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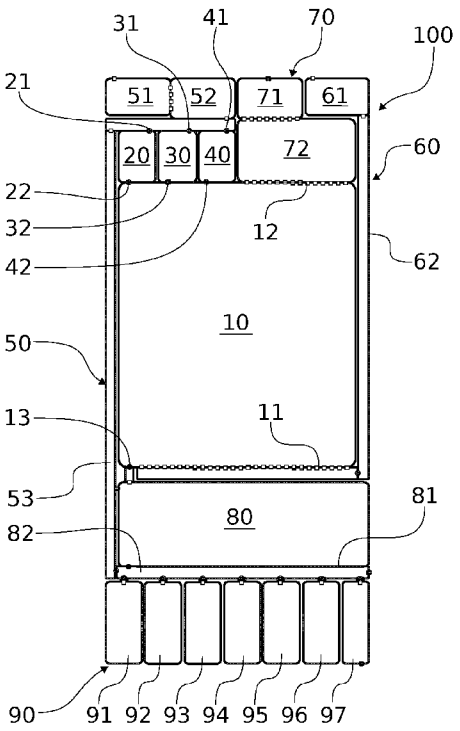

FIG. 1

(57) Abstract: A self-contained and easy-to-use cultivation unit for amateurs is herein proposed. The unit includes a sterile boundary isolating the cultivation unit from external contaminants, a cultivation chamber (10), a water inlet (50) configured to feed water into the cultivation chamber (10), and an air inlet (60) configured to feed air into the cultivation chamber (10). A first auxiliary chamber (20) is connected to the cultivation chamber (10) via a passageway (22), which may be manipulated from a closed state to an open state so as to introduce a substance contained in the cultivation chamber (10) and a substance contained in the first auxiliary chamber (20) to each other.

[Continued on next page]

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CULTIVATION UNIT AND SET FOR CULTIVATING CELLS

FIELD

[0001] The present disclosure relates to cultivation of plant cells, particularly for the production of food. More specifically, the disclosure relates to a cultivation unit and set for cultivating cells, particularly plant cells.

BACKGROUND

[0002] Cultivation bags for growing cells in a controlled and sterile environment have recently gained popularity. One example of a cultivation bag is proposed by US 20090233334 A1, which discloses a bag defining a cultivation chamber, which has been equipped with air inlets and outlets, which are simple ports made to the otherwise enclosed cultivation chamber. The ventilation of the cultivation bags has been further developed by US 20130082410 A1, which proposes sparger rings welded into the bag so as to transport gases from outside the cultivation chamber, along a pathway and through a porous sparger ring into the cultivation chamber.

[0003] While quite sophisticated, the known cultivation bags typically require trained professionals to set-up and execute cultivations. There is therefore a need for a cultivation unit, which would be self-contained and easy to use for amateurs. It is therefore an object of at least some herein disclosed embodiments to provide a cultivation chamber that is not only sterile but also usable in non-laboratory settings.

SUMMARY

[0004] A novel cultivation unit for cultivating cells is therefore herein proposed. The unit includes a sterile boundary isolating the cultivation unit from external contaminants, a cultivation chamber, a water inlet configured to feed water into the cultivation chamber, and an air inlet configured to feed air into the cultivation chamber. A first auxiliary chamber is connected to the cultivation chamber via a passageway, which may be manipulated from a closed state to an open state so as to introduce a substance contained in

the cultivation chamber and a substance contained in the first auxiliary chamber to each other.

[0005] The invention is defined by the features of the independent claims. Some specific embodiments are defined in the dependent claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Next, certain embodiments of the novel proposition are described in greater detail with reference to the accompanying drawings, in which:

FIGURE 1 illustrates a schematic layout of a cultivation unit according to one embodiment;

FIGURE 2A illustrates a schematic lateral cross-sectional view of the cultivation unit of FIGURE 1 showing the structure of a filter between two superposed film layers;

FIGURE 2B illustrates a schematic cross-sectional view of the cultivation unit of FIGURE 1 showing the structure of a filter between two superposed film layers as seen in direction of the flow;

FIGURE 3A illustrates a side elevation view an actuated passageway between the first and second chamber;

FIGURE 3B illustrates an explosion view of the device of FIGURE 3A;

FIGURE 3C illustrates a perspective view of the device of FIGURE 3A;

FIGURE 4 illustrates a schematic layout of a cultivation unit according to another embodiment;

FIGURE 5 illustrates a schematic layout of a variant of the cultivation unit according to FIGURE 4, and

FIGURE 6 illustrates a schematic layout of a variant of the cultivation unit according to FIGURE 5.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0007] As shown in FIGURE 1, the cultivation unit 100 according to one embodiment takes the form of a single-use unit intended to be used as a stand-alone device. The cultivation unit 100 is preferably constructed as a disposable bag that is self-contained in that it can be attached to air and water supplies at a household or other non-laboratory setting. For that purpose the cultivation unit 100 is delimited by a sterile boundary enabling the unit to be attached, for example, to a household water tap. In other words, all the chambers of the cultivation unit 100 are within the sterile boundary. In particular, all the components of the cultivation unit that cater for the production of cells are delimited by the sterile boundary. These components include namely the chambers and the inlets that reside within the sterile boundary. Firstly, the sterile boundary is provided by sealing the outer perimeter of the unit. Secondly, the inlets to the inside of the sealed perimeter have been provided with sterile filters. In other words, the filters are integrated into the cultivation unit so as to form part of the sterile boundary. In this context the term *sterile filter* refers to a filter, which has a pore size of at most 0.22 μm. The cultivation unit 100 contains an air inlet 60 for feeding air into the bag. The air inlet 60 has been provided with an inlet air filter 61, which is a sterile filter. The inlet air filter 61 may include a port for forced aeration (not shown), such as a tube connection to an air pump. The cultivation unit 100 also includes a water inlet 50 with a preferred two-stage filtering configuration. The water inlet 50 includes a carbon filter 51, which receives water from outside the unit 100 and passes the carbon filtered water onto a sterile filter 52 arranged in series with the carbon filter 51. Accordingly, the perimeter and inlets of the cultivation unit 100 form a sterile boundary.

[0008] As mentioned above, the cultivation unit 100 may be constructed as a bag. The material for the bag could be selected from a host of different materials non-permeable to water. Preferably the material is a food-grade polymer, which is pliable and transparent so that resulting bag will be supple and see-through. Such materials include, for example, polypropylene and polyethylene. In this context the expression *pliable* refers to a material or structure that is flexible enough to endure bending during transport and normal use. The unit is therefore supple enough to withstand handling and will experience flexible deformation when subjected to a load equal to its own weight. One particularly suitable material is low-density polyethylene film. The material may have the properties as set forth in Table 1.

Table 1: Material properties for the cultivation unit.

LDPE film	**Nominal value**	**Unit**	**Test Method**
Film Thickness - Tested	50	µm	
Secant Modulus - 2% Secant, (50 µm)	190	MPa	ASTM D882
Tensile Strength - (Yield, 50 µm)	10	MPa	ASTM D882
Tensile Strength - (Break, 50 µm)	22	MPa	ASTM D882
Tensile Elongation - (Break, 50 µm)	450	%	ASTM D882
Dart Drop Impact (50 µm)	110	g	ASTM D1709A
Elmendorf Tear Strength - (50 µm)	500	g	ASTM D1922
Vicat Softening Temperature	96	°C	ISO 306/A
Gloss (20°, 50.0 µm)	80		ASTM D2457
Haze (50.0 µm)	6,90	%	ASTM D1003
Water Vapour Transmission Rate (23°C, 50% RH) 50 µm	1.17	$g/m^2/day$	ASTME-96
Water Vapour Transmission Rate (38°C, 90% RH) 50 µm	9.58	$g/m^2/day$	ASTME-96
Oxygen Transmission Rate (23°C, 50% RH) 50 µm	4360	$mL/m^2/day$	ASTM D3985

[0009] The perimeter of the bag may be formed by superposing two layers of the sheet raw material and sealing the edges together by plastic welding, for example. Openings are left for the water and air supplies 50, 60. The filters 51, 52, 61 may be integrated into the cultivation unit 100 by enclosing the filters 51, 52, 61 by the superposed sheet layers of the cultivation unit 100. One such construction is presented in FIGURES 2A and 2B. As can be seen best from FIGURE 2A, there are two superposed layers 101, 102 attached to each other along a seam 103. The first film layer 101 is open at one end (left in FIGURE 2A) and a closed at the opposite end (right in FIGURE 2A). The second film layer 102 is also open at one end (right in FIGURE 2A) and closed at the opposite end

(left in FIGURE 2A), but reversed in respect to the first film layer 101. The filter 81 is arranged between the film layers 101, 102 and attached there to with the seam 103, which may be a plastic weld seam, adhesive seam, etc. The filter 81 therefore divides the inner volume of the bag in two. The filter may be any of the filters herein described. The entry flow indicated with a thickened dashed arrow line I entering the bag at one end cannot exit through the opposite end. Instead, it is forced to go through the filter. Conversely, the filtered flow must exit from the open end of the second film layer as an exit flow indicated thickened dashed arrow line O. FIGURE 2B shows the same structure from a different angle so as to demonstrate the bag-like shape of the unit.

