustrating the configuration of the fluidized bed reactor.

DETAILED DESCRIPTION

[0025] A more complete understanding of the processes and apparatuses disclosed herein can be obtained by reference to the accompanying drawings. These figures are merely schematic representations based on convenience and the ease of demonstrating the existing art and/or the present development, and are, therefore, not intended to indicate relative size and dimensions of the assemblies or components thereof.

[0026] Although specific terms are used in the following description for the sake of clarity, these terms are intended to refer only to the particular structure of the embodiments selected for illustration in the drawings, and are not intended to define or limit the scope of the disclosure. In the drawings and the following description below, it is to be understood that like numeric designations refer to components of like function.

[0027] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0028] Numerical values in the specification and claims of this application should be understood to include numerical values which are the same when reduced to the same number of significant figures and numerical values which differ from the stated value by less than the experimental error of conventional measurement technique of the type described in the present application to determine the value.

[0029] All ranges disclosed herein are inclusive of the recited endpoint and independently combinable (for example, the range of “from 2 grams to 10 grams” is inclusive of the endpoints, 2 grams and 10 grams, and all the intermediate values). The endpoints of the ranges and any values disclosed herein are not limited to the precise range or value; they are sufficiently imprecise to include values approximating these ranges and/or values.

[0030] As used herein, approximating language may be applied to modify any quantitative representation that may vary without resulting in a change in the basic function to which it is related. Accordingly, a value modified by a term or terms, such as “about” and “substantially,” may not be limited to the precise value specified, in some cases. In at least some instances, the approximating language may correspond to the precision of an instrument for measuring the value. The modifier “about” should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression “from about 2 to about 4” also discloses the range “from 2 to 4.”

[0031] The present disclosure relates to artificial or synthetic biofilms that can be used in a bioreactor. Generally speaking, microorganisms (such as bacteria, algae, or fungi) are immobilized in the artificial biofilm. The artificial biofilm can be placed in various configurations, shapes, or structures tailored to the specific design of the bioreactor. The bioreactor is used for the production of a valuable/desirable product from the microorganisms. For example, the microorganisms may be able to produce butanol from hydrogen gas.

[0032] The term “biofilm” refers to an aggregate of microorganisms that are embedded within a self-produced matrix of an extracellular polymeric substance. This extracellular polymeric substance generally contains extracellular DNA, proteins, and polysaccharides.

[0033] The present disclosure relates to an artificial biofilm composed of microorganisms immobilized in a matrix. The terms “artificial” and “synthetic” are used interchangeably to indicate that the matrix in which the microorganisms are embedded or immobilized is made of a material that is not naturally produced by the microorganisms.

[0034] The microorganisms in the artificial biofilm may be bacteria, algae, or fungi. One example of a suitable bacterium is *Ralstonia eutropha*. *R. eutropha* is a hydrogen-oxidizing bacterium that can grow in both anaerobic and aerobic environments. *R. eutropha* can perform aerobic respiration or anaerobic respiration by denitrification of nitrate and/or nitrite to nitrogen gas. It can also easily adapt between a heterotrophic lifestyle and an autotrophic lifestyle. Under autotrophic conditions, *R. eutropha* fixes carbon through the pentose phosphate pathway. *R. eutropha* can also produce and sequester polyhydroxyalkanoate (PHA) plastics when exposed to excess amounts of sugar substrate. PHA can accumulate to levels of approximately 90% of the cell's dry weight. Other examples of suitable bacteria include *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, and *Rhodospirillum rubrum*. These bacteria contain bacteriochlorophyll a/b.

[0035] The microorganisms are immobilized in a matrix (i.e. encapsulated) to form the artificial biofilm. The matrix is made of a material that is both gas-permeable and water-permeable. In particular embodiments, the matrix can be described as a gel. Exemplary materials suitable for the matrix include alginate, sol-gel silica, carrageenan, latex, polyvinyl alcohol, polystyrene sulfonate, and mixtures of these materials. Alginate is also known as algin or alginic acid (CAS# 9005-32-7), is commercially available, and can absorb a large quantity of water. Sol-gel silica refers to using silica in a sol-gel procedure to obtain a three-dimensional network containing both a liquid phase and a solid phase. Exemplary silicates include tetraethyl orthosilicate (TEOS). The microorganisms can be contained within the network. Carrageenan refers to polysaccharides which can gel, and which can be obtained from seaweed. Other materials, which are both gas-permeable and water-permeable, may also be used to immobilize the microorganisms.

