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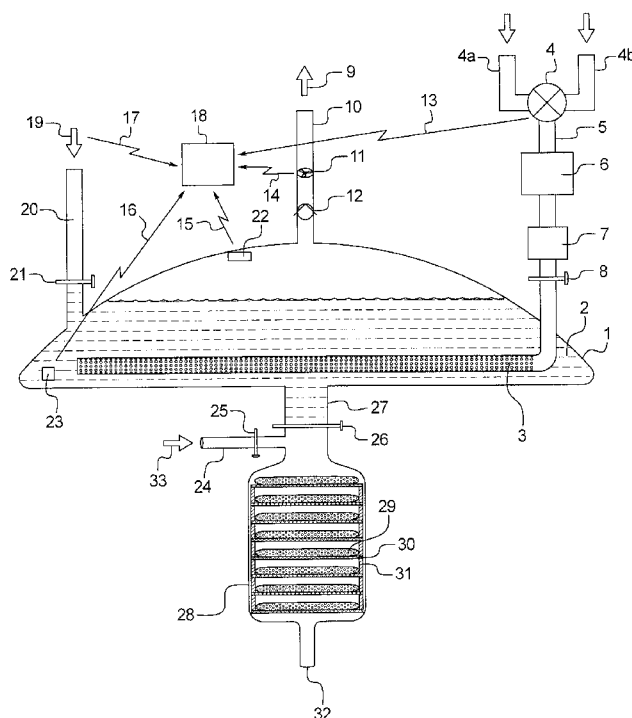
Declarations under Rule 4.17:

— as to the identity of the inventor (Rule 4.17(i))

[Continued on next page]

(54) Title: MULTIPURPOSE BIOREACTOR

FIG. 1



(57) Abstract: A multiuse bioreactor that is a single-use bioreactor, a development bioreactor, a commercial manufacturing bioreactor, a batch, a fed-batch, a perfusion and continuous bioreactor, a convective heat bioreactor, a product capture bioreactor, an ISO 9 bioreactor, a eukaryotic bioreactor, a prokaryotic bioreactor, a technology transfer-free bioreactor, and an inexpensive bioreactor is disclosed.

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- *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))*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12M 3/00, C12M 3/06, C12M 3/02, C12N 5/02, C12M 1/34, C12M 1/12 (2017.01)
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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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0024105 A1 (NIAZI) 23 January 2014 (23.01.2014) Claim 1; Claim 2; Claim 9; Claim 10; Claim 12; para [0003-0004]; para [0012]; para [0014]; para [0016]; para [0018-0019]	1-14
Y	US 2012/0077243 A1 (NIAZI) 29 March 2012 (29.03.2012) para [0014]; para [0019]; para [0028-0029]; para [0037]; para [0092]; Claim 1	1-14
Y	US 2005/0272146 A1 (HODGE et al.) 08 December 2005 (08.12.2005) para [0035]; para [0042]	1-14
Y	US 2014/0170635 A1 (NIAZI) 19 June 2014 (19.06.2014) para [0058]; Claim 1; para [0023]; para [0025]; para [0031]; para [0043]; para [0050]; para [0054]; para [0059]	1-14
Y	US 5,295,448 A (VICKERY) 22 March 1994 (22.03.1994) Abstract	2
Y	US 2015/0210974 A1 (NIAZI) 30 July 2015 (30.07.2015) Claim 1; para [0036]	7
Y	US 2014/0099700 A1 (ENERGAIA, INC) 10 April 2014 (10.04.2014) para [0157]; para [0161]; para [0163];	9

☒ 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/104963 A1 (BAYER HEALTHCARE LLC) 16 September 2010 (16.09.2010) Claim 2; para [0025]; para [0039]	14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

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 bioreactor

Group II: Claims 15-17, 19 drawn to a method for expressing and capturing a biological product

Group III: Claims 18, drawn to a method for optimizing bioprocess conditions

-----Please see continuation in next 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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

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

The inventions listed as Groups I-III 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:

Special Technical Features

Group I requires a bioreactor device, not required by Groups II-III.

Group II requires method steps for expressing and capturing a biological product, not required by Groups I and III.

Group III requires method steps for optimizing bioprocess conditions, not required by Groups I-II.

Common Technical Features

The feature shared by Groups I-III is the bioreactor set forth in claim 1.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2014/0024105 A1 to Niazi (hereinafter 'Niazi '105') in view of US 2012/0077243 A1 to Niazi (hereinafter 'Niazi '243'), US 2005/0272146 A1 to Hodge et al., (hereinafter 'Hodge'), and US 2014/0170635 A1 to Niazi (hereinafter 'Niazi '635').