[0010] The cultivation unit 100 contains several sub-volumes, which may be brought into fluid communication with one another. Firstly, the cultivation unit 100 includes a cultivation chamber 10 with dimensions set so as to be able to accommodate the substances needed for the cultivation process. The cultivation chamber 10 is also connected to the air inlet 60. The air inlet 60 may be a section of a supple bag divided into a conduit to pass air into the bag, as shown in FIGURE 1. According to an alternative embodiment, the air inlet 60 may be a separate tube or other profile attached to allow the passage of air into the cultivation chamber 10. The cultivation chamber 10 is therefore in fluid communication with the ambient space or with a connected air source through the inlet air filter 61. The inlet air filter 61 is connected to the cultivation chamber 10 via a channel 62, which leads the air to the cultivation chamber 10 through an inlet aeration port 11. In the illustrated example the inlet aeration port 11 is located at the bottom of the cultivation chamber 10. The inlet aeration port 11 may be provided by making a portion of the seam defining the cultivation chamber 10, i.e. the bottom portion, discontinuous so as to arrange small openings being permeable to air but impermeable to the cell mass inside the cultivation chamber 10. Accordingly, the bottom seam of the cultivation chamber 10 is perforated for aeration. The seam separating the cultivation chamber 10 from the channel 62 could alternatively or additionally be perforated. In the illustrated example, however, the channel 62 is separated from the cultivation chamber 10 with a solid seam and shaped to extend under the cultivation chamber 10 along its entire length to ensure sufficient air supply to the entire biomass being cultivated including the bottom part of the mass.

[0011] The cultivation unit 100 also has a first auxiliary chamber 20, which is or can be separated from the cultivation chamber 10. The purpose of the first auxiliary chamber 20 is to include a substance needed to cultivate the cells in the cultivation chamber 10. For

example, the cultivation chamber 10 could initially contain the inoculum and the first auxiliary chamber 20 could include a nutrient for the inoculum, or vice versa. It is preferable that the inoculum and nutrient are kept separate before the cultivation process. Indeed, it is particularly preferable to keep the inoculum and nutrient in a dehydrated or dry condition before cultivation. Accordingly, the cultivation and first auxiliary chamber 10, 20 should be kept separate before cultivation. For this purpose, the cultivation and first auxiliary chambers 10, 20 are initially separated from each other. The chambers may be formed into the bag by plastic welding a seam connecting the sheet materials so as to define to sub-volumes of the bag. There should, however, be an opening in the seam to allow flow between the sub-volumes in a controlled manner. A convertible passageway able to be converted from a closed state to an open state is therefore needed to connect the chambers 10, 20 to each other. To be more specific, fluid flow between the cultivation chamber 10 and the first auxiliary chamber 20 is prevented in the closed state of the passageway 22 and enabled only in the open state.

[0012] Such a passageway 22 may be provided in many different ways. According to one option, the convertible passageway 22 is constructed as floodgate. The floodgate may be set up as a sacrificial wall, separating the cultivation and first auxiliary chambers 10, 20 from each other in the closed state and being configured to be opened so as to enable fluid flow between the chambers 10, 20 in an open state. The wall may be made by connecting the two sheet layers of the raw material of the bag into a brittle or breakable seam, which may be broken. In a broken state, the two layers of the sheet material will be separated thus allowing for a fluid flow between the cultivation and first auxiliary chamber 10, 20. Such a sacrificial wall would be an irreversible floodgate.

[0013] According to another embodiment, the floodgate can be set up as reversible, whereby the two layers of sheet material would be separated from each other but perforated such to allow for a wall acting as the floodgate to be slid across to enable fluid flow. Such a construction would, however, require fine sealing between the floodgate and the layers of sheet material.

[0014] According to a further embodiment, the passageway 22 is constructed as two-component inter-engagement member. The inter-engagement member can include two mutually fitting components provided to the superposed layers of sheet material. One commercial option would be to use a so called MiniGrip® attachment. The components are

preferably configured to be manipulated from outside the cultivation unit 100 so as to fit each other in the closed state for separating the cultivation and first auxiliary chambers 10, 20 from each other, and to disengage from each other in the open state so as to enable fluid flow between the chambers 10, 20.

[0015] According to a yet further embodiment, the passageway contains an external divider including a female piece, which has a recess for receiving the bag, which in turn is supple enough to conform to the shape of the recess. The external divider also has a male piece designed to snap into the recess of the female piece and onto the bag and in doing so pressing the bag there between so as to block fluid flow in the back across the external divider. To ensure proper fit, the female piece is designed to be resilient enough to elastically deform upon insertion of the male piece, whereby the internal spring force will keep the female piece tightly on the male piece. To enable flow between the cultivation and first auxiliary chamber 10, 20, the male piece is removed from the female piece, whereby the pressure keeping the layers of sheet material against each other is removed and the substance kept separately in the cultivation and first auxiliary chambers 10, 20 may be brought into contact with each other.

[0016] In addition to an air source, the cultivation chamber 10 is connected to a water source via the water inlet 50. The water inlet 50 is constructed to feed water into the cultivation chamber 10 through the first auxiliary chamber 20. The water inlet 50 may be a section of a supple bag divided into a conduit to pass water into the bag, namely to the cultivation chamber 10, as shown in FIGURE 1. According to an alternative embodiment, the water inlet 50 may be a separate tube or other profile attached to allow the passage of water into the cultivation chamber 10. It is to be pointed out that water is only fed to the cultivation chamber 10 during the cultivation process. The water inlet 50 is particularly designed to flush the contents of the first auxiliary chamber 20 into the cultivation chamber 10 when the passageway 22 is open. For keeping the first auxiliary chamber 20 dry before the cultivation process is initiated, the contact point between the first auxiliary chamber 20 and the water inlet is provided with a convertible passageway 21, which in a closed state prevents water from entering the first auxiliary chamber 20 but enables water flow in an opened state. The inlet passageway 21 of the first auxiliary chamber 20 may be constructed similarly to outlet passageway 22. The water inlet 50 comprises a channel 53 running from the sterile filter 52 past the first auxiliary chamber 20, the inlet passageway 21 of which is tapped into the channel 53. The remaining portion of the channel 53 is discussed here after.

[0017] The cultivation unit may also include more auxiliary chambers connected to the cultivation chamber similarly to the first auxiliary chamber 20. In the embodiment of FIGURE 1, the cultivation unit also includes a second and third auxiliary chamber 30, 40 connected to the cultivation chamber 10 via passageways 32, 42, respectfully. On the other hand the second and third auxiliary chambers 30, 40 are tapped into the channel 53 of the water inlet 50 similarly to the first auxiliary chamber 20 via inlet passageways 31, 41, respectively. In this sense, the auxiliary chambers 20, 30, 40 are connected in parallel to the channel 53 of the water inlet 50. In use, the auxiliary chambers 20, 30, 40 may contain supplements to the inoculum contained in the cultivation chamber 10 that may be introduced to the cultivation chamber 10 in several stages. The use of the cultivation unit 100 will be discussed in greater detail here after.

[0018] FIGURE 1 also shows further connections of the cultivation chamber 10. As mentioned below, the air inlet 60 supplies air to the cultivation chamber 10 from below. The air is lead out of the cultivation chamber 10 through an outlet aeration port 12, which can have a similar structure to the inlet aeration port 11. The outlet aeration port 12 is designed to exhaust excess gases being produced by the cultivation process through an exhaust air port 70. The outlet aeration port 12 is connected to an exhaust air condenser 72, which prevents excess evaporation of moisture during cultivation. The exhaust air condenser 72 can be, for example a metal body cooled by Peltier element. The second stage of the exhaust air port 70 is an exhaust air filter 71 connected to the exhaust air condenser 72 for providing a sterile barrier between the cultivation volume and the outside environment. The exhaust air filter 71 may be a similar construction as the inlet air filter 61, for example.