[0036] It is contemplated that gases and liquids will be provided to the microorganisms on different sides of the artificial biofilm, e.g. liquids will pass on one side and gases will pass on the other side. Maintaining gas-liquid separation can be achieved in several different ways. In some embodiments, pressure differences and gas solubility in water at running conditions maintain the separation. For example, the gas can be provided at a pressure level that is sufficient to permit the liquid to contact the

artificial biofilm, but high enough to prevent the liquid from entering the gas pathway. In other embodiments, one or more membranes are provided to maintain the desired separation of gas and liquid flows, limiting their free interaction, while allowing the exchange of the required constituents to support metabolism and valuable/desirable product production within the organism-containing matrix. Such membranes can be micro-porous and hydrophobic, for example, which might be placed to prevent the liquid from entering the gas flow. The membranes could alternatively be micro-porous and hydrophilic.

[0037] If desired, a strengthening base may be added to the artificial biofilm. The strengthening base (and the microorganisms) is in the matrix of the artificial biofilm, and could alternatively be described as being generally dispersed throughout the biofilm. The strengthening base increases the structural stability of the artificial biofilm. An exemplary strengthening base is cloth. Another exemplary strengthening base is a metal screen. Yet another exemplary strengthening base is an open celled foam. In such foams, the microorganisms and their matrix could be described as being dispersed throughout the foam. For example, bacteria and alginate could fill the holes within the open celled foam.

[0038] The artificial biofilm can be shaped into any desired configuration or shape. For example, the artificial biofilm may be in the shape of a sphere, a sheet, or a tube. Spheres can be used in a packed bed or fluidized bed reactor format. Sheets, which are flat and planar, can be used in a reactor as a series of stacked sheets. A tube is a hollow cylinder, and can also be described as a rolled-up sheet. Tubes can be used to deliver one growth medium on the exterior of the tube, and to deliver a second different growth medium on the interior of the tube while keeping the two growth mediums from mixing. When the artificial biofilm is in the shape of a sphere, the sphere may have a diameter of from about 0.1 millimeter (mm) to about 20 mm. When the artificial biofilm is in the shape of a tube, the tube may have an inner diameter of from about 0.1 mm to about 30 mm, an outer diameter of from about 0.1 mm to about 30 mm (the outer diameter being greater than the inner diameter), and may have any length appropriate for the desired design.

[0039] The artificial biofilm can retain structural integrity and bacterial viability in different shapes, which allows it to be incorporated into many different types of reactors. The cell density, cell placement, and consistency of the artificial biofilm can also be controlled. The artificial biofilm can be formed quickly, without the need for a long incubation period while waiting for a natural biofilm to be created. In addition, the microorganisms are immobilized in the biofilm instead of living in the growth medium that typically circulates within the bioreactor vessel.

[0040] The artificial biofilm may be coated on or embedded within a supporting substrate. The supporting substrate may be in any desired shape, such as a sphere, a sheet, or a tube. The artificial biofilm can be applied as a layer upon a surface of the supporting substrate. For example, the artificial biofilm could be applied as a coating on the outer surface of a porous metal or ceramic tube. Alternatively, the artificial biofilm could be embedded within the supporting substrate. For example, the artificial biofilm can be surrounded by a porous foam. It is recognized that the supporting substrate and the strengthening base can be made from the same material. The distinction is that the strengthening base is dispersed within the artificial biofilm and generally cannot / could not be separated. The artificial biofilm maintains itself as a separate layer.

[0041] **FIG. 1** is a diagram illustrating the artificial biofilm coated upon a supporting substrate. The supporting substrate **100** has a surface **102** upon which the artificial biofilm **110** is coated. One side of the artificial biofilm contacts the supporting substrate, while the other side **114** of the artificial biofilm is exposed. The supporting substrate here may have a thickness such that the microorganisms in the artificial biofilm have access to all the necessary nutrients, delivered from both sides, to support metabolism and valuable/desirable product production.

