666567

SPRUSON & FERGUSON

AUSTRALIA

PATENTS ACT 1990

PATENT REQUEST: STANDARD PATENT

I/We, the Applicant(s)/Nominated Person(s) specified below, request I/We be granted a patent for the invention disclosed in the accompanying standard complete specification.

[70,71] Applicant(s)/Nominated Person(s):

Heraeus Sepatech GmbH, of Am Kalkberg, 3[illegible]2[illegible] Osterode, GERMANY

[54] Invention Title:

Culture Vessel for Cell Cultures

[72] Inventor(s):

Frank W. Falkenberg

[74] Address for service in Australia:

Spruson & Ferguson, Patent Attorneys
Level 33 St Martins Tower
31 Market Street
Sydney New South Wales Australia (Code **SF**)

Details of Basic Application(s):

[31] Appl'n No(s):	**[33] Country:**	**[32] Application Date:**
P 42 29 334.0	DE	2 September 1992

DATED this TWENTY FIFTH day of AUGUST 1993

Heraeus Sepatech GmbH

By:

Registered Patent Attorney

IRN: 247945

INSTR CODE: 55400

S040034 26/08/93

SPRUSON & FERGUSON

Australia

Patents Act 1990

NOTICE OF ENTITLEMENT

I, John Gordon Hinde, of Spruson & Ferguson, St Martins Tower, 31 Market Street, Sydney, New South Wales 2000, Australia, being the patent attorney for the Applicant(s)/Nominated Person(s) in respect of Application No 44938/93 state the following:-

The Applicant(s)/Nominated Person(s) has/have entitlement from the actual inventor(s) as follows:-

The Applicant(s)/Nominated Person(s) is/are the assignee(s) of the actual inventor(s).

The Applicant(s)/Nominated Person(s) is/are the applicant(s) of the basic application(s) listed on the Patent Request. The basic application(s) listed on the Patent Request is/are the first application(s) made in a Convention Country in respect of the invention.

DATED this Ninth day of November 19 93

.....

John Gordon Hinde

IRN: 247945

INSTR CODE: 55400

28

665P

AU9344938

(12) PATENT ABRIDGMENT (11) Document No. AU-B-44938/93
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 666567

(54) Title
CULTURE VESSEL FOR CELL CULTURES

(51)[5] International Patent Classification(s)
C12M 003/06 C12M 003/00

(21) Application No. : 44938/93 (22) Application Date : 26.08.93

(30) Priority Data

(31) Number	(32) Date	(33) Country
4229334	02.09.92	DE GERMANY

(43) Publication Date : 10.03.94

(44) Publication Date of Accepted Application : 15.02.96

(71) Applicant(s)
HERAEUS SEPATECH GMBH

(72) Inventor(s)
FRANK W. FALKENBERG

(74) Attorney or Agent
SPRUSON & FERGUSON , GPO Box 3898, SYDNEY NSW 2001

(56) Prior Art Documents
US 4748124

(57) Claim

1. Culture vessel, similar to a roller bottle, for cell cultures with a cell density of at least 10^7 cells per millilitre, which, forming a supply chamber, contains a nutrient medium in which is arranged at least one production chamber which receives the cell cultures and is bathed by the nutrient medium, and is separated from the nutrient medium by a dialysis membrane through which nutrients are transported from the supply chamber into the production chamber, while metabolic products are transported from there into the supply chamber, and with a gas feed and discharge system into the supply chamber, characterised in that there is provided as a gas feed and discharge system a silicone gas exchange membrane having a thickness between 0.1 mm and 1mm, that is impermeable to liquids and to microorganisms contaminating the cell cultures, and permeable to the gases required and produced during cell culture, which forms at least a part of the outer wall of the culture vessel and whose total surface area, material, and thickness are selected so that the permeability to oxygen is at least 0.01mg/h for each 10^7 cells.