Niazi '105 teaches a bioreactor comprising:

- a. at least one single-use container with an inner volume, capable of holding a cell culture and culture medium to express a biological product, a top surface, and a bottom surface (Claim 1 "A disposable bioprocessing apparatus intended for receiving a fluid in need of agitation and sparging using a gas, comprising: a first sheet of film material having a first peripheral region; a second sheet of film material having a second peripheral region and overlapping said first sheet of film material such that said first and second peripheral regions of said first and second sheets of film material are joined together so as to form a peripheral region of a bag and to define an interior space within said bag between said first and second sheets of film material"; Claim 12 "The apparatus according to claim 1, wherein said apparatus is used to express recombinant proteins and antibodies"; para [0012] "FIG. 1 is a side view of the apparatus"; Note, Fig.1 shows a top surface and a bottom surface);
- b. at least one liquid inlet disposed in the top surface of the container in (Claim 1 "at least one fluid port in said bag"; para [0012] "FIG. 1 is a side view of the apparatus"; para [0014] "Reference is now made to FIG. 1 . . . The bag may be hermetically sealed and may have one or more openings or fittings 9 for introducing or recovering a fluid 2"; Note, Fig.1 shows one liquid inlet disposed in the top surface of the container);
- c. at least one gas inlet disposed in the top surface of the container (Claim 1 "at least one gas inlet port in said bag"; para [0012] "FIG. 1 is a side view of the apparatus."; para [0014] "Reference is now made to FIG. 1 . . . The bag may be hermetically sealed and may have one or more openings or fittings . . . [for] introducing gas 10 through a sparger 4"; Note, Fig.1 shows one gas inlet disposed in the top surface of the container); d. an inline gas heater or cooler connected to the gas inlet (Claim 10 "The apparatus according to claim 1, further including a means of heating or cooling said fluid in said bag.");
- f. at least one gas sparging unit connected to the gas inlet and disposed in the culture medium (Claim 1 "at least one sparging rod disposed in said middle compartment and connected to a source of sterile compressed gas"; para [0014] "Reference is now made to FIG. 1 . . . The bag may be hermetically sealed and may have one or more openings or fittings . . . [for] introducing gas 10 through a sparger 4");
- g. at least one exhaust gas outlet disposed in the top surface of the container and in fluid communication with outside environment (Claim 1 "at least one gas exhaust port in said bag"; para [0012] "FIG. 1 is a side view of the apparatus."; para [0014] "Reference is now made to FIG. 1 . . . The bag may be hermetically sealed and may have one or more openings or fittings . . . [for] exhausting a gas 8"; Note, Fig.1 shows one gas outlet disposed in the top surface of the container);
- i. at least one liquid outlet (para [0014] "The bag may be hermetically sealed and may have one or more openings or fittings 9 for introducing or recovering a fluid 2");
- j. a movable raised support platform to hold the container (Claim 1 "a hard surface to support said bag");
- k. a mechanical device connected to the platform for shaking, rotating, rocking or vibrating the platform (Claim 1 "a means of agitating said fluid in said bag"; Claim 2 "The apparatus according to claim 1, wherein said means of agitating said fluid inside said bag comprises rocking said hard surface to such an angle as to cause movement of said fluid from said right compartment to said left compartment passing through said middle compartment and vice versa");
- l. a plurality of sensors in communication with an electronic controller to allow control of the condition of a liquid in the container (Claim 9 "The apparatus according to claim 1, further including a sensor for sensing a condition of said fluid in said bag."; para [0019] "It may also be desirable to provide means in the bag to facilitate sensing characteristics of the fluid, such as the pH, oxygen content, temperature, etc. Preferably these sensors are of disposable type and embedded in the bag and remote receivers monitor the response to the sensors").

Niazi '105 does not expressly state the liquid inlet is in fluid communication with a plurality of sources of liquids including a culture medium, a cell culture, a pH adjusting solution, and a nutritive solution. Niazi '243 teaches the liquid inlet is in fluid communication with a nutritive solution such as culture medium (para [0037] "a liquid port 17 connected to a source of nutrient medium through inlet 5, a liquid media sterilizing filter 6 disposed between the inlet 5 and liquid port 17 and a stopcock 12 disposed between the sterilizing filter 6 and the liquid port 17."). Since it is well known that cell culture in a bioreactor requires a means to adjust pH to maintain optimal pH in the bioreactor, it would have been obvious to one of ordinary skill that additional inlets for a pH adjusting solution and for introducing the cell culture into the bioreactor, could be connected to the bioreactor in a manner analogous to that of the liquid medium inlet port.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

