

A method for continuous, real-time monitoring of chemical concentrations in bioreactors and apparatus and probe therefor

5 The invention relates to an apparatus comprising a reactor and a probe for measuring and/or monitoring the chemical composition of a fluid medium through automated, continuous sampling techniques. Moreover, the present invention deals with the probe, and a kit of components comprising the reactor and the probe. Furthermore, the present invention relates to the use of a probe, and a method for measuring and/or monitoring the chemical composition of a fluid medium.

10 Sensors are typically utilized in bioreactors and fermenters for real-time monitoring and the control of spatiotemporal changes of relevant process parameters such as pH value, temperature and oxygen concentrations. However, most of the currently existing sensors have major shortcomings.

15 Importantly, these sensors do not allow the measurement of other critical elements such as amino acids, proteins (such as growth factors), fatty acids and/or minerals. One may try to solve this problem by including numerous sensors to measure concentrations of each one of such compounds individually but this is often not feasible. The available space to integrate/immerse sensors into a bioreactor is limited and large numbers of inserted sensors into a vessel will affect the medium
20 mixing and fluid dynamics within the reactors. In addition, most sensors lose sensitivity over time, which affects long-term real-time monitoring and precision of the measurements. Moreover, for many of the compounds of interest, there are no sensors available yet.

25 To overcome these issues, bioreactor users often collect samples manually and analyze them using external devices. Such approaches can often lead to contamination of the bioreactor content and of the collected samples, especially if sampling is carried out repeatedly, and only provide a temporal snapshot of an extended, dynamic process. Therefore, a continuous, automated measurement of the chemical composition of the fluid medium within the bioreactor is required to
30 provide an accurate estimate of the state of the process and an opportunity to control the operations, while reducing the probability of contamination.

Microdialysis is a method e.g. known in neuroscience applications. It has been used for many decades to determine concentrations of neurotransmitters (including amino acids), proteins, fatty acids and other components in the brain of freely

moving animals (particularly rodents). Conceptually, a probe with a semi-permeable membrane is to be inserted into the medium of interest.

The compounds of interest passively diffuse into the probe and are collected at defined intervals. The samples can be analyzed in real-time or stored to be analyzed at a later time-point. "Overview of Brain Microdialysis" (Vladimir I. Chefer et al., Curr. Protoc. Neurosci. 2009 Apr; Chapter: Unit 7.1), and "Microdialysis in Rodents" (Augustin Zapata et al., Curr. Protoc. Neurosci. 2009 Apr; Chapter: Unit 7.2); describe methods for carrying out Microdialysis in living organisms.

The *in vitro* use of microdialysis probes is less common, but has also been described, e.g. in "Microdialysis-bioreactor for on-line monitoring of glucose in food samples" (Saverio Mannino et al., Electroanalysis 2005, 9(17), pp. 1337-1340) and "In vivo and in vitro microdialysis sampling of free fatty acids" (Signe Mølhøj Jensen et al., J. Pharm. Biomed. Anal. 2007, 43(5), pp- 1751-1756). However, the employed methods are used for selectively detecting individual compounds such as glucose or free fatty acids, respectively, and require complex configurations and/or radiolabeled analytes to enable such detection of individual compounds.

WO 2004/111209 describes a method of monitoring the formation of tissue in a tissue construct, which comprises contacting a micromembrane probe with the tissue construct, carrying out microdialysis and/or ultrafiltration to extract a marker into the dialysate and analysing the marker in the dialysate. For detection of individual analytes, different methods such as enzymatic procedures, radioactive labelling and fluorescent labelling are employed.

WO 2021/237036 discloses an apparatus and method for automatic and continuous glucose monitoring based on binding proteins. The apparatus comprises a sampling device, a microfluidic column device involving a solid support comprising immobilized binding protein, and a fluorometer. As the sampling device, a microdialysis device is used, which is inserted into a cell-culture medium within the reactor.