[0042] **FIG. 2** is a diagram illustrating an artificial biofilm embedded within a supporting substrate. Here, the supporting substrate is illustrated in the form of a tube **200** formed from a porous foam. The artificial biofilm is embedded within the tube as a distinct layer **210**. Both inner surface **212** and outer surface **214** of the artificial biofilm contact the supporting substrate. The tube could also be described as an inner annulus **202** and an outer annulus **204**. It should be noted that the microorganisms are not dispersed within the porous foam, but are rather within a distinct layer. The tube may

have an inner diameter of from about 0.1 mm to about 30 mm, an outer diameter of from about 0.1 mm to about 30 mm (the outer diameter being greater than the inner diameter), and may have any length appropriate for the desired design.

[0043] The combination of the artificial biofilm with a supporting substrate is referred to herein as a bioreactor. Generally, the purpose of the bioreactor is to control the location of the microorganisms and permit the collection of products secreted by the microorganisms. In this regard, the shape of the artificial biofilm may aid in collecting the product. For example, if the artificial biofilm is in the shape of a sphere, the products may accumulate within the sphere, and could be collected by removal of the spheres. Alternatively, the artificial biofilm could be made into a thin sheet so that the product could migrate or otherwise collected in a liquid growth medium that is flowed past the sheet.

[0044] Materials for making the various components of the artificial biofilm and the supporting substrate as disclosed herein are known in the art, as are methods for making them.

[0045] The following examples are for purposes of further illustrating the present disclosure. The examples are merely illustrative and are not intended to limit processes or devices made in accordance with the disclosure to the materials, conditions, or process parameters set forth therein.

EXAMPLES

[0046] **FIG. 3** is a picture of a porous metal or ceramic tube, which has been coated with an artificial biofilm in a glass bioreactor.

[0047] **FIG. 4** is a picture of a an artificial biofilm in the shape of a tube. Here, the microorganisms were immobilized using an alginate matrix, with an open celled foam used as a strengthening base. The microorganisms and alginate were embedded in the holes within the open cell foam.

[0048] **FIG. 5** is a picture showing the foam reinforced alginate tube of **FIG. 4** (i.e. an artificial biofilm) with water flowing through the interior.

[0049] **FIG. 6** is a picture of other synthetic biofilms in the shape of tubes. Again, the microorganisms were immobilized using an alginate matrix and a foam strengthening

base. This artificial biofilm surrounds a porous metal screen tube which is used as a supporting substrate.

[0050] **FIG. 7** is a picture of an artificial biofilm that includes a strengthening base. Here, the microorganisms are in an alginate matrix with cloth serving as the strengthening base.

[0051] **FIG. 8** is a picture showing the artificial biofilm in the form of spheres. The spheres are made from microorganisms and alginate.

[0052] **FIG. 9** is a picture showing the alginate spheres of **FIG. 8** being used in a fluidized bed reactor.

[0053] **FIG. 10** is a diagram illustrating the configuration of the fluidized bed reactor. Liquid medium flows into the bioreactor through an inlet tube (labeled "Media In"), carrying the liquid medium to the bottom of the bioreactor vessel. The medium outlet tube (labeled "Media Out") has foam around the opening to assist in retaining the spheres within the reactor. The outlet tube is located at a higher elevation compared to the inlet tube. The gas enters the bioreactor (labeled "Gas In") through a frit in the bottom of the reactor vessel and exits via the medium outlet tube. The buoyancy of the gas bubbles, the flow of the liquid medium, and the density of the spheres induce motion of the spheres, ensuring sufficient mixing and opportunity for nutrient/waste exchange between the spheres, the gas, and the medium.

[0054] The present disclosure has been described with reference to exemplary embodiments. Obviously, modifications and alterations will occur to others upon reading and understanding the preceding detailed description. It is intended that the present disclosure be construed as including all such modifications and alterations insofar as they come within the scope of the appended claims or the equivalents thereof.