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

Niazi '105 also does not expressly state the gas inlet is in fluid communication with a source of at least one nutritive gas and at least one inert gas. Niazi '243 teaches the gas inlet is in fluid communication with a source of at least one nutritive gas (para [0037] "a fresh air inlet 18 to which is connected at least one means of sparging 4, a source of fresh gas through fresh air inlet 8, a gas sterilizing filter 7 disposed between gas inlet 18 and fresh air inlet 8"). Niazi '243 further teaches recirculating an inert gas into the bioreactor (para [0014] "Since there are only two gases that are of importance, oxygen and carbon dioxide, there is no reason why nitrogen and argon cannot be re-used coming out of the exhaust of a bioreactor. This is an inventive step wherein only the useful components in the air are utilized while allowing use of a completely closed system."). It would have been obvious to one of ordinary skill that as an alternative to recirculating inert gases, inert gases could be provided along with the oxygen supplied through the gas inlet.

Niazi '105 also does not expressly state the means for heating or cooling the liquid comprises an inline gas heater or cooler connected to the gas inlet. Niazi '243 teaches recirculating an inert gas into the bioreactor (para [0014] "Since there are only two gases that are of importance, oxygen and carbon dioxide, there is no reason why nitrogen and argon cannot be re-used coming out of the exhaust of a bioreactor. This is an inventive step wherein only the useful components in the air are utilized while allowing use of a completely closed system."). Since the recirculating gas also has the same temperature as the liquid contained in the bioreactor, it would have been obvious to one of ordinary skill in the art that temperature of the liquid in the bioreactor could be modulated via modulating the temperature of the gas passing through the liquid, and could be accomplished by using an inline gas heater or cooler connected to the gas inlet.

Niazi '105 also does not expressly state the use of an inline gas sterilizing filter connected to the gas inlet. Niazi '243 teaches the use of an inline gas sterilizing filter connected to the gas inlet (Claim 1 "e. a gas sterilizing filter installed between the source of fresh gas and the gas inlet"). Given that Niazi '105 teaches that contamination of the bioreactor leads to degradation of the product (para [0003] "One significant limitation or shortcoming of such an arrangement is the danger of contamination or leakage during mixing. . . . This opening provides an opportunity for bacteria or other contaminants to enter, which of course can lead to the degradation of the product. A corresponding danger of environmental contamination exists in applications involving hazardous or toxic fluids, or suspensions of pathogenic organisms, since dynamic seals or bearings are prone to leakage.") and suggests use of pre-sterilized containers for the bioreactor (para [0004] "0004) In an effort to overcome these problems, the recent trend in the biotechnology industry is to use disposable plastic bags for a number of bioprocessing steps. Pre-sterilized disposable plastic bags eliminate the need for cleaning, sterilization and validation of the containers after each bioprocessing batch"), one of ordinary skill in the art would have found it obvious to use an inline gas sterilizing filter, as taught by Niazi '243, in order to maintain the sterile environment within the bioreactor and avoid unwanted contamination.

Niazi '105 also does not expressly state the gas exhaust further comprises a oneway exhaust gas flow control valve and an inline vent fan. Niazi '243 teaches the gas exhaust further comprises a oneway exhaust gas flow control valve (para [0037] "The exhaust air escapes through gas outlet 11 and moved by using a peristaltic pump 9 and through a stopcock 12 for recirculation through gas inlet 18 and sparger 4"). Since a peristaltic pump enables a unidirectional gas flow, it would have been obvious to one of ordinary skill in the art that a device such as a peristaltic pump or an inline vent fan to ensure a unidirectional air flow could be used.

Niazi '105 also does not expressly state the use of h. a pressure sensor disposed in the container and connected to an electronic controller to adjust exhaust gas flow to maintain a positive pressure inside the container continuously. Hodge teaches the use of a pressure sensor (para [0035] "Pressure may be monitored and controlled by standard pressure transducers upstream of an inlet air filter and downstream of an exhaust air filter. Alternatively disposable pressure sensors can be used inside the sterile envelope of the disposable bioreactor, either by taking off the shelf devices (e.g. Utah Medical or Honeywell) or by creating a tee on the air inlet and/or exhaust line."). Given that Niazi '105 teaches use of sensors for sensing conditions in the bioreactor (para [0019] "It may also be desirable to provide means in the bag to facilitate sensing characteristics of the fluid . . . Preferably these sensors are of disposable type and embedded in the bag and remote receivers monitor the response to the sensors"; claim 9 "a sensor for sensing a condition of said fluid in said bag"), one of ordinary skill in the art would have found it obvious that the bioreactor of Niazi '105 can further comprise the pressure sensor of Hodge.

