Liquid-gas-phase exposure reactor for cell culturing

Description

[0001] The invention relates to a method and a device for initiation of cell growth and for cultivation of cells in high densities, wherein the cells to be cultivated are located in hollow-filament membranes and are brought alternately into a liquid nutrient medium and a gas phase present thereabove.

Prior art

[0002] Mammalian cell cultivation for the synthesis of biopharmaceutical drugs is operated mainly in stirred reactors. Heretofore airlift reactors have been used less frequently and hollow-fiber reactors very rarely for servicing the market with drugs based on mammalian cells. To improve the volumetric product yields in stirred reactors, the cell density and the effective production time of the cells are increased by optimizing the methods and using nutritional regimens specific to the cell lines in fed batch methods. The production technology is laid out in bioreactor trains containing three to four stirred reactors, each with a volumetric capacity of approximately five times that of the preceding bioreactor. The largest available stirred reactor for cultivation of mammalian cells currently has a volumetric capacity of 20,000 liters. Fed-batch processes in stirred reactors are robust, can be scaled up to the cited volumes and long ago were accepted by the authorities for drug synthesis. Disadvantages are the long dwell times of the products in the culture chamber, the need for separation of cells from the harvest supernatant, the cleaning and sterilization expenses incurred during multiple use and the high investment and operating expenses for plants equipped with this technology.

[0003] For proteins such as factor VII, which are susceptible to degradation and thus impose a short dwell time in the bioreactor during synthesis, there have been developed devices and systems that permit perfusion of the culture chamber and thus continuous operation of the stirred reactors. For this purpose, efficient cell retention with continuous media feed and product harvesting is necessary. Spin filters are used here in the interior chamber of the stirred reactor, while support materials in the form of fluidized or stationary beds are used in the traps, where the production cells can adhere to surfaces. The continuous mode of operation of stirred reactors can

also be achieved via external cell-retention systems, such as cell sedimentation, continuous cell centrifugation or ultrasonic cell collection. Advantages of the continuous mode of operation are short product dwell times in the bioreactor, constant product quality during synthesis, increase of the volumetric productivity and greater flexibility of batch volume as a function of the cultivation time to be defined. Disadvantages are contamination of the harvest with residual cells, the cleaning and sterilization expenses incurred for multiple use and the high investment and operating costs for the corresponding plants.

[0004] Besides the hollow-fiber bioreactors of ACUSYST® X Cell Generation, which have proved effective for the synthesis of biopharmaceuticals, other reactor systems are available in which all components coming in contact with the cell culture are designed as disposable components. Thus they can be discarded once they have been used to synthesize a batch. Expensive cleaning and sterilization procedures are not required. Commercially available systems of this type are membrane-based systems such as Cell-Pharm®, Cellmax®, Technomouse®, CELLine®, miniPERM® or OptiCell®. Membrane methods have several advantages. In perfusion operation they can achieve very high cell densities (10^7-10^8 cells/ml) - by virtue of a large membrane surface per unit volume. Moreover, the cells are protected by the membranes from shearing forces. In principle, they are designed for one-time use, so that cleaning and sterilization after use are not necessary. In the art of disposable bioreactors, the wave bioreactor has also proved effective heretofore in the trial phase for the synthesis of biopharmaceuticals. In the system, the cells are cultivated in a bag system, which is systematically agitated in order to improve intimate mixing. One advantage of this reactor technology is the one-time usability of the culture system. Disadvantages are the low achievable densities and the limited scale-up capability.

[0005] In all cited methods and devices, uniform nutrient supply and in particular oxygen supply at high cell densities is problematic. Neither the attempt to solve this problem via complex process steps involving pressurization (1989, US Patent 4804628 A) nor the direct introduction of oxygen into the cell culture chamber via a further membrane system 1986, German Patent 2431450 A1 and 1995, German Patent 4230194 A1) led to culture systems whose scale could be increased as desired and in which the cells could be uniformly supplied. In hollow-fiber bioreactors, in which the cells are cultivated between the hollow fibers and the nutrients are transported in the lumen of the fibers, scale-up is limited by the length of the hollow fibers. However, the length of the hollow fibers is limited by consumption of the oxygen from the hollow fibers. Thereby scale-up is possible only by the use of parallel units. In practice, however, this leads to unprofitable

processes. In other words, the scale-up capability of the hollow-fiber reactors is defeated by the lack of adequate homogeneous supply of the cells with fresh gas and liquid nutrient components.