[0019] The bottom of the cultivation chamber 10 includes an outlet 13 for the cultivated biomass. The outlet 13 may simply be an opening equipped with a convertible passageway, such as one described above. The outlet 13 will preferably lead to a harvest chamber 80 located below the cultivation chamber 10 in the illustrated embodiment. The harvest chamber 80 gathers the biomass produced in the cultivation chamber 10 via the outlet 13 and distributes the biomass to be collected to several collecting containers.

[0020] A collector 90 is provided to the harvest chamber 80 for collecting the ready product there from. According to the embodiment of FIGURE 1, the collector 90 comprises several collecting chambers, namely seven collecting chambers 91 – 97, which

are connected to the harvest chamber 80 via a particular feeder channel 82. As shown in FIGURE 1, the feeder channel 82 may simply be the bottom section of the harvest chamber 80 separated by a filter 81 for enabling a flush of the harvested product. When the cultivation unit 100 is constructed as a supple bag, the cultivated biomass may be pushed into the collecting containers 91 – 97 by applying pressure to the harvest chamber 80 by hand, for example. To enhance hygiene the channel 53 of the water inlet 50 leads water to the feeder channel 82 for washing the product. Accordingly, it is preferable to equip the connection between the water channel 53 and the feeder channel 82 with a convertible passage way, such as on described above. The collecting containers 91 – 97 connected to the feeder channels 82 may be bags made from the same material as the rest of the cultivation unit 100 but such seamed as to be separated from one another and from the feeder channel 82. The seam surrounding the collecting containers 91 – 97 may therefore be made strong enough to keep the collecting containers 91 – 97 attached during normal use but weak enough to break under fatigue.

[0021] A cultivation unit 100 such as that shown in FIGURE 1 may be used as part of a cultivating kit produced as a self-contained source of biomass and intended to be use by amateurs in a non-laboratory environment. The kit includes a cultivation unit and the ingredients for producing biomass except for water and air, which is to be sourced from said non-laboratory environment, such as tap water and ambient air. The cultivation unit may be single-use unit, which is to be discarded after use, or it may be returned to the seller after use for replenishment depending on the structure of the cultivation unit. Preferably the ingredients are stored in the cultivation unit separately in different chambers so as to prevent the cultivation process from starting during storage. Ideally, the ingredients are dry, which means that the dry weight content of the ingredients is 25 weight per cent or more, preferably 30 weight per cent or more.

[0022] The ingredients of the kit include an inoculum for cultivating cells, preferably edible cells, particularly plant cells. Examples of suitable inocula include:

arctic bramble (*Rubus arcticus*): several lines,

cloudberry (*Rubus chamaemorus*),

stone berry (*Rubus saxatilis*),

raspberry (*Rubus idaeus*),

strawberry (*Fragaria x ananasa*): two lines,

bilberry (*Vaccinium myrtillus*),

cranberry (*Vaccinium oxycoccos*),

lingonberry (*Vaccinium vitis-idaea*),

crowberry (*Empetrum nigrum*),

rowanberry (*Sorbus aucuparia*),

birch cells (*Betula pendula*),

honeysuckle (*Lonicera caerulea var. kamtschatica*),

scurvy-grass (*Cochlearia*),

barley (*Hordeum vulgare*),

oat (*Avena sativa*),

cress (*Lepidium sativum*, *Cardamine amara*, *Nasturium officinale*, *Barbarea vulgaris*, *Cochlearia officinalis*, *Trapaeolum maius*),

corn salad (*Valerianella locusta*),

dandelion (*Taraxacum officinale*),

taro (*Colocasia esculenta*),

sago palm (*Metroxylon sagu*),

jackfruit (*Artocarpus heterophyllus*),

sugar maple (*Acer saccharum*),

bean (*Vicia faba, V. spp.*),

chick pea (*Cicer arietinum*),

olive tree (*Olea europaea*),

Raphanus spp., *Eruca vesicaria,*

sesame (*Sesamum indicum*),

cotton (*Gossypium* spp.),

flax (*Linum usitatissimum*),

hazel (*Corylus avellana*),

beech (*Fagus sylvatica*),

parsnip (*Pastinaca sativa*),

black salsify (*Scorzonera hispanica*),

salsify (*Tragopogon porrifolium*),

turnip-rooted chervil (*Chaerophyllum bulbosum*),

japanese artichoke (*Stachys affinis*),

florence fennel (*Foeniculum vulgare*),

bamboo (*Bambusa vulgaris*, *Dendrocalamus asper*, *Gigantochloa* spp., *Phyllostachys pubescens*, *Dendrocalamus latiflorus*),

seakale (*Crambe maritima*),

cardoon (*Cynara cardunculus*),

rhubarb (*Rheum rhabarbarum*),

garden sorrel (*Rumex rugosus*, *R. patientia*),

New Zealand spinach (*Tetragonia tetragonioides*),

sweet chestnut (*Castanea sativa*),

water chestnut (*Trapa biscornis*),

Sechium edule, *Lagenaria siceraria*, *Luffa aegyptiaca*, *Momordica charantia*, *Trichosanthes cucumerina,*

Pinus spp.,

Picea abies,

Larix spp.,

Tilia spp.,

Quercus spp.,

Acer spp.,

pomegranate (*Punica granatum*),

granadille (*Passiflora edulis*),

litchi (*Litchi chinensis*, *Nephelium lappacum*),

mangosteen (*Garcinia mangostana*),

goosberry, red currant (*Ribes uva-crispi*, *R. rubrum*),

water melon (*Citrullus lanatus*),

guava (*Psidium guajava*),

persimmon (*Diospyros kaki*),

Chinese Goosberry (*Actinidia chinensis*),

Cornelian cherry (*Cornus mas*),

sea buckthorn (*Hippophae rhamnoides*),

elder (*Sambucus niger*),

West Indian cherry (*Malpighia glabra*),

quince (*Cydonia oblonga*),

loquate (*Eriobotrya japonica*),

Annona spp.,

medlar (*Mespilus germanica*),

pineapple (*Ananas comosus*),

fig (*Ficus carica*),

mulberry (*Morus alba*, *M. nigra*),

Paraguay tea (*Ilex paraguariensis*),

cola tree (*Cola* spp.),

guarana (*Paullinia cupana*),

liquorice (*Glycyrrhiza glabra*),

Stevia reboudiana,

ginger (*Zingiber officinale*, *Alpinia officinarum*),

laurel (*Laurus nobilis*),

common rue (Ruta graveolens),

dill (*Anethum graveolens*),

salad chervil (*Anthriscus cerefolium*),

lovage (*Levisticum officinale*),

purslane (*Portulaca oleracea*),

borage (*Borago officinalis*),

hyssop (*Hyssopus officinalis*),

lemon balm (*Melissa officinalis*),

summer savory (*Satureja hortensis*),

Artemisia abrotanum, *A. absinthium*, *A. dracunculus,*

clove tree (*Syzygium aromaticum*),

black cummin (*Nigella sativa*),

nutmeg (*Myristica fragrans*),

cardamome (*Elettaria cardamomum*),

allspice (*Pimenta dioica*),

caraway (*Carum carvi*),

coriander (*Coriandrum sativum*),

anise (*Pimpinella anisum*),

star anise (*Illicium verum*),

juniper (*Juniperus communis*),

capers (*Capparis spinosa*),

white mustard (*Sinapis alba*),

woodruff (*Asperula odorata*), and

stinging nettle (*Urtica dioica*) as well as

Algae, such as *Chlorella sorokiniana* and *Chlorella vulgaris*, and

Cyanobacterium, such as *Arthrospira platensis* and *Arthrospira maxima*.

[0023] In addition to edible plant cells the kit may also be set up for cultivating a host of different biological matter including bacteria, yeast, fungus, mould, etc. by including an appropriate inoculum in the cultivation unit 100.

[0024] Initially, the inoculum may be included in the cultivation chamber 10 or the inoculum may be contained in one of the auxiliary chambers 20 – 40. The ingredients also include a nutrient contained in the same chamber as the inoculum or preferably in a separate chamber, where from the nutrient may be introduced to the inoculum for cultivation by opening the separating passageway. For example, the inoculum may be contained in the cultivation chamber 10 and the nutrient may be contained in the first auxiliary chamber 20, whereby the two ingredients may be mixed by converting the passageways 21, 22 to an open state thus enabling water flow to the first auxiliary chamber 20. The water flowing through the first auxiliary chamber 20 flushes the nutrient to the cultivation chamber 10 and the cultivation process may begin. The cultivation chamber 10 may receive air from the air inlet 60 constantly or upon connecting the air inlet to an external pump (not shown). Naturally, the water inlet 50 must be connected to a water source, such as a tap, before use.