In the apparatuses and methods for *in vitro* microdialysis known in the art, the employed microdialysis probes are often needle-shaped or rod-shaped and are typically inserted into the medium of interest in an orientation, thus that the probe longitudinally protrudes into the interior of a bioreactor, which allows the sampling portion of the probe (i.e. the semi-permeable membrane) to be located at a central position within the reactor. This enables sampling of the medium of interest at a predetermined position, e.g. at a position proximate to or in contact with an

immobilized cell culture, and/or at a position of increased concentration of a certain component of the medium of interest.

While such orientation may be beneficial for determining local concentrations of specific analytes at a predetermined position, it is often desirable to agitate the medium of interest, e.g. in order to provide a homogeneous distribution of the components throughout the medium. However, it has been found that agitation (e.g. stirring) of the medium surrounding the microdialysis probe leads to disadvantages. On the one hand, large particles and/or debris present in the medium of interest may collide with the probe due to agitation, which can cause damage to the semipermeable membrane. On the other hand, the positioning of the probe in the medium of interest as known in the art affects the fluid dynamics of the agitated medium inside the reactor, causing undesired turbulences in the medium of interest. These turbulences can lead to increased shear stress both on the semipermeable membrane of the probe and on potentially sensitive components of the medium of interest, and increase the risk of mechanically damaging both the probe and the potentially sensitive components of the medium.

US 2007/0292958 relates to apparatuses and methods for analyzing enzyme reactions, including large, industrial-scale reactions. The method involves the use of microdialysis probes connected to an analytic device, e.g. for HPLC analysis, introduced into stirred reactors. To protect the probe from damage, by collision with large particles or debris, an outer sleeve made from polypropylene mesh can be provided around the probe. However, while such a sleeve protects the probe from collisions, the disadvantages arising from altered fluid dynamics of the agitated medium are not addressed.

It now has been surprisingly found that the effects of the probe on the fluid dynamics of an agitated medium, and the disadvantages associated therewith, can be substantially reduced or eliminated by selection of a specific shape, orientation and/or position of the probe within a reactor.

One aspect of the present invention is an apparatus comprising a reactor, preferably a bioreactor; and at least one probe comprising a first surface and a second surface and at least one hollow conduit for transporting fluids, formed between the first surface and the second surface, running along the first surface and the second surface, wherein the hollow conduit has an inlet opening for receiving fluids, and an outlet opening for releasing fluids received through the inlet opening and transported through the hollow conduit, wherein the first surface is made at least partially from a semipermeable membrane, and the semipermeable membrane forms a wall of a

semipermeable portion of the hollow conduit, and wherein the first surface is exposed to the interior volume of the reactor, and wherein the probe is fixed permanently or non-permanently to a portion of an inner surface of a wall of the reactor with the second surface of the probe facing the portion to which the probe is fixed.

Another aspect of the invention is a kit of components comprising the reactor and probe as defined above, and optionally at least one fluid source and/or at least one instrument for collecting and/or analyzing fluids, wherein the probe is configured to be fixed permanently or non-permanently to a portion of an inner surface of a wall of the reactor with the second surface of the probe facing the portion to which the probe is fixed.

A further aspect of the invention is a probe comprising a first surface and a second surface and a hollow conduit for transporting fluids, formed between the first surface and the second surface, running along the first surface and the second surface, wherein the hollow conduit has an inlet opening for receiving fluids, and an outlet opening for releasing fluids received through the inlet opening and transported through the hollow conduit, wherein the first surface is made at least partially from a semipermeable membrane, and the semipermeable membrane forms a wall of a semipermeable portion of the hollow conduit, and wherein the first surface and the second surface of the probe deviate from being planar, and the curvatures of the first surface and the second surface of the probe are essentially concentric, and wherein the probe is configured to be fixed permanently or non-permanently to a portion of an inner surface of a wall of a reactor with the second surface of the probe facing the portion to which the probe is configured to be fixed.