Culture Vessel for Cell Cultures

The invention concerns a culture vessel, similar to a roller bottle, for cell cultures with a cell density of at least 10^7 cells per millilitre, which, forming a supply chamber, contains a nutrient medium in which is arranged at least one production chamber which receives the cell cultures and is bathed by the nutrient medium, and is separated from the nutrient medium by a dialysis membrane through which nutrients are transported from the supply chamber into the production chamber, while metabolic products are transported from there into the supply chamber; and with a bacteria-tight gas feed system into the supply chamber. The invention furthermore concerns a method for cell culturing wherein a nutrient medium that bathes a production chamber that is sealed on all sides and contains the cell culture being cultured is placed in a culture vessel similar to a roller bottle, and a continuous rolling motion is imparted to the culture vessel.

Culture vessels of this kind can be used, for example, for in vitro production of monoclonal antibodies. Monoclonal antibodies are currently produced for a number of purposes in diagnosis, treatment, and biomedical research, usually using hybridoma technology methods. "Hybridoma cells" is the term for immortalised hybrids of antibody-producing cells and myeloma cells. The antibodies produced by the hybridoma cells, which are characterised by high specificity, are referred to as "monoclonal" antibodies.

For *in vitro* antibody production, these hybridoma cells are cultured in certain liquid media whose composition corresponds as exactly as possible to that of blood. These media contain ingredients which include salts, sugar, vitamins, amino acids, and a buffer system based on sodium hydrogen carbonate ($NaHCO_3$). Usually the hybridoma cells are cultured in an incubator atmosphere with high atmospheric humidity and a CO_2 content that is at equilibrium with the $NaHCO_3$ present in the medium.

The monoclonal antibodies produced in this manner with conventional stationary *in vitro* methods, in the form of tissue culture products, are very well suited for many purposes in basic biomedical research and in clinical diagnosis. However, the monoclonal antibodies produced in this manner are suitable for a number of applications in which monoclonal antibodies are needed in highly pure and concentrated form only after laborious further processing. In the *in vivo* production form (ascites fluid), the monoclonal antibodies are already present in very high concentrations (up to 20mg/mL) in the final product. When monoclonal antibodies are produced with the usual stationary in vitro production methods, however, concentrations of only approximately 0.01 to 0.10mg/mL are achieved.

To allow the production of monoclonal antibodies at higher concentration and higher purity in vitro as well, a method and an apparatus in the form of a culture vessel similar to a roller bottle have been proposed in which a "supply chamber" with nutrients for the cells

being supplied, and a plurality of "production chambers" arranged therein in which cell growth occurs and in which the monoclonal antibodies are produced, are separated from one another by semipermeable dialysis membranes. Cells are supplied with nutrients from the "supply chamber" through the semipermeable dialysis membrane, while waste products and metabolic products are discharged, again through the dialysis membrane, from the "production chambers" into the "supply chamber." This apparatus has become known as the "Bochum glass mouse." This culture vessel for cell cultures is described, for example, in the manuscript for the poster entitled "The Glassmouse: A Rollerbottle-like Apparatus for Culturing Hybridomas in Dialysis Bags," presented by T. Hengelage, F. Haardt, and F.W. Falkenberg at the 1991 World Congress on Cell and Tissue Culture in Anaheim, California on June 16-20, 1991. The known culture vessel for cell cultures consists of a glass tube with an outside diameter of 120mm, the ends of which are turned outward to form flanges. The length of the glass tube including the flange is 320mm. The ends of the glass tube are sealed with 15mm thick polymethyl methacrylate (PMMA) disks. One of the PMMA disks has 5 through holes, one of them along the long axis of the vessel and sealed with a stopper that in turn has two smaller openings which are used to admit a CO_2/air mixture and to equalise pressure. For this purpose, a stainless steel tube with an inside diameter of 1mm is passed through one of the two openings and extends to the opposite end of the glass tube; through this, the CO_2/air mixture is fed through a sterile filter into the interior of the vessel. The four remaining holes in the PMMA disk surrounding the central hole are used to introduce dialysis sacks, which project into the culture vessel and whose walls in each case consist of semipermeable dialysis membranes. The cell culture mixtures being cultured are placed in these dialysis sacks, which act as production chambers, while the interior of the culture vessel additionally serves as the supply chamber for the cells, and is filled with nutrient medium to approximately 40% of its volume. The cells are supplied with nutrients from the supply chamber through the semipermeable dialysis membranes, while waste products and metabolic products are also discharged through the dialysis membrane. To allow the culture vessel to rotate about its long axis, it can be equipped with a sealed rotary leadthrough through which the supply line for the CO_2/air mixture passes.