[0025] The kit may also include other ingredients, which are preferably kept separate from the above-mentioned ingredients before actively introduced to one another. For example, the kit may include a second nutrient contained originally in a separate chamber, such as the second auxiliary chamber 30. Similarly to the first nutrient, the second nutrient may be flushed into the cultivation chamber 10 by converting the inlet and outlet passageways 31, 32 into an open state thus allowing water to flow through the second auxiliary chamber 30. That way, the second nutrient may be introduced to the cultivation process later than the first nutrient. In the beginning of the cultivation process the sugar (carbon source) concentration in the cultivation may not be very high (such as >200 g/L) since it may inhibit growth of the cells. The same may apply to other nutrients as well such as ammonium (nitrogen source). Thus, it is possible to prolong the cultivation and produce more cell material by adding more nutrients during the cultivation.

[0026] Further foreseeable ingredients include additional formulations, which may be used in later stages of the cultivation process. Such additional formulations may be included in yet further chambers, such as the third auxiliary chamber 40. The additional formulation may take the form of substances regulating the flavor or texture (e.g. viscosity) of the produced biomass. Exemplary additional formulations include natural or synthetic flavours, fragrances, pigments (colours) and/or their respective precursors for example: flavor/fragrance vanillin; flavor precursor betuligenol; pigment anthocyanin, carotenoid etc. Similarly to the nutrients, additional formulations will be flushed from the third auxiliary chamber 40 to the cultivation chamber 10.

[0027] After the biomass has been cultivated, the mass is harvested into the harvesting chamber 80 by opening the outlet 13 of the cultivation chamber 10. The product is distributed along the width of the harvesting chamber 80 by pressing the supple bag by hand or by shaking the unit or by a large chamber-sized actuator. The filter 81 will perform a final cleansing to the biomass before it is collected to the collector 90, such as a series of collecting containers 91 – 97, which may then be removed from the unit for delivering the biomass for consumption.

[0028] In the example above, the passageways between different compartments of the cultivation unit 100 have been presented as manually opened members controlling the flow between compartments. However, it is also possible to equip the unit with actuators, which may of course be manipulated by hand or by an automation system. One such actuator option is presented in FIGURES 3A to 3C. Turning first to FIGURE 3A, which

shows as an exemplary fashion the passageway 22 between the cultivation chamber 10 and the first auxiliary chamber 20. The passageway 22 may take the form of an actuator comprising two cooperating components, the operation of which is more apparent in FIGURE 3A. The first component 22A is arranged on one side of the cultivation unit 100 and the second component 22B is arranged on the other side of the cultivation unit 100. The first component 22A of the actuator includes a plunger, which is configured to be pushed from a closed state as shown in FIGURE 3B into an open state (not shown). The second component is configured to connect to the first component 22A so as to clamp the cultivation unit 100 there between. The clamping brings the two film layers in tight contact with one another thus preventing flow through the actuator. The push of the plunger from the closed state into the open state makes the plunger to penetrate the cultivation unit, or vice versa. The plunger therefore may act as a valve. The actuator may include a biasing spring biasing the actuator toward the closed state. Such valves are known *per se* in the medical field. The actuator may operate in response to a magnetic, electromagnetic, pneumatic or manual input.

[0029] The embodiment of FIGURE 1 may be varied in to simplify or otherwise adapt the cultivation unit to a particular purpose. One such of a varied embodiment is shown schematically in FIGURE 4. According to the illustrated embodiment a cultivation unit is provided in a simplified modification from the cultivation unit of FIGURE 1. The cultivation unit comprises only a cultivation chamber 10 and an isolated first auxiliary chamber 20. Naturally, further auxiliary chambers are foreseeable. The air inlet 60, water inlet 50, and exhaust air port 70 differ from those of the embodiment of FIGURE 1 in that they are constructed as tubes having external filters 51, 61, 71 attached to the tubes. The tubes have been attached to the cultivation unit by adhesive connection or the tubes may have been sealed during the lamination of the parallel layers of the sheet forming the bag. Despite being distanced from the cultivation chamber 10 by the tubes, the filters 51, 61, 71form part of the sterile boundary. The filters 51, 61, 71 are therefore the contact point of the cultivation unit to external supplies of air and water and to the ambience. Similarly, the collector shown in FIGURE 1 has been replaced by an outlet 98 which may take the form of a tube or other protruding volume. The outlet 98 is preferably sealed by means of a cap 99 or other seal. In the shown embodiment, the aeration port of FIGURE 1 may be replaced by a sloping bottom end of the cultivation chamber 10. The bottom end of the cultivation chamber 10 is made to slope towards the bottom end of the channel 62 of the

air inlet 60 so as to feed air to the bottom-most part of the cultivation chamber 10.

[0030] The different constructions may be combined or varied further. One such combination is shown in FIGURE 5. According to this embodiment, air feed of FIGURE 4 is replaced with that of FIGURE 1, i.e. with an aeration port 11.

[0031] A further variant is shown in FIGURE 6, wherein the cultivation unit of FIGURE 5 is modified by replacing the inlets and air exhaust port of FIGURE 4 with those of FIGURE 1. Also, a collector 90 is added between the outlet 98 and the cultivation chamber 10 to provide for a larger volume.

[0032] The cultivation unit or kit as the ones described above may be part of a cultivation system including also a dock including the necessary framework for operating the cultivation unit. The cultivation system may include dock for one or more cultivation units. The cultivation unit and the dock preferably contain a standardized interface for connecting the two with a simple motion. The dock may include, for example, ready water and/or air inlets preferably equipped with respective pumps for feeding water and/or air into the bag, automated manipulators for operating the passageways, particularly the actuated passageways, as well as an illuminator for providing sufficient light to the cultivation chamber in an appropriate wavelength. The dock may also be equipped with sensors, such as absorbance, image recognition, spectrometers, etc., for monitoring the progress of the cultivation. The dock also preferably includes a user interface for controlling the system. The user interface may be, for example, a network connection enabling remote control through web-based services, apps, etc.

[0033] It is to be understood that the embodiments of the invention disclosed are not limited to the particular structures, process steps, or materials disclosed herein, but are extended to equivalents thereof as would be recognized by those ordinarily skilled in the relevant arts. It should also be understood that terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting.

[0034] 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 of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment. Where reference is made to a numerical value using a term such as, for

example, about or substantially, the exact numerical value is also disclosed.

[0035] As used herein, a plurality of items, structural elements, compositional elements, and/or materials may be presented in a common list for convenience. However, these lists should be construed as though each member of the list is individually identified as a separate and unique member. Thus, no individual member of such list should be construed as a de facto equivalent of any other member of the same list solely based on their presentation in a common group without indications to the contrary. In addition, various embodiments and example of the present invention may be referred to herein along with alternatives for the various components thereof. It is understood that such embodiments, examples, and alternatives are not to be construed as de facto equivalents of one another, but are to be considered as separate and autonomous representations of the present invention.

[0036] Furthermore, the described features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. In this description, numerous specific details are provided, such as examples of lengths, widths, shapes, etc., to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that the invention can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the invention.

[0037] While the forgoing examples are illustrative of the principles of the present invention in one or more particular applications, it will be apparent to those of ordinary skill in the art that numerous modifications in form, usage and details of implementation can be made without the exercise of inventive faculty, and without departing from the principles and concepts of the invention. Accordingly, it is not intended that the invention be limited, except as by the claims set forth below.

[0038] The verbs “to comprise” and “to include” are used in this document as open limitations that neither exclude nor require the existence of also un-recited features. The features recited in depending claims are mutually freely combinable unless otherwise explicitly stated. Furthermore, it is to be understood that the use of "a" or "an", that is, a singular form, throughout this document does not exclude a plurality.

REFERENCE SIGNS LIST

10	cultivation chamber
11	aeration port
12	aeration port
13	outlet
20	first auxiliary chamber
21	passageway
22	passageway
22A	first component
22B	second component
30	second auxiliary chamber
31	passageway
32	passageway
40	third auxiliary chamber
41	passageway
42	passageway
50	water inlet
51	carbon filter
52	sterile filter
53	channel
60	air inlet
61	inlet air filter
62	channel
70	exhaust air port
71	exhaust air filter
72	exhaust air condenser
80	harvest chamber

81	filter
90	collector
91	first collecting container
92	second collecting container
93	third collecting container
94	fourth collecting container
95	fifth collecting container
96	sixth collecting container
97	seventh collecting container
98	outlet
99	cap
100	cultivating unit
101	first film layer
102	second film layer
103	seam
I	entry flow
O	exit flow

CITATION LIST

US 20090233334 A1

US 20130082410 A1

CLAIMS

1. A cultivation unit (100) for cultivating cells, comprising:
   - a sterile boundary delimiting the cultivation unit (100),
   - a cultivation chamber (10),
   - a water inlet (50) configured to feed water into the cultivation chamber (10), and
   - an air inlet (60) configured to feed air into the cultivation chamber (10),

   characterized by a first auxiliary chamber (20) connected to the cultivation chamber (10) via a convertible passageway (22), which may be converted from a closed state to an open state so as to introduce a substance contained in the cultivation chamber (10) and a substance contained in the first auxiliary chamber (20) to each other.