Yet another aspect of the invention is the use of the probe according to the invention for measuring and/or monitoring the chemical composition of a fluid medium in a reactor, wherein the probe is fixed permanently or non-permanently to a portion of an inner surface of a wall of a reactor with the second surface of the probe facing the portion to which the probe is fixed, thereby reducing disturbances in the fluid dynamics of the fluid medium in the reactor.

Furthermore, an object of the present invention is a method for measuring and/or monitoring the chemical composition of a fluid medium comprising the steps:

a) providing an apparatus as described herein;

- b) introducing a fluid medium into the reactor of the apparatus, thus that the first surface of the at least one probe exposed to the interior volume of the reactor is immersed in the fluid medium,
- c) optionally agitating, preferably stirring the fluid medium;
- 5 d) feeding a perfusion fluid into the hollow conduit through the inlet opening at a predetermined rate;
- e) analyzing the chemical composition of the sample fluid released from the outlet opening at predetermined intervals, preferably by chromatographic means, more preferably by high performance liquid chromatography,
- 10 wherein the semipermeable membrane is configured to permit diffusion of at least one component, preferably selected from proteins, enzymes, minerals and fatty acids, of the fluid medium and/or the perfusion fluid between the fluid medium in the reactor and the perfusion fluid in the hollow conduit, thus generating the sample fluid in the hollow conduit, and wherein preferably the chemical composition of the
- 15 perfusion fluid fed into the hollow conduit through the inlet opening corresponds essentially to the initial chemical composition of the fluid medium as introduced into the reactor.

The invention and embodiments thereof are illustrated in more detail in the following.

A fluid medium in the context of the present invention is not particularly limited and
20 may be any fluid comprising at least one component that is capable of passing through a semipermeable membrane. Preferably, the fluid medium is an aqueous solution, more preferably an aqueous cell culture medium or nutrient solution, more preferably an aqueous nutrient solution, preferably comprising at least one component selected from proteins, enzymes, minerals and fatty acids.

25 The reactor type used in the present invention is not particularly limited, and may be any type of reactor, preferably bioreactor, known in the art, which can accommodate fluid media, provided that it allows permanent or non-permanent fixture of a probe as described herein to a portion of an inner surface of a wall of the reactor. Preferably, the reactor is suitable for carrying out chemical, biochemical and/or
30 biological processes. More preferably, the reactor is a bioreactor suitable for growing and/or cultivating cell tissue, in particular edible cell tissue.

For example, the reactor may be a container or vessel, such as a bottle, a flask, a vial or a tank, configured to contain a fluid medium, or a conduit, such as a hollow structural section, a tube, a pipe or a hose, through which a fluid medium is passed.
35 Preferably, the reactor is a container or vessel.

The shape of the reactor is not particularly limited, and may be any reactor shape known in the art. For example the reactor may have an essentially prismatic (such as cuboid) shape, an essentially ellipsoid (such as spherical) shape or an essentially cylindrical shape.

- 5 Preferably, the reactor has an essentially ellipsoid shape or an essentially cylindrical shape, more preferably an essentially spherical shape or an essentially cylindrical shape, more preferably an essentially cylindrical shape.

In the present description, the terms “essentially prismatic”, “essentially ellipsoid”, “essentially spherical” and “essentially cylindrical” mean that the walls of the reactor
10 surround an internal space that can be described by a prism, an ellipsoid, a sphere or a cylinder, respectively, which may contain irregularities, and which may deviate from being a prism, an ellipsoid, a sphere or a cylinder, respectively, to provide the required or preferred functions to the reactor. For example, the space may deviate from being the respective shape by including features allowing fixture of the probe
15 as described herein, connection of the reactor to other components of the apparatus as described herein, introduction and removal of solids and/or fluids, e.g. liquids and/or gases, into and from the reactor, inclusion of agitating means into the reactor, etc.