CLAIMS:

1. A bioreactor, comprising a synthetic biofilm and a supporting substrate;
wherein the synthetic biofilm comprises bacteria immobilized in a matrix,
wherein the matrix is water permeable and gas permeable.
2. The bioreactor of claim 1, further comprising a membrane on a surface of
the synthetic biofilm.
3. The bioreactor of any of claims 1-2, wherein a liquid flow is provided on
one side of the synthetic biofilm, and a gas flow is provided on an opposite side of the
synthetic biofilm.
4. The bioreactor of any of claims 1-3, wherein the matrix contains alginate,
sol-gel silica, carrageenan, latex, polyvinyl alcohol, polystyrene sulfonate, or mixtures
thereof.
5. The bioreactor of any of claims 1-4, further comprising a strengthening
base dispersed throughout the biofilm.
6. The bioreactor of claim 5, wherein the strengthening base is cloth, metal
or a foam.
7. The bioreactor of any of claims 1-6, wherein the biofilm is in the shape of a
sphere, a sheet, or a tube.
8. The bioreactor of any of claims 1-7, wherein the biofilm is coated on or
embedded within the supporting substrate.
9. The bioreactor of any of claims 1-8, wherein the supporting substrate is in
the shape of a tube or a sheet.

10. The bioreactor of any of claims 1-9, wherein the supporting substrate is a porous metal or ceramic tube.

11. The bioreactor of any of claims 1-10, wherein the supporting substrate is a porous foam in which the biofilm is embedded as a distinct layer.

12. The bioreactor of any of claims 1-11, wherein the microorganisms are bacteria.

13. The bioreactor of any of claims 1-12, wherein the synthetic biofilm comprises bacteria immobilized in an alginate matrix and a porous foam strengthening base, the synthetic biofilm is in the shape of a tube, and a membrane is located on a surface of the synthetic biofilm.

14. A synthetic biofilm, comprising microorganisms immobilized within a matrix, wherein the matrix is water permeable and gas permeable.