2. The cultivation unit (100) according to claim 1, wherein fluid flow between the cultivation chamber (10) and the first auxiliary chamber (20) is prevented in the closed state of the passageway (22) and enabled in the open state.

3. The cultivation unit (100) according to claim 1 or 2, wherein the passageway (22) is constructed as a floodgate, for example a sacrificial wall, separating the cultivation chamber (10) and first auxiliary chamber (20) from each other in the closed state and being configured to be opened manually, for example broken manually, so as to enable fluid flow between the chambers (10, 20) in an open state.

4. The cultivation unit (100) according to claim 1, 2 or 3, wherein the passageway (22) is constructed as two-component inter-engagement member, the components of which are configured to be manipulated from outside the cultivation unit (100) so as to:
   - fit each other in the closed state for separating the cultivation chamber (10) and first auxiliary chamber (20) from each other, and to
   - disengage from each other in the open state so as to enable fluid flow between the chambers (10, 20).

5. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit (100) is a single-use unit.

6. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit (100) is a bag, particularly a disposable bag

7. The cultivation unit (100) according to any of the preceding claims, wherein the unit is made of a polymer material, particularly transparent polymer material preferably food grade polymer material, such as polypropylene or polyethylene.

8. The cultivation unit (100) according to any of the preceding claims, wherein the unit is made of a pliable material.

9. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit has been formed by superposing at least two layers of a sheet material.

10. The cultivation unit (100) according to any of the preceding claims, wherein the chambers (10, 20) are formed by plastic welding.

11. The cultivation unit (100) according to any of the preceding claims, wherein the passageway (22) comprises an actuator, such as a magnetic, electromagnetic or manual actuator.

12. The cultivation unit (100) according to any of the preceding claims, wherein the air inlet (60) comprises a sterile filter (61).

13. The cultivation unit (100) according to claim 12, wherein the filter (61) has been integrated into the cultivation unit (100) by enclosing the filter (61) by the superposed sheet layers of the cultivation unit (100).

14. The cultivation unit (100) according to any of the preceding claims, wherein the air inlet (60) is connected to the bottom of the cultivation chamber (10) through aeration pores or holes or opening (11).

15. The cultivation unit (100) according to any of the preceding claims, wherein the water inlet (50) comprises a sterile filter, preferably an at least two-stage filter comprising a carbon filter and a sterile filter (51, 52).

16. The cultivation unit (100) according to claim 15, wherein the filter (61) has been integrated into the cultivation unit (100) by enclosing the filter (61) by the superposed sheet layers of the cultivation unit (100).

17. The cultivation unit (100) according to any of the preceding claims, wherein the water inlet (50) is configured to feed water into the cultivation chamber (10) during cultivation.

18. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit (100) comprises a second auxiliary chamber (30), which is connected to the cultivation chamber (10) via a passageway (32), which may be manipulated from a closed state to an open state, wherein the inoculum and nutrients or a nutrient mixture may be contained in the first and second auxiliary chamber (20, 30) and flushed into the cultivation chamber (10) for cultivation.

19. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit (100) comprises a third auxiliary chamber (40), which is connected to the cultivation chamber (10) via a passageway (42), which may be manipulated from a closed state to an open state, wherein the inoculum, the first nutrient and the second nutrient may be contained in the first, second and third auxiliary chamber (20, 30, 40), respectively, and flushed into the cultivation chamber (10) for cultivation so as to feed nutrients or additional formulations to the cultivation.

20. The cultivation unit (100) according to any of the preceding claims, wherein the water inlet (50) is connected to the cultivation chamber (10) via the first auxiliary chamber (20) or any combination thereof so as to flush the contents of said first auxiliary chamber (20) into the cultivation chamber (10).

21. The cultivation unit (100) according to any of the preceding claims, wherein the water inlet (50) is connected to the cultivation chamber (10) via the first, second or third auxiliary chamber (20, 30, 40) or any combination thereof so as to flush the contents of said first, second or third auxiliary chamber (20, 30, 40) into the cultivation chamber (10).

22. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit comprises a harvest chamber (80) connected to the cultivation

chamber (10) and to the water inlet (50) for feeding water into the harvest chamber (80) so as to wash the harvested cells.

23. The cultivation unit (100) according to claim 22, wherein the cultivation unit (100) comprises at least one, preferably a plurality of, collecting container(s) (91–97) connected to the harvest chamber (80) for collecting the washed cells.

24. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit comprises an exhaust air port (70) comprising exhaust air condenser (72) for preventing excess evaporation of moisture during cultivation.

25. The cultivation unit (100) according to any of the preceding claims, wherein the cultivation unit (100) is for cultivating edible cells, particularly plant cells.

26. The cultivation unit (100) according to any of the preceding claims, wherein the sterile boundary delimits the chambers (10, 20) as well as the inlets (50, 60).

27. A set for cultivating cells comprising:
- a cultivating unit (100) according to any of the preceding claims,
- an inoculum contained in the cultivation or first auxiliary chamber (10, 20), and
- a nutrient contained in the chamber not occupied by the inoculum,

wherein the inoculum and nutrient may be introduced to each other by manipulating the passageway (22) between the cultivation chamber (10) and the first auxiliary chamber (20) from closed state to open state.

28. The set according to claim 27, wherein the inoculum is a vegetative inoculum.

29. The set according to claim 27 or 28, wherein the set comprises a second nutrient contained in a second auxiliary chamber (30) of the cultivating unit (100), from where it may be introduced to the inoculum or the other nutrient or both by manipulating a convertible passageway (32) between the second auxiliary chamber (30) and cultivation chamber (10) from the closed state to open state.

30. The set according to claim 27, 28, or 29, wherein the inoculum or nutrient(s) or both are in a dehydrated form for improved shelf-life.

31. The set according to any of the preceding claims 27 to 30, wherein the dry weight content of the inoculum or nutrient(s) or both is 25 weight per cent or more, preferably 30 weight per cent or more.

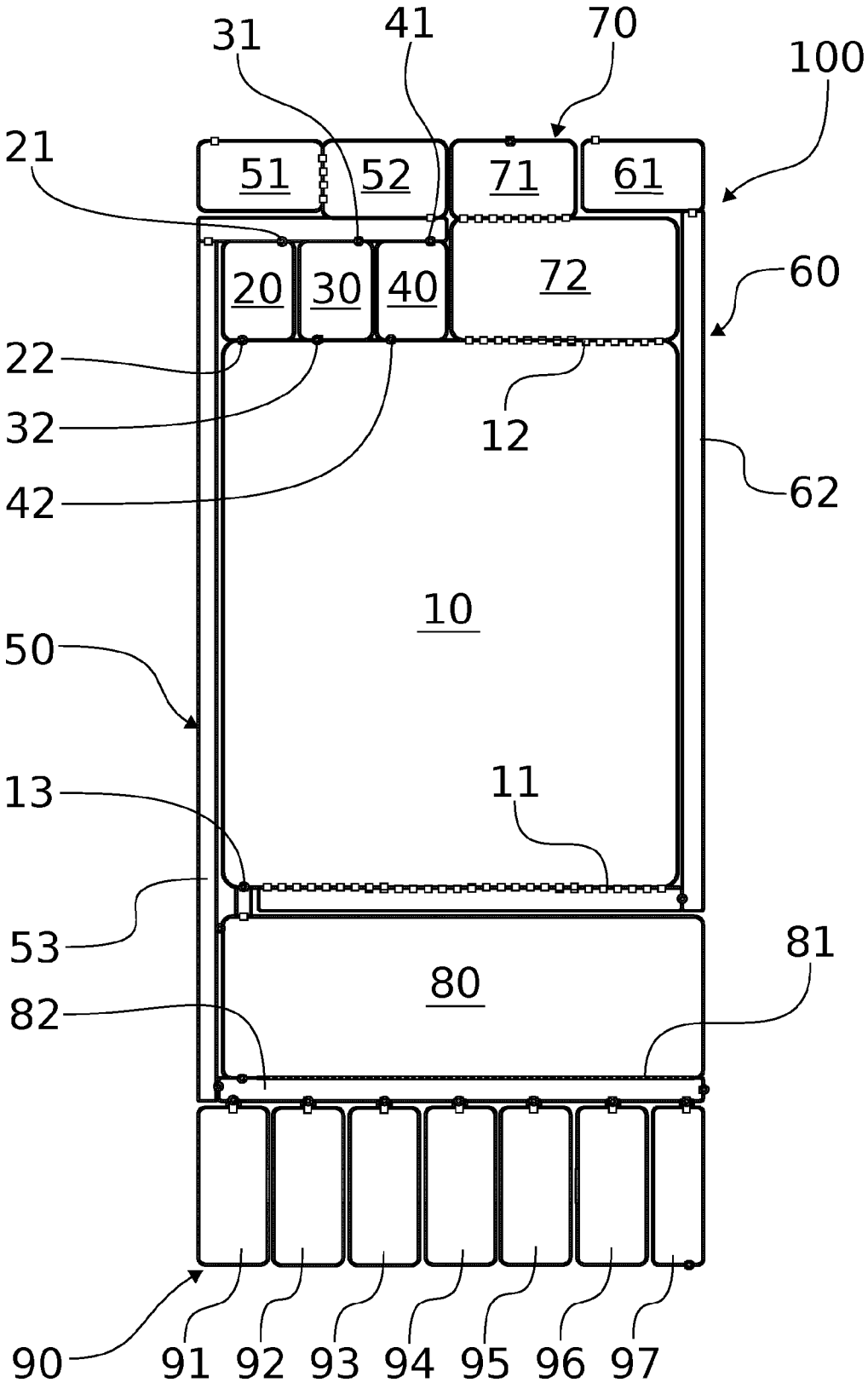
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FIG. 1