[0006] In International Patent Application WO 03/064586 A2, it was proposed that cells be cultivated in high density in compartments, the dimension of which compartments is not to exceed 5 mm in length. The interior chamber of the compartments forms a culture chamber, which is partitioned from the supply chamber by a semipermeable element. The cells are retained in the compartments, and oxygen exchange takes place via hollow-fiber membranes. Supply of the cells with nutrients and with oxygen is ensured by means of a variably adjustable mixture of gas and cell-culture media. Although the culture device and the method solve the problem of nutrient and oxygen supply and guarantee scale-up capability, the method described in WO 03/064586 A2 suffers from a disadvantage in that cells of high density must be introduced into the compartments. To overcome this disadvantage, it is proposed in International Patent Application WO 03/102123 A2 that biodegradable gels be used to reduce the inoculation density at the beginning of cell cultivation.

[0007] A liquid-gas-phase exposure bioreactor has been developed in principle by the Zellwerk® Co. and is being sold by the Sartorius® Co. In this bioreactor, the cells that adhere to surfaces are immobilized on disks of carrier material. The disks are disposed in series on a shaft, and are rotated in a cylinder that is half-filled with medium and half-filled with gas. An advantage of this arrangement is the cyclic exposure of these cells to both phases. Disadvantages are the limitation of the system and method to adhering cells, the presence of detached cells in the harvest fluid and the limitation of scale-up capability.

Object of the invention

[0008] The object of the invention is to further improve the method described in WO 03/064586 A2.

Achievement of the object

[0009] The object was achieved as specified in the claims. According to the invention, the initial growth and cultivation of cells is undertaken in a liquid-gas-phase exposure bioreactor containing a supply chamber in which there are disposed hollow-filament membranes having an inside diameter of no larger than 5 mm and whose inner volume forms culture compartments. The following process steps take place:

- introduction of the cells into the culture compartments
- filling approximately one half of the supply chamber with nutrient medium and the other half with a gas mixture
- turning on perfusion of medium and gas simultaneously or separately
- cyclic exposure of the hollow-filament membranes and of the cells contained therein in the gas or liquid phase

[0010] According to a preferred alternative embodiment of the inventive method, the hollow-filament membranes are oriented horizontally in the bioreactor. After the reactor has been filled, half of the membranes are covered with nutrient medium. By rotating the reactor 360° in one direction and then in the opposite direction, cyclic exposure of the hollow-filament membranes and thus of the cells in the gas or liquid phase is achieved.

[0011] Rotation in one direction and then in the opposite direction prevents the tubing connected to the reactor from becoming twisted.

[0012] According to the invention, the rotation is stopped for a certain time after 180° in order to achieve equal exposure times in the gas and liquid phases. The holding times can be variably adjusted. Thereby it is ensured that the cells are supplied sufficiently with nutrients during the dwell time of the membranes in liquid nutrient medium and sufficiently with oxygen during the dwell time in the gas phase. By varying the holding times, it is simultaneously possible to adapt to the individual metabolic requirements of the individual cell lines.

[0013] Alternatively, the cyclic exposure of the hollow-filament membranes can be achieved by immersing the hollow-filament membranes in the nutrient medium and then lifting them into the gas phase. Different dwell times of the cells in the two phases can be achieved by this procedure.

[0014] To implement the inventive method, cells of low density are first introduced into the culture chamber, whereupon they grow to cells of high density. By using gels -as described in International Patent Application WO 03/102123 A2 -it is possible to introduce, into the culture

chamber, cells of the lowest cell density together with gels of cross-linked polypeptides, which have a high glutamine content, and/or with semisolid media of viscous fluids or fluids composed of microscopically small gel fragments.