This apparatus, in which the cells enclosed in the production chambers are surrounded by the semipermeable dialysis membranes, allows hybridoma cells to be cultured over longer periods and at high densities (more than 10^7cells/mL). The known culture vessel, however, is a relatively complex apparatus which is difficult to handle, the construction of which requires a certain skill that not every laboratory shop can provide. In the known culture vessel, the gases necessary for cell culture metabolism and to create physiological conditions are supplied by introducing into the supply chamber the gas mixture which constitutes the surrounding atmosphere; oxygen physically dissolves in the nutrient

medium, and is transported from there through the dialysis membrane into the production chamber. Although the transport of oxygen from the supply chamber through the dialysis membrane into the cell culture chamber is not very efficient, it is sufficient for cell densities up to about 10^7cells/mL. Higher cell densities require an improvement in oxygen supply. Since at higher cell densities the cells' oxygen requirement is so high that the oxygen content in the cell culture chamber is exhausted in a few minutes, and the oxygen in the supply chamber is used up in less than one hour, additional oxygen must be delivered from the gas phase into the nutrient medium in the supply chamber. The weak point of the known culture vessel has proven to be the fact that continuous feeding of the CO_2/air mixture through the rotary leadthrough can cause infections of the cell cultures.

The underlying object of the present invention is to make available a culture vessel for the generation of cell cultures at high cell densities that can be produced economically and is easy to handle, and in which the danger of infections is reduced. A further underlying object of the invention is to indicate a cell culture method that is easy to implement and with which high cell densities with concurrently high vitality can be generated in a short time.

With regard to the culture vessel, according to the invention this object is achieved, based on the culture vessel already described, by the fact that there is provided as a gas feed and discharge system a silicone gas exchange membrane that is impermeable to liquids and to microorganisms contaminating the cell cultures, and permeable to the gases required and produced during cell culture, which forms at least a part of the outer wall of the culture vessel and whose total surface area, material, and thickness are selected so that the permeability to oxygen is at least 0.01mg/h for each 10^7cells. Selection of a gas exchange membrane that is impermeable to liquids and to microorganisms contaminating the cell cultures, and permeable to the gases needed for cell respiration, means that cell culture infections brought in via the gas feed and discharge system can be almost ruled out. The gases required for cell respiration are primarily oxygen, and the carbon dioxide that is produced by cell culture when oxygen is utilised. Since the gas exchange membrane is permeable to oxygen and carbon dioxide, it is possible to influence the concentrations of these gases inside the culture vessel, especially inside the supply chamber, by adjusting the concentrations in the atmosphere surrounding the culture vessel. The minimum oxygen requirement for cell culture survival depends on factors that include the type of cell. A minimum oxygen requirement of 0.01mg/h for each 10^7cells in the cell culture is generally assumed. The total surface area, material, and thickness of the gas exchange membrane must be selected so as to ensure this "minimum oxygen supply." Suitable membrane geometries and gas permeabilities can be determined with a few experiments.

Because the gas feed and discharge system forms at least a part of the outer wall of the

AUSTRALIAN PATENT OFFICE

culture vessel, gas feed and discharge lines in the form of tubes or hoses are not required. The culture vessel can therefore be manufactured economically and made easy to handle, being designed for example as a roller bottle.

Suitable cell cultures include, for example, hybridoma cells, tumour cells, or transfixed tumour cells.

A culture vessel in which the permeability of the gas exchange membrane for oxygen is at least 0. 05mg/h for each 10^7cells has proven successful, especially with regard to the highest possible cell density.