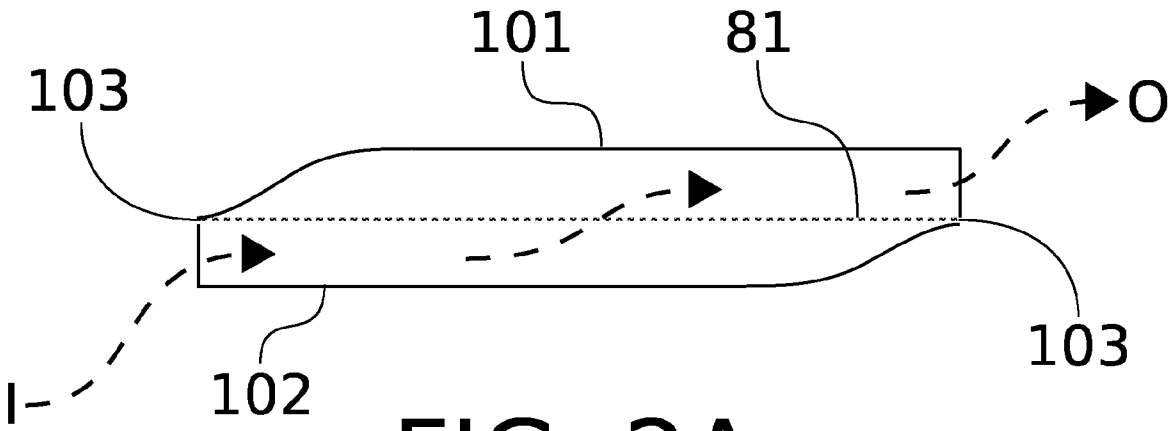

FIG. 2A

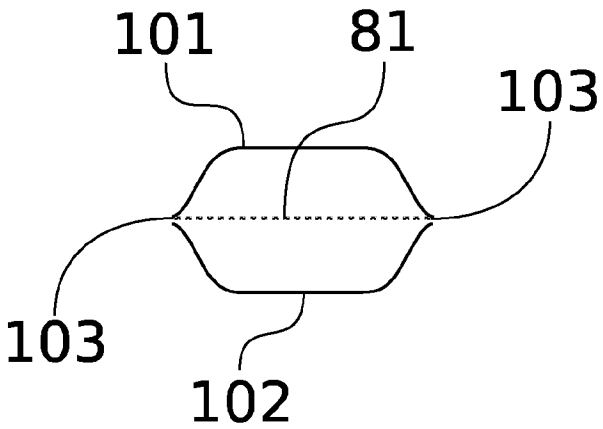

FIG. 2B

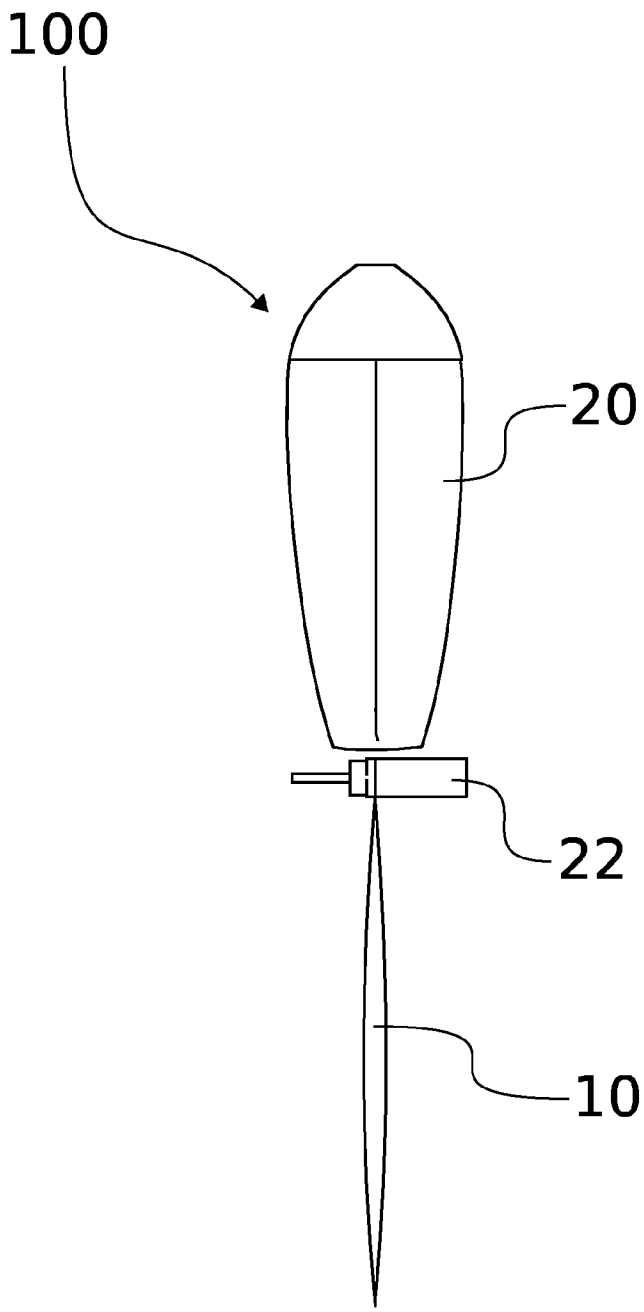

FIG. 3A

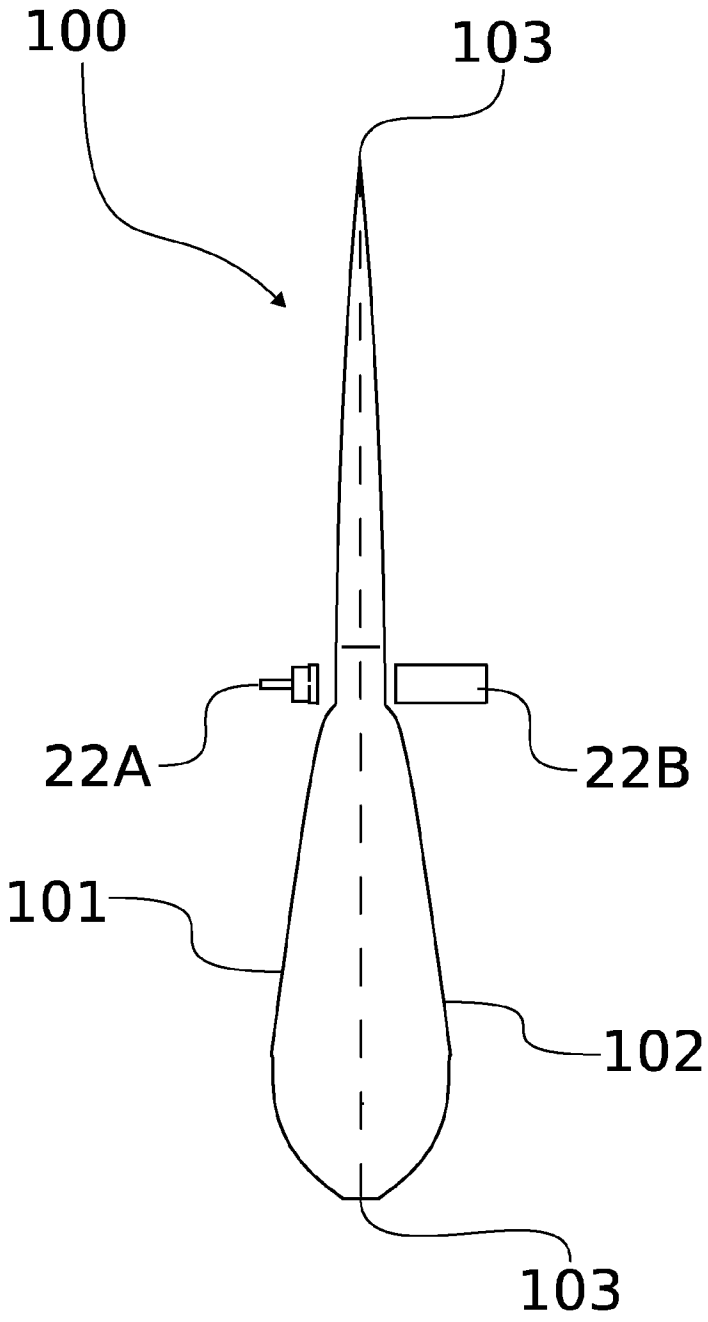

FIG. 3B

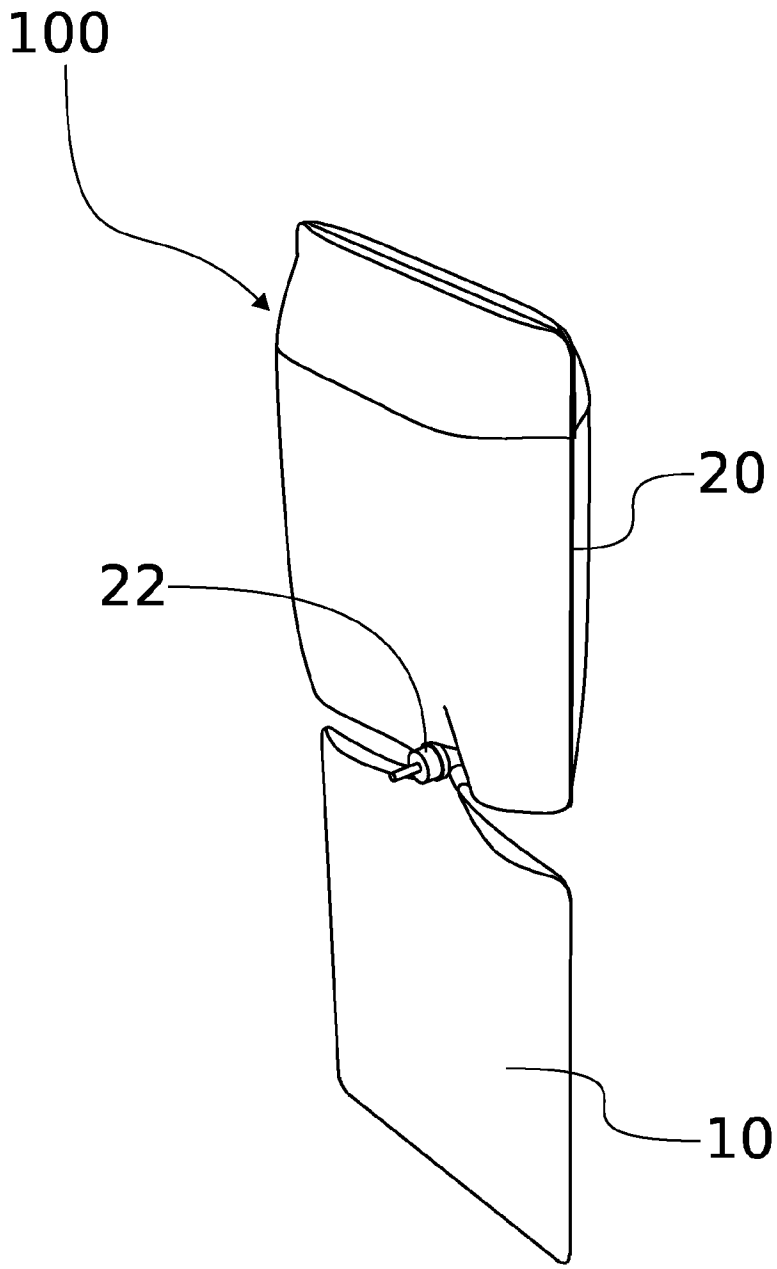

FIG. 3C

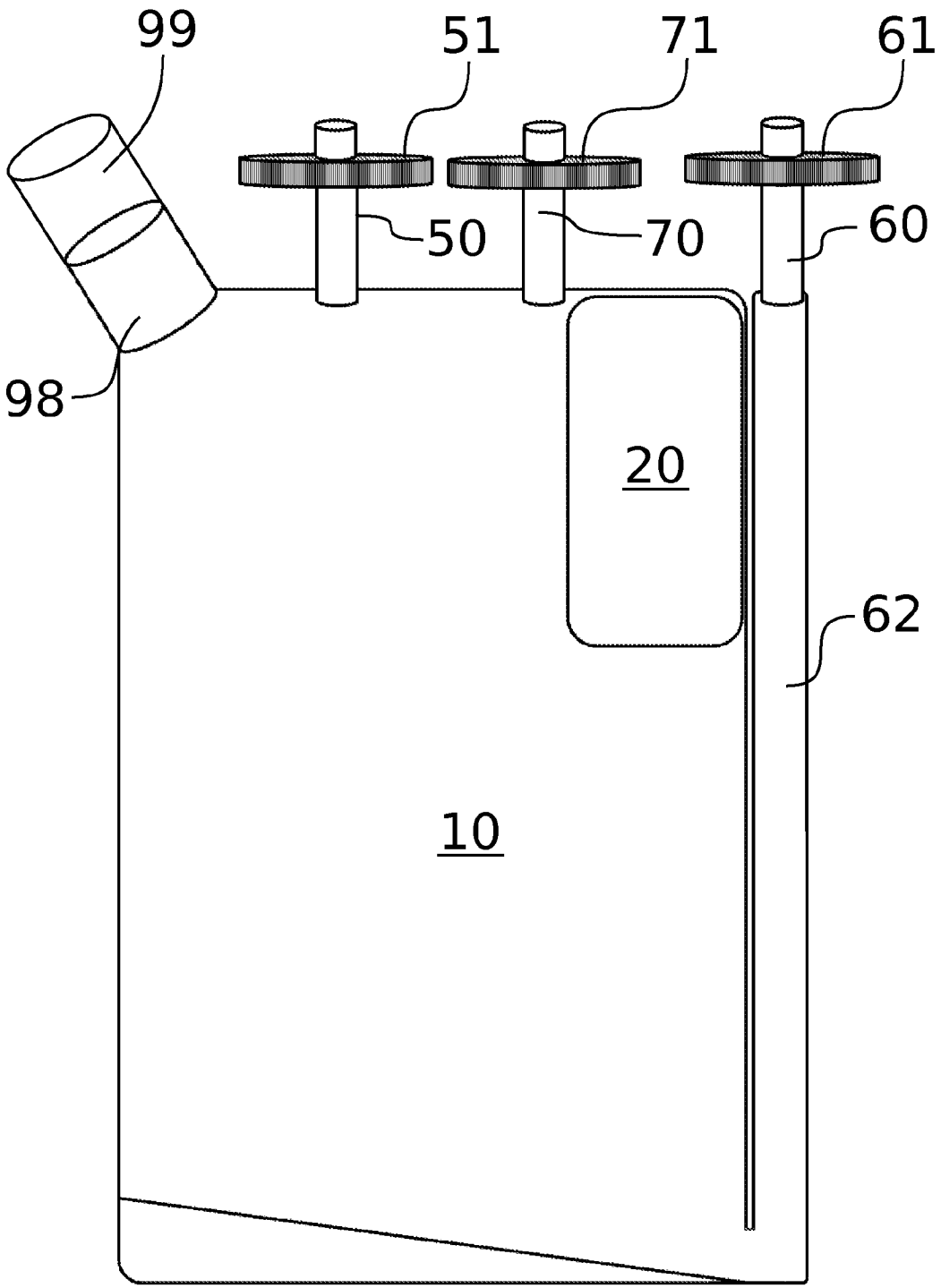

FIG. 4

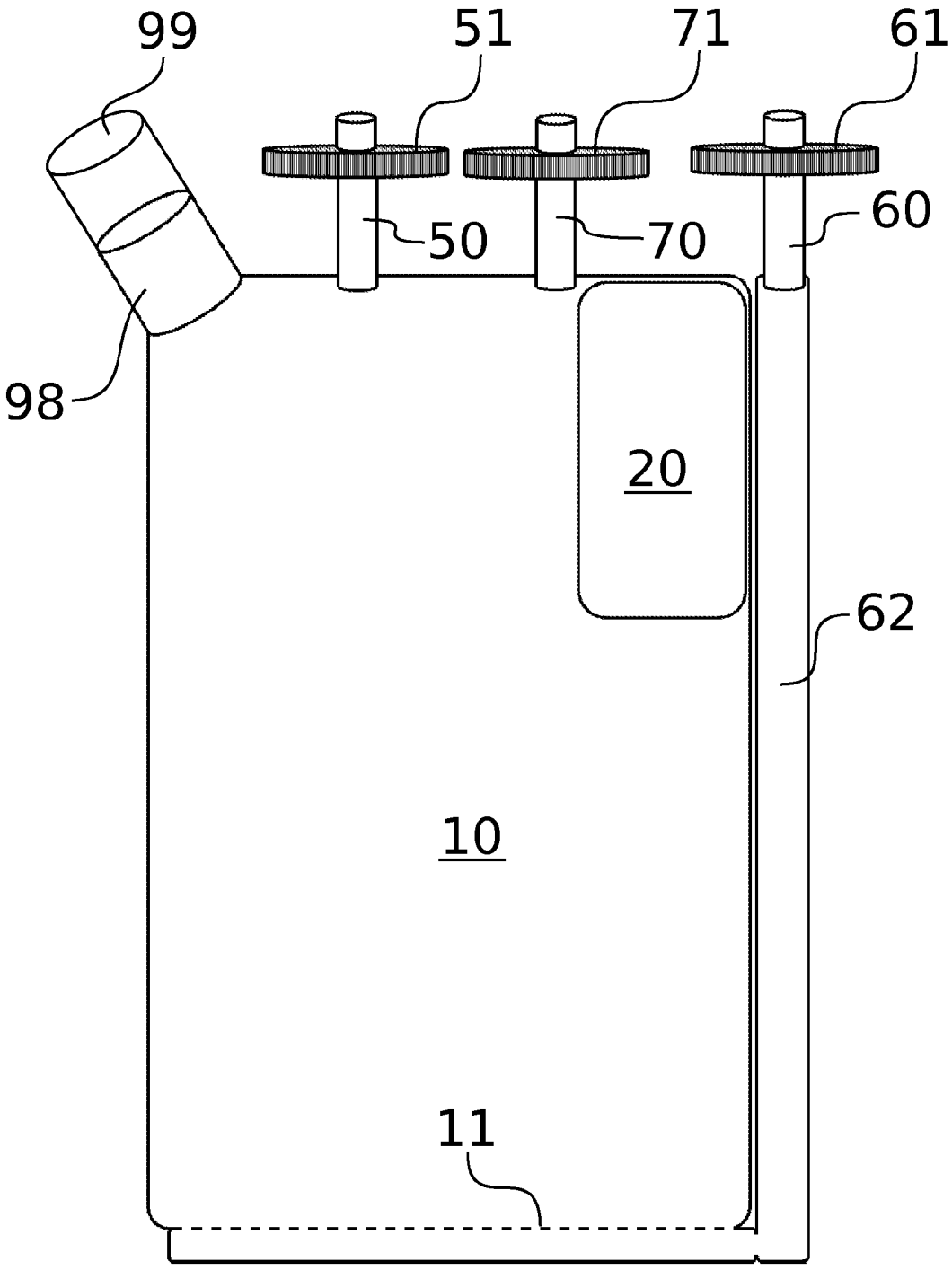

FIG. 5

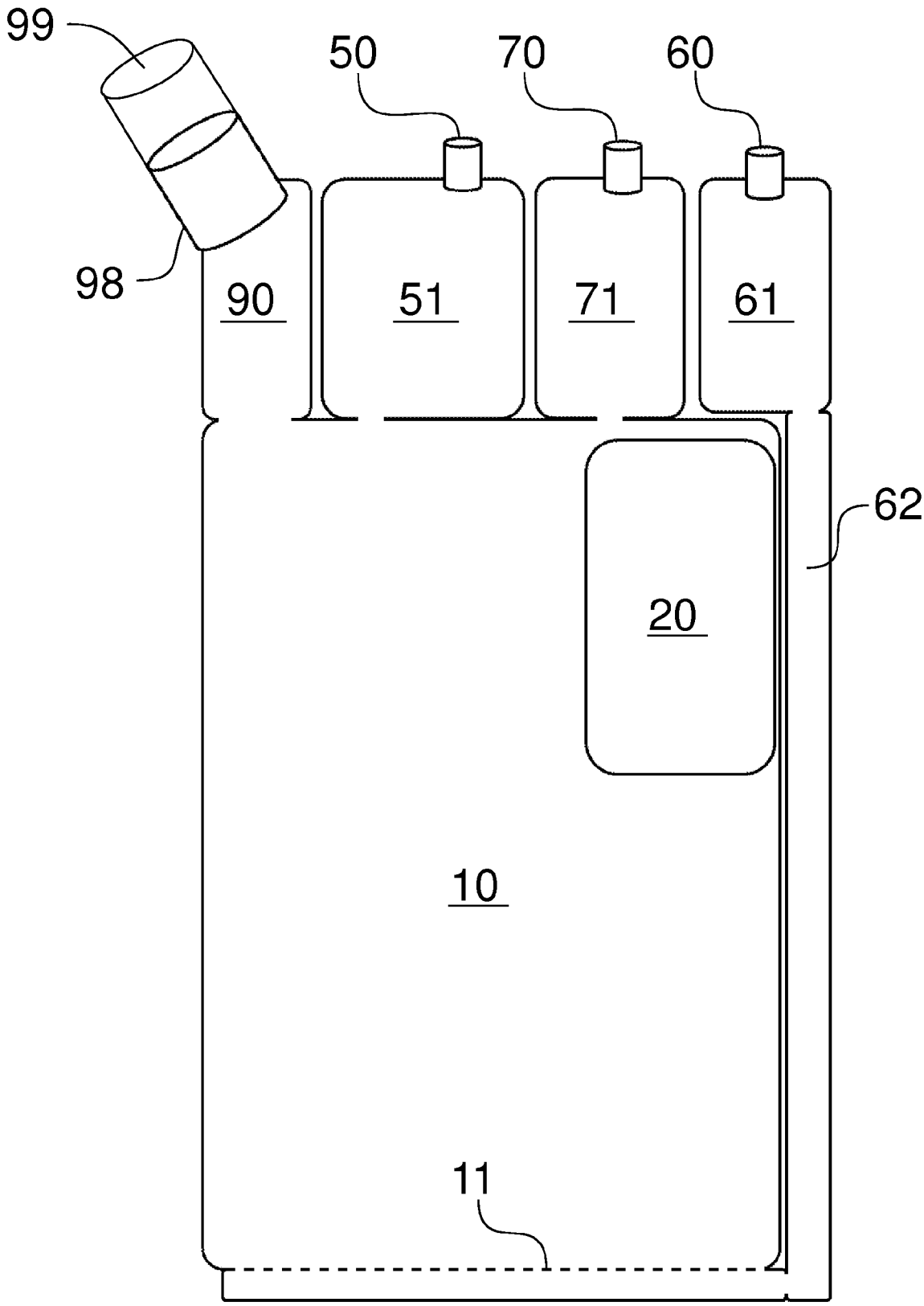

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2017/050717

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M1/00 A01H4/00
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)
C12M A01H A01N C12N

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DE 197 07 497 A1 (BOEHRINGER MANNHEIM GMBH [DE]) 19 February 1998 (1998-02-19) column 8, line 6 - column 11, line 27; figures 1,2 -----	1-26 27-31
X	WO 2015/180908 A1 (GE HEALTHCARE BIO SCIENCES AB [SE]) 3 December 2015 (2015-12-03) page 4, line 6 - page 5, line 19 page 5, line 28 - page 6, line 4 page 7, line 10 - line 24 page 8, line 19 - page 9, line 6 page 9, line 24 - line 33 figures 1-3 ----- -/--	1-26

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
2 January 2018	16/01/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cubas Alcaraz, Jose

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2017/050717

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP H06 327461 A (TOKIMEC INC) 29 November 1994 (1994-11-29) paragraphs [0002], [0003] paragraph [0014] - paragraph [0020]; figure 1 -----	27-31

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/FI2017/050717

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		CA 2264206 A1	26-02-1998
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WO 2015180908 A1	03-12-2015	CN 106459873 A	22-02-2017
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