[0015] The cells are introduced into the compartments via a central charging system outside the supply chamber, so that simultaneous uniform input of the cells into all compartments is possible via one port.

[0016] The inventive method is suitable for cultivating protozoa, bacteria, yeasts, fungi and plant or mammalian cells.

[0017] The method is novel compared with the method described in International Patent Application WO 03/064586 A2, since therein there is provided no exposure of the cells in two different phases but merely exposure of the cells in a variably adjustable mixture of gas and cell culture media. Also, no movement of the reactor or of the membranes was described therein. Not even a hint that the membranes containing the cells can be moved for certain times in the corresponding phases is obtained from WO 03/064586 A2. This lies in the fact that, in the cited publication, there is needed a device for production of the variably adjustable mixture of gas and cell culture media, and so movement of the membranes - especially by rotation - would necessitate further complicated provisions with respect to the connections.

[0018] The inventive device is composed of a cylindrical or spherical two-phase supply chamber (which can be charged with gas and medium respectively), in which -parallel to the longitudinal axis of the cylinder shell- polymeric, cell-retaining, micro filtering, hollow-filament membranes having an inside diameter of no more than 5 mill are fixed in the end plates, the inner volumes of which form culture compartments, in which the cells to be cultivated are disposed, the supply chamber containing a gas phase through which a gas mixture can flow and a liquid phase through which a culture medium can flow, each hollow-filament membrane having a spacing of at least 5 mm to the neighboring hollow-filament membrane over the length of the cylinder, the hollow-filament membranes being symmetrically disposed relative to an imaginary cross section along the axis of rotation of the cylinder and no membrane being disposed on an imaginary cross-sectional plane along the axis of rotation of the cylinder.

[0019] The membranes are permeable for all substances but not for whole cells. The culture chamber, composed of the total volume of the compartments, is partitioned from the supply

chamber by the membrane. This permits the supply substrates to pass into the culture chamber and supply the cells. The partition system also permits products to pass out of the cell compartments into the supply environment.

[0020] The membrane is composed of polymers, such as polysulfone, polyether sulfone or polycarbonate. Hollow-filament membranes that are composed of poly ether sulfone, which is a biocompatible material, and that have membrane wall thicknesses smaller than 300 μm, water permeabilities of greater than 6 m^3/m^2*h*bar and pore diameters of 0.1 to 1.0 μm have proved to be particularly suitable.

[0021] To prevent the formation of liquid films and thus to ensure uniform exposure of the membranes in the gas phase, the membranes have a minimum spacing relative to one another.

[0022] The membranes are preferably disposed in a hexagonal array. This means that every membrane -with the exception of those located at the outer peripheries -is surrounded by 6 membranes with the same spacing relative to the central membrane. Thus the most uniform possible packing density can be ensured in the supply chamber. Further space-saving devices are not necessary.

[0023] The hollow-filament membranes are symmetrically arranged relative to an imaginary cross section along the axis of rotation of the cylinder -which for practical purposes represents the phase boundary. No membranes are located on the imaginary cross-sectional plane along the axis of rotation of the cylinder. In this way it is ensured that, during the holding times, all membranes are either completely in the gas phase or completely in the liquid phase.

[0024] The inventive device is novel compared with the device described in WO 03/064586 A2, since two-phase operation is not provided therein. Furthermore, it does not need any special device for production of a mixture of gas and media or for collection of liquid from the spent mixture of gas and media.

[0025] For input and removal of gas, every end plate of the cylinder contains at least two ports which are respectively disposed above and below the imaginary cross-sectional plane, so that continuous supply with gas is ensured even during rotation of the cylinder around its axis of rotation.

[0026] Furthermore, at least one tubing port for media perfusion and at least one inlet for introduction of seed cells into the culture chamber are disposed on the head faces.

[0027] In addition, the device contains tubings, gas humidifiers, a medium trap in the gas line, an ultrafiltration unit in a product-harvesting line, a hardware unit, pumps, measuring and control units as well as a drive motor and a frame, to permit mounting and rotation of the device.