Materials that have a permeability coefficient of at least 1 x $10^{19}m^2/s$ x Pa, advantageously at least 5 x $10^{19}m^2/s$ x Pa, have proven suitable for the gas exchange membrane. Silicone has proven to be a particularly suitable material for the gas exchange membrane. To guarantee sufficient oxygen supply, gas membranes that are as thin as possible are preferred; membranes with a thickness between 0.1 and 1mm have proven suitable. A silicone membrane is especially economical and can be manufactured in any shape by injection moulding. Silicone is available commercially in many thicknesses, shapes, and specific gas permeabilities. It has high tear resistance and good chemical resistance to the media ordinarily used in cell culturing, and is therefore also especially easy to handle. The easy sterilisability of a silicone gas exchange membrane is also especially advantageous; in particular, it can be sterilised in an autoclave very effectively and with no substantial changes in shape. It can therefore also be used several times. For a given gas permeability, the required geometry of the gas exchange membrane depends on the gas requirement resulting from cell respiration, and on the partial pressures of the gases involved in cell respiration, especially the oxygen partial pressure acting on it from outside. With an external pressure of 1atm and an incubator atmosphere with an oxygen partial pressure corresponding approximately to that of air, gas exchange membranes with a surface area of at least $5cm^2$ have proven suitable for cell cultures of 35mL and 10^7cells per millilitre of cell culture mixture.

It has been found to be advantageous to provide the culture vessel with a gas exchange membrane whose surface area is at least $5cm^2$. Gas exchange membranes that have a surface area of at least $5cm^2$ have proven successful, for example, in culture vessels that accommodate a volume of about 300mL, wherein the cell culture comprises about 35mL with a cell density of approximately 10^7cells/mL.

A culture vessel that is designed as a hollow cylinder, both of whose ends are sealed with a gas exchange membrane, has proven especially successful. In this context the peripheral surface of the hollow cylinder guarantees the mechanical stability of the culture vessel, while the gas exchange membrane at the two ends provides a good supply of gas to, and

discharge of gas from, the cell culture.

A culture vessel in which the gas exchange membrane is folded has proven to be advantageous, especially with regard to a high cell density within the cell culture. This increases the total surface area of the gas exchange membrane for a given area covered by the gas exchange membrane, thus improving gas exchange and in particular the supply of oxygen to the cells. With hollow cylindrical culture vessels, for example, the peripheral surface can be designed in the form of a bellows. Such bellows are easy to manufacture.

An embodiment of the culture vessel in which the entire outer wall, except for support elements which mechanically stabilise the outer wall, is designed as a gas exchange membrane is especially preferred. High cell densities can be achieved quickly and with high vitality with a culture vessel of this kind, which ensures very good gas exchange. The support elements stabilise the shape of the culture vessel and the geometrical arrangement of its parts with respect to one another. They can be designed, for example, as a metal grid or one made of a stable plastic, the meshes of which are covered by the gas exchange membrane; or as thickenings of the outer wall made of the same material as the gas exchange membrane. The culture vessels similar to roller bottles that have proven especially advantageous are those in which the ends of the culture vessel also serve as support elements for the gas exchange membrane, the peripheral surface being substantially designed as a gas exchange membrane.

With regard to the method, the object derived from the method described above is achieved, according to the invention, by the fact that the production chamber is filled with the cell culture being cultured in such a way that it contains an air bubble whose volume occupies at least 10% of the volume of the production chamber, and that a tumbling movement of the culture vessel is superimposed on the rolling movement in such a way that the air bubble moves in the cell culture due to buoyancy. Because of the tumbling movement of the culture vessel and its buoyancy in the liquid cell culture, the air bubble moves back and forth within the production chamber. This back-and-forth movement of the air bubble makes a significant contribution to good mixing of the cell culture mixture and thus to a steady supply of nutrients thereto, and in particular to steady oxygen input and continuous discharge of metabolic products. The tumbling movement can be achieved, for example, by cyclically tilting the long axis of the culture vessel in a seesaw movement; the pivot point for the seesaw movement can be provided, for example, in the region between the ends of the culture vessel. This can be achieved, for example, by providing the ends of a culture vessel similar to a roller bottle with cams projecting beyond the peripheral surface, which act in cross section as elliptical rolling contact surfaces, the major axes of the two ovals being offset from one another.

Advantageously, the period of the tumbling movement is made precisely long enough so that in each movement cycle the air bubble is allowed enough time to rise to the respective elevated end of the production chamber.

The mixing effect of the air bubble is especially pronounced with air bubbles having a volume between 20% and 50% of the volume of the production chamber.