The material from which the reactor is made is not particularly limited and may be
20 any reactor material known in the art that is suited for being in contact with a fluid medium as described herein. For example, the reactor can be made from metal, glass, polymer(s) and composites thereof, provided the portions of the reactor that come in contact with the fluid medium, e.g. the inner wall of the reactor, are inert toward the fluid medium and any components thereof. Preferably, the reactor is
25 made from glass or metal, more preferably from stainless steel. In some embodiments, the inner surface of the reactor walls may be coated with a further material that is inert toward the fluid medium and any components thereof, or prevents adhesion thereof to the inner surface of the reactor walls.

Inert in the present context means that no detectable reaction of the material with
30 the fluid medium or components thereof takes place, and no detectable dissolution of the material in the fluid medium takes place under the conditions present in the reactor over a period of at least 2 months.

The size of the reactor is not particularly limited and depends on the amount of fluid medium to be accommodated therein, or the scale of the process carried out therein.
35 Preferably, the internal volume of the reactor is at least 0.25 L, more preferably at

least 5 L, more preferably at least 20 L, more preferably 25 to 250 L, more preferably 30 to 100 L.

Moreover, the reactor may contain functional features.

Typically, the reactor will contain at least one opening for introducing and/or
5 removing fluid media and/or other components into and from the reactor.

In addition, the reactor may contain at least one sensor for determining at least one
parameter of a fluid medium and/or a process in the reactor. For example, the
reactor may contain a thermometer for determining the temperature of a fluid
medium in the reactor, a pressure gauge for determining the pressure within the
10 reactor, a pH-meter for determining the pH value of a fluid medium, electrodes for
determining the conductivity of a fluid medium in the reactor and/or transparent
windows allowing visual inspection of the fluid medium.

Moreover, the reactor may contain components allowing manipulation of the
conditions within the reactor. For example, the reactor may contain heating and/or
15 cooling means for manipulating the temperature within the reactor. Furthermore, the
reactor may contain at least one agitator, such as a stirrer, for agitating a fluid
medium within the reactor.

The apparatus according to the present invention comprises a reactor, e.g. as
described above, and at least one probe. The probe comprises a first surface and a
20 second surface and at least one hollow conduit for transporting fluids, formed
between the first surface and the second surface.

In the apparatus of the present invention, the first surface of the probe is exposed
to the interior volume of the reactor, and thus to the fluid medium when said fluid
medium is introduced into the reactor. The first surface of the probe is made at least
25 partially from a semipermeable membrane. The material of the semipermeable
membrane is not particularly limited and may be any material known in the art for
semipermeable membranes, in particular those commonly used for microdialysis,
provided it is inert toward the fluid medium to be introduced into the reactor and any
components thereof. The material and the further properties, such as the pore size,
30 of the membrane are typically selected depending on the fluid medium that is to be
introduced into the reactor, and the components thereof, the concentration of which
is to be monitored.

For example, the material of the semipermeable membrane may be selected from
silicones, polysulfones, polyethersulfones, polyacrylonitrile, cellulose, polyamides,
35 polycarbonates, chemically modified derivatives thereof, copolymers thereof and

composites thereof. Preferably, the material of the semipermeable membrane is selected from silicones, since silicones are typically durable and are essentially not susceptible to adhesion of, e.g. cell tissue. Furthermore, the pore size of the semipermeable membrane may be, e.g. selected to have a molecular weight cut-off
5 range from 0.1 to 500 kDa, preferably 1 to 200 kDa, more preferably 5 to 100 kDa, more preferably 10 to 50 kDa.

In particular, the pore size is selected to have a molecular weight cut-off above the molecular weight of the components of the fluid medium, the concentration of which is to be measured and/or monitored. When multiple probes are used in the
10 apparatus of the invention, these may comprise semipermeable membranes with different molecular weight cut off ranges and/or made from different materials.