[0028] The purpose of the ultrafiltration unit in a product-harvesting line is to concentrate the respective product.

[0029] Surprisingly, it has been found that a higher cell density and thus a higher yield of cell products can be achieved with the inventive device than with the device according to WO 03/064586 A2. This can be attributed on the one hand to the optimal use of space and on the other hand to the improved supply of the cells by cyclic exposure of the hollow-filament membranes in the two phases.

[0030] Alternatively, the ports for the gas supply are mounted not on the head faces but on the cylinder shell, above and below the imaginary cross-sectional plane.

[0031] The inventive use of the device lies in the cultivation of cells at high densities and in the recovery of cell products, cell constituents, viruses, proteins or low molecular weight substances, such as drugs as well as diagnostic and research reagents.

[0032] The features of the invention follow from the elements of the claims and from the description, both individual features and also pluralities of features in the form of combinations representing advantageous embodiments for which protection is applied for with this specification.

[0033] The substance of the invention comprises a combination of known elements (partitioning of culture and supply chambers by membranes) and new elements (arrangement of the membranes in the supply chamber, rotatability of the device, alternating exposure of the cells in the gas and liquid phases respectively), which influence one another mutually and in their overall effect lead to an advantage in use and to the desired success, which lies in the fact that there is achieved the capability of effective continuous cultivation of cells in high densities and of recovery of products from these cells with simultaneous cell retention.

[0034] The invention and its function will be explained hereinafter with reference to figures. The purpose of the figures is to permit better understanding, but they are not to be construed as the only constructions with which the claims can be implemented.

Figure 1: Schematic diagram of the bioreactor system

[0035] The figure represents one version of the bioreactor system on the basis of a cylindrical supply chamber (12). The alignment but not the real dimensions and actual number of hollow-filament culture compartments disposed in the supply chamber is represented by the black lines in the supply chamber. The cylindrical vessel is driven by a rotary device (23) such that its direction of movement alternates periodically at a suitable rhythm. The flow of gas phase through the supply chamber is ensured by a gas line, which is composed of a gas-mixing station (13) and a gas humidifier (14) for the gas feed into the cylinder and of a media trap (15) and a contamination trap (16) for the gas discharge out of the cylinder. The flow of liquid phase through the cylinder is ensured by a media line, which is composed of the media reservoir and a pump (17) for the media feed and of a pump (19) and the product-collecting vessel (20) for the discharge from the cylinder. Furthermore, a measuring-sensor train (18) for measuring the oxygen, pH and temperature is integrated in the discharge line. A circulation containing a pump (21) and an ultrafiltration module (22) is connected to the product-collecting vessel for concentration of the product in the product-collecting vessel (20). The product-free filtrate is discharged at the bottom of this ultrafiltration module and discarded, while the concentrated product is recycled to the product-collecting vessel. Advantageously, all elements of the bioreactor system, beginning with the port on the gas-mixing station, are disposable materials The pumps are designed as hose pumps.

Figure 2: Longitudinal section of the cylindrical two-phase supply chamber

[0036] During reactor operation, the cylindrical supply chamber (12) contains a gas phase (5) in the upper part and a liquid phase (6) in the lower part, the two phases forming a phase boundary (7). On the left, the supply chamber is terminated by an end plate (1) for the feed of gas and medium, and on the right it is terminated by an end plate (2) for discharge thereof. On the right terminated which terminates. Gas is passed through ports (3, 4) in the end plates. Media transport takes place via central ports (8, 9) -located on the axis of rotation of the cylinder -in the respective end plates. The individual culture compartments for the cells are designed as identical hollow-filament membranes and are represented in the supply chamber by parallel black lines. Input of the cell suspension takes place separately from the gas and media supply, via ports (10) shown in solid black in the end plates. For uniform seeding with the cells in all hollow-filament

membranes, the ports end in cell-distributing chambers (11), which are in communication with the interior space of every individual hollow-filament membrane.