The speed of the rolling movement of the culture vessel is advantageously, on the one hand made so fast that the cell cultures cannot settle in the production chamber, and on the other hand made so slow that the cells can still follow the rolling motion despite their inertia. The speed satisfying these conditions depends on the settling velocity of the cell cultures, their mass, and the size of the production chamber. Values of between 20mm/s and 50mm/s have proven successful as linear velocities for a point on the circumference of the production chamber.

An exemplary embodiment of the invention is depicted in the drawings and will be explained in more detail below. In the single Figure of the drawings, a culture vessel for cell cultures according to the invention is depicted in lengthwise section as a schematic illustration.

The reference number 1 is assigned to the entirety of the culture vessel. It has a substantially hollow and cylindrical supply chamber 2, the outer wall of which consists of a silicone tube 3 that is provided, in the region of its ends, with external threads 4, 5 and flanges 6, 7 pointing inward. The wall thickness of the silicone tube 3 is approximately 3 mm. It has, evenly distributed over its entire peripheral surface, openings that are covered on the outside with a 0.38mm thick silicone film 8, so that the webs 9 remaining in the peripheral surface of the silicone tube 3 project from the silicone film 8 into the interior of the supply chamber 2 and form a cohesive grid that gives the culture vessel 1 sufficient mechanical stability for the stresses that occur during culturing. The supply chamber 2 has a length of approximately 15cm, an outside diameter of approximately 5cm, and can contain a total volume of approximately 300mL. The total peripheral surface area of the silicone tube 3 is approximately 240cm^2, approximately two thirds of which is occupied by the openings covered with the silicone film 3. The ends of the silicone tube 3 are each sealed with an annular disk 12, 13 with a centre hole 10, 11, made of stable plastic; the centre hole 10 of the annular disk 12 is surrounded by a sleevelike collar 14 that extends from the annular disk 12 toward the interior of the supply chamber 2, forming a kind of hose nipple. One free end of a dialysis sack 15 (Visking 20/32) is pulled over the collar 14 and fastened onto it with a silicone sleeve 16. The other end of the dialysis sack 15 projects into the supply chamber 2 and is sealed. The dialysis sack 15, which consists of a thin, semipermeable dialysis membrane (also reference number 15), can hold a total

volume of about 60mL. The two annular disks 12, 13, the centre holes 10, 11 of which can each be sealed with a rubber stopper 17, 18, are pressed in a fluid-tight manner against the flange 6, 7 of the supply chamber 2 by means of annular screw caps 19, 20 that engage in the external threads 4, 5 of the supply chamber 2. The rubber stoppers 17, 18 are accessible through the openings in the screw caps 19, 20. The culture vessel 1 can rotate about its long axis 21, as indicated by the directional arrow 22.

Approximately 35mL of cell culture mixture 23 is placed in the dialysis sack 15, which acts as the production chamber, and the production chamber is then sealed with the rubber stopper 17. As a result, an air bubble 24 with a volume of about 25mL is enclosed in the dialysis sack 15. At the same time, the supply chamber 2 is filled to about half its height (when oriented horizontally) with nutrient medium 25 for the cell culture mixture 23. Cell culturing occurs in a medium that depends on a $NaHCO_3$ buffer. To maintain the buffer system, culturing takes place in an incubator (not shown in the Figure) with a predefined CO_2 and O_2 atmosphere, high atmospheric humidity, and defined temperature. As the culture vessel 1 rotates, the dialysis sack 15 is bathed on all sides in the nutrient medium 25. As a result, nutrients are transported through the semipermeable dialysis membrane 15 from the supply chamber 2 into the cell culture (also reference number 23), and at the same time metabolic products are transported out from there into the supply chamber 2. The oxygen present in the incubator atmosphere passes through the silicone film 8, which has sufficient permeability to oxygen to meet the oxygen requirement of the cell culture 23 (at least 0.01mg/h per 10^7cells), directly into both the nutrient medium 25 and the supply chamber atmosphere 26 located above it. It is physically dissolved in the nutrient medium 25, gradually passes through the dialysis membrane 15, and is thus brought into the cell culture 23. The carbon dioxide gas produced as the oxygen is used up is discharged through the dialysis membrane 15 from the cell culture 23 into the nutrient medium 25 of the supply chamber 2, and removed from the culture vessel 1 through the silicone film 8. The permeability of the silicone film 8 to carbon dioxide gas is substantially greater than its permeability to oxygen, so that excess pressure cannot build up inside the supply chamber 2. The silicone film 8 is, on the other hand, impermeable to liquids and to microorganisms that might contaminate the cell culture, such as bacteria, fungi, or spores. During culturing, samples can be removed, the cell culture 23 can be inoculated, or the nutrient medium 25 can be checked or replaced through the respective rubber stoppers 17, 18.