The first surface of the probe may optionally comprise a mesh of a more durable material than the membrane material, e.g. to protect the membrane from large particles and/or debris present in a fluid medium. In this case, the first surface of the
15 probe that is exposed to the interior volume of the reactor is partially made of the semipermeable membrane and partially made of the more durable material. The mesh may e.g. be a polymeric or a metallic mesh.

The second surface of the probe may be made from any material that is inert toward the fluid medium or any components thereof. For example, the material of the
20 second surface may be the same as the material of the first surface, or may be different therefrom. For example, the second surface may be made from a semipermeable membrane as disclosed for the first surface, or may be made from the same material as the semipermeable membrane, while being impermeable (i.e. lacking the pores). Alternatively, an entirely different material may be used for the
25 second surface. In some embodiments of the apparatus of the invention, which are described in more detail further below, the probe may be integrated into the wall of the reactor.

In such embodiments, the second surface of the probe may correspond to the interface formed by the interior volume of the probe and the portion of the inner
30 surface of the wall of the reactor, to which the probe is fixed. In these embodiments, the second surface of the probe is not necessarily made from a separate material as such, but is defined by the portion of the inner surface of the wall of the reactor, to which the probe is fixed.

The hollow conduit for transporting fluids, formed between the first surface and the
35 second surface of the probe and comprising an inlet opening and an outlet opening, runs along the first surface and the second surface of the probe. The expression

“runs along” in the present context means that at least a portion of the hollow conduit, preferably at least 80%, more preferably at least 90% of the conduit is oriented in a direction concurrent with the first and second surfaces of the probe.

5 The hollow conduit comprises at least one semipermeable portion, in which a wall of the hollow conduit is formed by the semipermeable membrane of the first surface of the probe.

10 This means that the wall of the hollow conduit in said semipermeable portion at least partially corresponds to the first surface of the probe, and thus is exposed to the interior volume of the reactor. During operation of the apparatus of the invention, this enables diffusion of components between the fluid medium in the reactor and the perfusion fluid in the hollow conduit through the semipermeable membrane forming the wall of the hollow conduit in the semipermeable portion.

15 In the apparatus of the present invention, the probe is fixed permanently or non-permanently to a portion of an inner surface of a wall of the reactor with the second surface of the probe facing the portion to which the probe is fixed.

20 Permanent fixture in the present context means that, after fixture, the probe cannot be removed from the reactor wall without causing damage to the probe or the reactor. Permanent fixture may be obtained e.g. by using permanent adhesives or by welding the probe to the inner surface of the reactor wall. Permanent fixture reduces the risk of the probe being accidentally detached from the inner surface of the reactor wall, e.g. due to intense agitation, which in turn may lead to damaging of the probe itself and other internal components of the reactor due to potential collisions therewith, or sensitive components of the process being monitored (e.g. cell tissue). Moreover permanent fixture reduces the risk of leakage.

25 In contrast, non-permanent fixture means that, the probe is reversibly fixed to the inner surface of the reactor wall. Non-permanent fixture may be obtained e.g. by using non-permanent adhesives, magnets, or mechanical fixture, e.g. by screws, clamps, bolts, etc.. Non-permanent fixture is beneficial, e.g. if the probe needs to be removed for cleaning, maintenance or replacement of the probe or reactor.

30 In the apparatus of the present invention, the second surface of the probe faces the portion of the inner surface of the wall of the reactor, to which the probe is fixed. The second surface may be fixed to the said portion directly, thus that essentially no gap is provided between the inner surface of the wall and the second surface of the probe, or at a distance from said portion, for example using separators. If the second surface of the probe is fixed at a distance from the portion of the inner surface of the
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reactor wall, this distance is preferably not more than 10%, preferably not more than 5%, more preferably not more than 2.5% of the diameter of the reactor.

Keeping the distance low is useful for reducing the effect of the probe on the fluid dynamics of an agitated fluid medium in the reactor. In addition, it has been found
5 that this also has benefits on the measurement and/or monitoring of the chemical composition of a fluid medium in the reactor, since the agitation of the fluid medium leads to movement of the fluid medium in the direction of the reactor walls (e.g. due to centrifugal movement during stirring), and thus in the direction of the probe that is located at the inner surface of the reactor walls, improving the probability of
10 diffusion between the fluid medium and a perfusion fluid inside the hollow conduit, which in turn is beneficial for the measurement accuracy.