Figure 3: Top view of the end plates of the supply chamber

[0037] In the top view of the feed end plate (1) there is illustrated one of the arrangements used for the inlets for gas -shown as small circles -and for medium (8) into the supply chamber. The top view of discharge end plate (2) shows the central port for product discharge (9) and an arrangement used for the gas-discharge ports, which are shown as small circles. In this example four ports, represented by black dots, for seeding with the cell suspension are integrated in each of the two end plates, which can be charged via a merged tubing connection.

[0038] The invention will be explained by means of practical examples, without being limited to these examples.

Practical examples:

Example 1: Cells of high density

[0039] Two bioreactor systems were constructed according to the scheme illustrated in Fig. 1. The cylindrical supply chamber had a total volumetric capacity of 14 liters. During the process, the liquid phase contained 7 liters. In both cases, 144 hollow-filament membranes each 500 mm in length were disposed in axially symmetric arrangement in the supply chamber. Seeding with cells in the interior spaces of the hollow-filament membranes took place with cells of high density in PBG1.0 basic medium containing 0.02% of added human serum albumin via the seeding ports in the end plates. The cell line produces a human protein, which can be isolated from the culture supernatant by a one-step chromatographic method and then assayed exactly as to its content. The culture time was 10 and 23 days. Over this time, a mixture of air and 5% CO_2 was passed continuously through the gas phase of the supply chamber. In total, a quantity corresponding to 11 liters in 10 days and 24 liters in 23 days was used to supply the cells in the runs. During the

experiment, the liquid-phase and gas-phase exposure cycles were each 30 seconds between the phase alternations. After completion of culturing, the cells were harvested from the hollow-filament membranes via the seeding ports and the cell density and viability were determined. The protein was isolated from an aliquot of the cell-free product harvest and its content was assayed. The following table shows the cell density and viability achieved in the hollow-filament culture compartments.

	Bioreactor run 1 (10 days)	Bioreactor run 2 (23 days)
Total cell count in inoculum [cells per ml of culture chamber]	1.5E7	1.8E7
Viability of inoculum [%]	67	80
Total cell count of harvest	2.4E7	2.25E7
Viability of harvest (%)	54	29
Total quantity of protein (mg)	48	168

[0040] High cell densities were successfully achieved in the system. Furthermore, 48 mg and 168 mg of protein were formed during the process and were collected from the cell-free culture supernatant into the corresponding product-collecting vessel.

Example 2: Cells of low density

[0041] Cells in living cell densities of 1.3E5 cells per milliliter of culture chamber were used for inoculation in two two-phase exposure reactors, each containing 12 hollow-filament membrane shaving a length of 200 mm, and were cultivated for 4 days while both plastic reactors were being rotated. Prior to inoculation, the cells were mixed with microscopically small gel fragments of HSA. Media exchange was effected discontinuously. The metabolic activity was measured via the glucose consumption. After completion of the runs, the cell densities were determined by harvesting the gel together with the cells contained therein and counting via Trypan Blue. Within

the short culture time, expansion of the cells to 6E5 living cells per milliliter of culture chamber (4.6 times) and 5E5 living cells per milliliter of culture chamber (3.8 times) was successfully achieved.

Example 3: Functioning principle of the bioreactor system

[0042] The supply principle of the bioreactor is based on exposing the cells alternately in medium and in a gas mixture, thus making it possible to improve the supply of the cells with oxygen compared with conventional systems.

Example 4: Construction of the system

[0043] The core piece of the bioreactor is a cylindrical plastic reactor mounted horizontally. In this vessel, hollow-filament membranes are clamped over the length, parallel to the axis of rotation, as illustrated in Fig. 2. Hereby two chambers separated from one another by the membranes are created in the cylinder. One is the supply chamber, which surrounds the hollow-filament membranes and is composed of a liquid phase and a gas phase. The boundary between these two phases is sketched in Figs. 2 and 3. The other is the space inside each hollow-filament membrane. The sum of all hollow-filament internal spaces represents the culture chamber for the cells. Via the number of hollow-filament membranes disposed symmetrically around the axis of rotation, the system can be scaled-up to any desired size in terms of its culture chamber. The two chambers have separate inlets, and are partitioned from one another. The only communication between the supply chamber and the culture chamber is represented by the pores of the membrane. With an advantageous pore diameter of 0.1 to 1.0 µg per milliliter, these pores are permeable for small molecules and proteins, but not for the cells. The overall device cited and described in Fig. 1 ensures that gas and liquid can be passed continuously through the system.