In the interest of good mixing of both the cell culture 23 and the nutrient medium 25, the culture vessel 1 can be rotated about its long axis 21 at a rotation speed of about 34rpm; superimposed on this rotation 22 is a slow, cyclical tumbling movement of the culture vessel 1, in which the ends of the silicone tube 3 undergo a continuous back-and-forth movement in the manner of a seesaw. As a result of their buoyancy in the cell culture 23, the air bubbles 24 therefore also move back and forth inside the dialysis sack 15, thus

promoting mixing of the cell culture 23, and supplying it steadily with nutrients (especially oxygen), in a particularly effective manner.

The culture vessel 1 according to the invention is particularly easy to handle; it can produce high-purity cell cultures with cell densities of more than 10^7cells/mL, and, in the case of hybridoma cells, concentrations of monoclonal antibodies that are at least 10 times greater than the concentrations attainable with standard stationary cultures.

The claims defining the invention are as follows:

1. Culture vessel, similar to a roller bottle, for cell cultures with a cell density of at least 10^7 cells per millilitre, which, forming a supply chamber, contains a nutrient medium in which is arranged at least one production chamber which receives the cell cultures and is bathed by the nutrient medium, and is separated from the nutrient medium by a dialysis membrane through which nutrients are transported from the supply chamber into the production chamber, while metabolic products are transported from there into the supply chamber, and with a gas feed and discharge system into the supply chamber, characterised in that there is provided as a gas feed and discharge system a silicone gas exchange membrane having a thickness between 0.1 mm and 1mm, that is impermeable to liquids and to microorganisms contaminating the cell cultures, and permeable to the gases required and produced during cell culture, which forms at least a part of the outer wall of the culture vessel and whose total surface area, material, and thickness are selected so that the permeability to oxygen is at least 0.01mg/h for each 10^7 cells.

2. Culture vessel according to Claim 1, characterised in that the permeability of the gas exchange membrane to oxygen is at least 0.05mg/h for each 10^7 cells in the cell culture.

3. Culture vessel according to claim 1 or claim 2, characterised in that the gas exchange membrane covers a total surface area of at least $5cm^2$.

4. Culture vessel according to any one of claims 1 to 3, characterised in that the culture vessel is designed as a hollow cylinder sealed at both ends with the gas exchange membrane.

5. Culture vessel according to any one of claims 1 to 4, characterised in that the gas exchange membrane is folded.

6. Culture vessel according to any one of claims 1 to 5, characterised in that the entire outer wall of the culture vessel, except for support elements which mechanically stabilise the outer wall, is designed as a gas exchange membrane.

7. Culture vessel, similar to a roller bottle, for cell cultures with a cell density of at least 10^7 cells per millilitre, which, forming a supply chamber, contains a nutrient medium in which is arranged at least one production chamber which receives the cell cultures and is bathed by the nutrient medium, and is separated from the nutrient medium by a dialysis membrane through which nutrients are transported from the supply chamber into the production chamber, while metabolic products are transported from there into the supply chamber, and with a gas feed and discharge system into the supply chamber, characterised in that there is provided as a gas feed and discharge system a silicone gas exchange membrane, said culture vessel being substantially as hereinbefore described with reference to the accompanying drawings.