Niazi '105 also does not expressly state the liquid outlet is disposed in the bottom surface of the container, further comprising a liquid flow control valve or that the support platform is movable and raised with an opening to allow passage of the liquid outlet to pass through the support platform. Niazi '635 teaches a bioreactor design wherein the liquid outlet is disposed in the bottom surface of the container, further comprising a liquid flow control valve (para [0058] "The bioreactor consists of a two dimensional disposable flexible bag (1) resting on a support surface (10), which is capable of being tilted and further resting on a frame (9), a means of gassing consisting of a gas inlet (2), a gas filter (3) and a sparging tube (4). The flexible bag (1) further contains a gas outlet (5), a nutrient medium inlet (6), a liquid drain (14) controllable by a stopcock (13)"). Since the device of Niazi '635 could also be used exclusively for its purification utility in a manner analogous to a chromatography column well known in the art, it would have been obvious to one of ordinary skill in the art that the device of Niazi '635 could be connected to the bioreactor of Niazi '105 to combine the advantages of both devices in a single design.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/13228

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

Niazi '105 also does not teach m. a capture column disposed under the support platform and above ground, holding a binding resin and connected to the liquid outlet to receive liquid from the container further comprising a process liquid inlet, a process liquid outlet and a process liquid outlet control valve. Niazi '635 teaches a device that serves to hold a binding resin and further comprises a process liquid inlet, a process liquid outlet and a process liquid outlet control valve (Claim 1 "A method for producing and separating a biological product from a liquid comprising: a. providing a separative bioreactor comprising: (1) a container suitable for growing a biological culture, wherein the container further comprises a liquid inlet and a drain; and (2) at least one pouch comprising a porous material with a plurality of pores and capable of housing a binding resin, wherein the pouch is placed inside the container, and wherein the pouch optionally comprises at least one resin inlet/outlet port directly connected to the pouch; b. introducing nutrient medium into the container; c. introducing biological culture into the container; d. growing of the biological culture by agitating the liquid; e. determining the concentration of biological product in the liquid at periodic intervals; f. introducing resin into the pouch, when the concentration of biological product reaches a desired amount, through the resin inlet sufficient to bind the biological product determined in step (e); g. determining the concentration of biological product in the liquid until it reaches a predetermined low value; h. removing the liquid from the container through the drain and discarding it; and (1) adding to the container a sufficient quantity of a buffer capable of removing the biological product from the resin and collecting the buffer for further purification of the biological product; or (2) removing the resin by aspirating the resin through the resin port and subjecting the resin to further steps of purification outside the container.")). Since the device of Niazi '635 could also be used exclusively for its purification utility in a manner analogous to a chromatography column well known in the art, it would have been obvious to one of ordinary skill in the art that the device of Niazi '635 could be connected to the bioreactor of Niazi '105 to combine the advantages of both devices in a single design. Since Niazi '105, Niazi '243 and Niazi '635 teach various design aspects of a single use bioreactor and Hodge teaches a pressure sensor that could be used with such a bioreactor, it would have been obvious to one of ordinary skill in the art of biotechnology, to combine the aspects of Niazi '105, Niazi '243 and Niazi '635 with Hodge, to create an improved design for a single use bioreactor.

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

The feature shared by Groups II-III are the method steps of expressing and collecting a biological product.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Niazi '635.

Niazi '635 discloses expressing and collecting a biological product in a bioreactor (para [0003] "The present invention relates to a novel bioreactor design for expressing and separating a biological product from other components in a bioreaction broth, which combines the step of expressing and separating within the bioreactor by binding the biological product with a resin within a bioreactor"; para [0046] "the instant invention proposes a bioreactor capable of growing all types of cells and organisms and additionally provides a ready means of harvesting of biological products in a bioreactor"; para [0049] "the instant invention provides a means of continuously removing expressed biological product from a culture media to enhance the level of expression that may be depressed because of the higher concentration of biological product in the mixture").

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Another feature shared by Groups II-III is a plurality of containers.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Niazi '243.

Niazi '243 teaches the use of a plurality of containers as bioreactors (para [0015] "using disposable flexible containers as bioreactors"; para [0092] "The containers useful in the various embodiments of the invention may be of any size suitable for containing a nutrient medium"; para [0094] "It should also be understood that many of the components described herein also are desirably flexible, e.g., the containers desirably comprise flexible biocompatible polymer containers (such as collapsible bags)").

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-III therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.