[0044] The bioreactor system also includes a mobile hardware unit, the pumps and compressors, as well as measuring and control units. Furthermore, this unit also includes a drive motor and a rotary device, which permits mounting and rotation of the plastic reactor.

Example 5: Functioning principle

[0045] By means of the rotary device, the plastic reactor is turned around its axis of rotation in a rotation cycle that can be adapted to the respective cell line. This rotation cycle is advantageously repeated without interruption over the entire bioreactor run time. The eight phases of a rotation cycle are listed below by way of example.

Phase: rotation to the right by 180°
Phase: holding time
Phase: rotation to the right by 180°
Phase: holding time
Phase: rotation to the left by 180°
Phase holding time
Phase: rotation to the left by 180°
Phase: holding time

[0046] As the result of a rotation cycle, the reactor has performed one full revolution in one direction and one full revolution in the opposite direction and is once again disposed in the original starting position. The alternation of direction of rotation permits media and gas to flow through ports, which are integrated in fixed position in the reactor and to which plastic tubes are fixed. In contrast to the majority of mammalian-cell bioreactors, therefore, the system operates effectively without mobile structural components that project into the sterile supply or culture zone, such as impeller shafts or media and gas feed tubes. Thus the associated contamination risk does not exist, and no expenses are incurred for safeguarding corresponding rubbing surfaces, for example by double rotating mechanical seals.

[0047] The system construction and mode individual of operation simultaneously ensure that every hollow-filament membrane is subjected to identical exposure conditions in both phases over the entire bioreactor run time, regardless of the number of such membranes in the system. The exposure in the gas phase primarily achieves the supply of oxygen, while the exposure in the liquid phase achieves primarily the uptake of dissolved nutrients and the discharge of metabolic products. Both nutrient medium and gas mixture can be fed continuously.

Advantages / novel features:
Reactor with integrated cell retention system as disposable article

Membrane is permeable for protein, permitting cell-free harvesting

Identical exposure conditions for every individual hollow-filament membrane in the system

Short diffusion paths for oxygen during exposure in the gas phase

No gradient formation in the gas phase of the exposure reactor over the length of the reactor

A plurality of gas ports, which are distributed appropriately over the end caps and which permit gas to flow through continuously even during rotation

Definitions:

Cells of high density: A culture with high cell density is achieved at cell densities greater than 1E7 cells per milliliter of culture chamber.

Cells of low density are achieved when the cell density in the culture chamber lies between 1E4 and 1E7 per milliliter of culture chamber.

Cells of lowest density: A culture with the lowest cell density is achieved at densities lower than 1E4 cells per milliliter of culture chamber.

Nutrient medium: Nutrient medium is an aqueous solution containing the nutrients essential for the cells, such as glucose, amino acids and trace elements.

Gas mixture: Within the meaning used here, a gas mixture advantageously describes a mixture of air and carbon dioxide with variable mixing ratio. Furthermore, the present meaning of the term gas mixture also includes variable mixing ratios of nitrogen, oxygen and CO_2.

List of reference numerals:

1	Feed end plate	11	Cell-distributing chambers
2	Discharge end plate	12	Cylindrical supply chamber
3, 4	Ports for gas supply and removal	13	Gas-mixing station
5	Gas phase	14	Gas humidifier

6	Liquid phase	15	Media trap
7	Phase boundary	16	Contamination trap
8	Inlet for medium (central port)	17, 19, 21	Pumps
9	Central port for product discharge	22	Ultrafiltration module
10	Ports for the culture chamber	23	Rotary device

Claims

1. A method for initiation of growth and cultivation of cells in a liquid-gas-phase exposure bioreactor containing a supply chamber in which there are disposed hollow-filament membranes having an inside diameter of no larger than 5 mill and whose inner volume forms culture compartments, characterized by the following steps:
   - introduction of the cells into the culture compartments
   - filling approximately one half of the supply chamber with nutrient medium and the other half with a gas mixture
   - turning on perfusion of medium and gas simultaneously or separately
   - cyclic exposure of the hollow-filament membranes and of the cells contained therein in the gas or liquid phase

2. A method according to claim 1, characterized in that the hollow-filament membranes are oriented horizontally, after the reactor has been filled, half of the membranes are covered with nutrient medium, and cyclic exposure of the hollow-filament membranes is achieved by rotating the reactor 360° in one direction and then in the opposite direction.