8. Method for cell culturing wherein a nutrient medium that bathes a production

AUSTRALIAN PATENT OFFICE

chamber that is sealed on all sides and contains the cell culture being cultured is placed in a culture vessel, similar to a roller bottle, according to any one of claims 1 to 6, and a continuous rolling motion is imparted to the culture vessel, characterised in that the production chamber is filled with the cell culture being cultured in such a way that it contains an air bubble whose volume occupies at least 10% of the volume of the production chamber, and that a tumbling movement of the culture vessel is superimposed on the rolling movement in such a way that the air bubble moves in the cell culture due to buoyancy.

9. Method for cell culturing wherein a nutrient medium that bathes a production chamber that is sealed on all sides and contains the cell culture being cultured is placed in a culture vessel, substantially as hereinbefore described with reference to the accompanying drawings.

Dated 5 December, 1995
Hereaus Sepatech GmbH

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

AUSTRALIAN PATENT OFFICE

Culture Vessel for Cell Cultures

Abstract

There is a known culture vessel [1], similar to a roller bottle, for cell cultures[23] which, forming a supply chamber[2], contains a nutrient medium[25] in which is arranged at least one production chamber[15] which receives the cell cultures[23] and is bathed by the nutrient medium[25], and is separated from the nutrient medium[25] by a dialysis membrane[15] through which nutrients are transported from the supply chamber[2] into the production chamber[15], and with a gas feed and discharge system into the supply chamber[2]. In order to make available, on this basis, a culture vessel [1] for generating cell cultures[23] with a high cell density which is economical to manufacture and easy to handle, and in which the danger of infections is diminished, it is proposed that there be provided as a gas feed and discharge system for the gases required and produced during cell culturing a gas exchange membrane[8] that forms at least a part of the outer wall of the culture vessel [1] and whose total surface area, material, and thickness are selected so that the permeability to oxygen is at least 0.01mg/h for each 10^7 cells. Also known are methods for cell culturing wherein a nutrient medium[25] that bathes a production chamber[15] that is sealed on all sides and contains the cell culture[23] being cultured is placed in a culture vessel [1] and a continuous rolling motion is imparted to the culture vessel [1]. In order to indicate, on this basis, a method that is easy to implement and with which high cell densities with concurrently high vitality can be generated in a short time, the production chamber[15] is filled with the cell culture[23] being cultured in such a way that it contains an air bubble whose volume occupies 10% of the volume of the production chamber[15], and a tumbling movement of the culture vessel is superimposed on the rolling movement in such a way that the air bubble moves in the cell culture[23].

Figure 1.

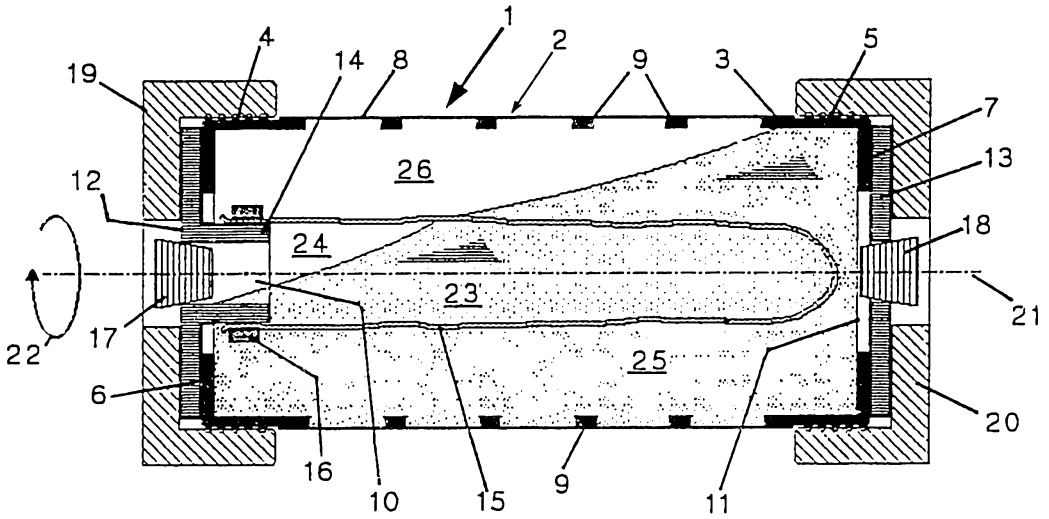

FIG. 1