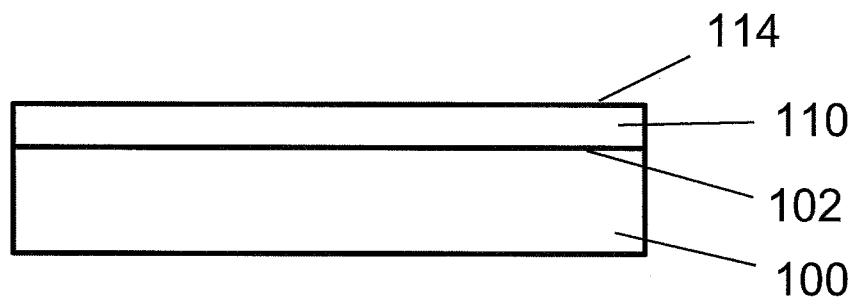


FIG. 1

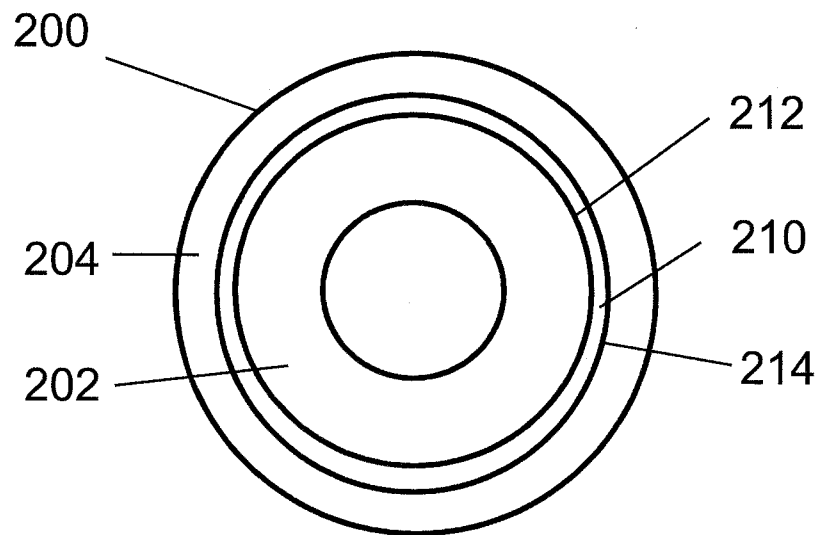


FIG. 2



FIG. 3



FIG. 4



FIG. 5

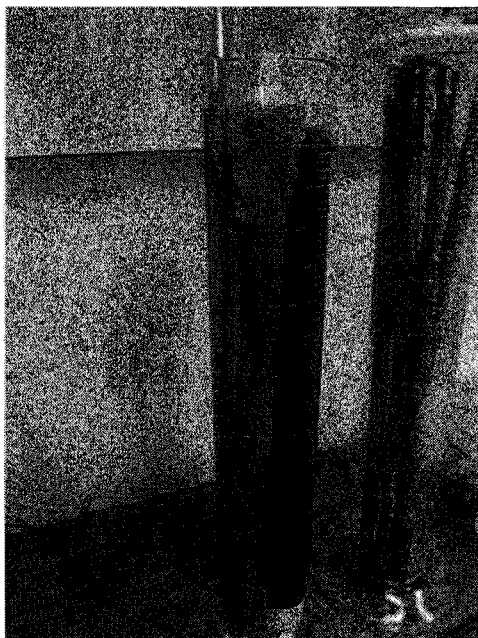


FIG. 6

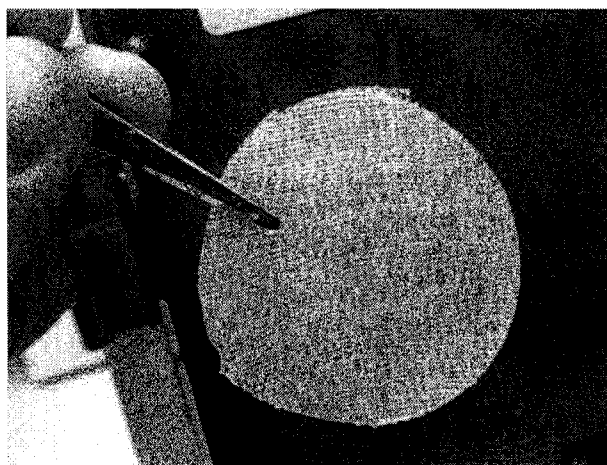


FIG. 7



FIG. 8



FIG. 9

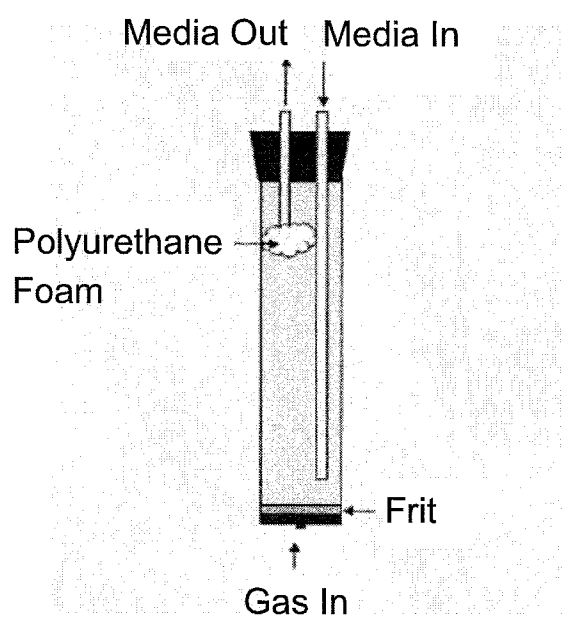


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/53339

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12M 1/00; C12M 3/00 (2013.01)

USPC - 435/298.1, 298.2, 299.1, 299.2

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 C12M 1/00; C12M 3/00 (2013.01)

USPC 435/298.1, 298.2, 299.1, 299.2

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

USPC 435/298.1, 298.2, 299.1, 299.2 (Keyword limited, terms below)

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

PatBase, Google Patents, Google Scholar (NPL); Keywords: BIOFILM BIOREACTOR BACTERIA, synthetic, matrix, membrane, permeable, gas, liquid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0168403 A1 (Corcho-Sanchez et al.) 11 September 2003 (11.09.2003) para [0034], [0039], [0041], [0058], [0068], [0099], [0100]	1-3, 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

06 December 2013 (06.12.2013)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/53339

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.: 4-13
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:

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.:

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.