3. A method according to claim 2, characterized in that the rotation takes place in two 180° steps, which are separated from one another by variably adjustable waiting times, so that each individual hollow-fiber membrane spends the same time in the liquid phase as in the gas phase.

4. A method according to claim 1, characterized in that the cyclic exposure is achieved by immersing the hollow-filament membranes in the nutrient medium and then lifting them into the gas phase.

5. A method according to claim 1, characterized in that cells of low density are introduced into the culture chamber and grow to cells of high density.

6. A method according to claim 5, characterized in that cells of the lowest cell density are introduced into the culture chamber together with gels of cross-linked polypeptides, which have a high glutamine content, and/or with semisolid media of viscous fluids or fluids composed of microscopically small gel fragments.

7. A method according to claim 1, characterized in that the cells are protozoa, bacteria, yeasts, fungi and plant or mammalian cells.

8. A method according to claim 1, characterized in that the cells are introduced into the compartments via a central charging system outside the supply chamber and in that simultaneous (homogeneous) input of the cells into all compartments is possible via one port.

9. A device for initiation of growth and cultivation of cells in a cylindrical or spherical two-phase supply chamber (which can be charged with gas and medium respectively), in which parallel to the longitudinal axis of the cylinder shell -polymeric, cell-retaining, micro filtering, hollow-filament membranes having an inside diameter of no more than 5 mm are fixed in the end plates, the inner volumes of which form culture compartments, in which the cells to be cultivated are disposed, characterized in that

- the supply chamber contains a gas phase through which a gas mixture can flow and a liquid phase through which a culture medium can flow
- each hollow-filament membrane has a spacing of at least 0.5 mm to the neighboring hollow-filament membrane over the length of the cylinder
- the hollow-filament membranes are symmetrically disposed relative to an imaginary cross section along the longitudinal axis of the cylinder
- no membrane is disposed on the imaginary cross-sectional plane along the longitudinal axis of the cylinder.

10. A device according to claim 9, characterized in that, for input and removal of gas, every end plate of the cylinder contains at least two ports, which are respectively disposed above and below the imaginary cross-sectional plane, and so the supply chamber containing the hollow-filament membranes can be rotated around its longitudinal axis during supply of medium and gas.

11. A device according to claim 9 and 10, characterized in that the ports for the gas supply are mounted not on the head faces but on the cylinder shell, above and below the imaginary cross-sectional plane.

12. A device according to claim 9, characterized in that the hollow-filament membranes have a wall thickness smaller than 300 µm, a water permeability of greater than 6 $m^3/m^2*h*bar$ and a pore diameter of 0.1 to 1 µm.

13. A device according to claim 9, characterized in that the membranes are disposed in a hexagonal array in the supply chamber.

14. A device according to claim 9, characterized in that at least one tubing port for medium perfusion and at least one inlet to the culture chamber are disposed on the head faces.

15. A device according to at least one of claims 10 to 14, characterized in that it additionally contains tubings, gas humidifiers, a medium trap in the gas line, an ultrafiltration unit in a product-harvesting line, a hardware unit, pumps, compressors, measuring and control units as well as a drive motor and a frame, to permit mounting and rotation of the device.

16. The use of the device according to claims 9 to 15 for cultivation of cells in high densities and for the recovery of cell products, cell constituents, viruses, proteins or low molecular weight substances.

17. The use according to claim 16 for recovery of drugs.

18. The use according to claims 16 and 17 for synthesis of diagnostic reagents.