Preferably, the second surface of the probe is fixed directly to said portion of the inner surface of the reactor wall. In this case, the probe may optionally be fixed to the portion of the inner surface of the wall of the reactor by integrating the probe into
15 the wall of the reactor, thus that the second surface of the probe corresponds to the interface formed by the interior volume of the probe and the portion of the inner surface of the wall of the reactor, to which the probe is fixed.

It is further possible to provide structural elements to the wall of the reactor to facilitate fixture of the probe. For example, such structural elements may be
20 indentations, grooves, recesses, or channels formed within the inner surface of the wall at the portion to which the probe is fixed, into which the probe can be fitted.

Although this leads to higher complexity of the reactor design, such structural elements allow fixing the probe in a way that the first surface of the probe, which is exposed to the interior volume of the reactor, and the inner surface of the wall of the
25 reactor around the portion to which the probe is fixed, have a smooth transition, thus that the effects of the probe on the fluid dynamics of an agitated fluid medium within the reactor are reduced even further.

In some preferred embodiments of the apparatus of the invention, the second surface of the probe and the portion of the inner surface of the wall of the reactor to
30 which the probe is fixed, deviate from being planar, and a curvature of the second surface of the probe is essentially concentric with a curvature of said portion to which the probe is fixed. The curvatures are considered "essentially concentric" in the present context, if two centers of curvature are offset by not more than 10%, preferably by not more than 5%, more preferably by not more than 2% of the greater
35 of the respective curvature radii.

In these embodiments, it is preferred that the curvature radius of the second surface of the probe deviates from the curvature radius of the portion of the inner surface of the wall of the reactor to which the probe is fixed by not more than 10%, more preferably by not more than 5%, more preferably by not more than 2%. Particularly
5 preferably, the curvature radius of the second surface of the probe essentially corresponds to the curvature radius of the portion of the inner surface of the wall of the reactor to which the probe is fixed, meaning that essentially the entire second surface of the probe touches the inner surface of the wall in the portion to which the probe is fixed.

10 In the present description, this means that at least 90%, preferably at least 95%, more preferably at least 98% of the second surface is in contact with the inner surface of wall of the reactor. In embodiments where the probe is fixed to the portion of the inner surface of the wall of the reactor by integrating the probe into the wall of the reactor, thus that the second surface of the probe corresponds to the interface
15 formed by the interior volume of the probe and the portion of the inner surface of the wall of the reactor, to which the probe is fixed, the curvature of the second surface may be considered identical to the curvature of said portion.

It is also preferred that the first surface of the probe, too, deviates from being planar and the curvature thereof is essentially concentric with the curvature of the second
20 surface of the probe.

The curvature of the second surface of the probe can be provided by a rigid element which is attached to the second surface. The rigid element may be any structural element, which holds its shape when not exposed to external mechanical forces or when exposed to the conditions within the reactor, and can be made from any solid
25 material that is inert to, or is coated with a coating inert to, the fluid medium and components thereof, preferably a metal or a polymer, more preferably a metal, more preferably stainless steel. For example, the rigid element may be a band, rod, tube, profile, hollow structural section, plate or similar structural element that has a curvature concentric with the curvature of the second surface of the probe.

30 For example, the second surface of the probe itself may comprise or be the rigid element and/or the rigid element may be attached to the second surface on the inside of the probe and/or the rigid element may be attached to the second surface on the outside of the probe. For example, in cases where the second surface of the probe is in direct contact with the inner surface of the wall of the reactor, or where
35 the probe is integrated into the wall of the reactor, the wall itself may be the rigid element. For example, in cases where a distance from the second surface of the

probe to the inner surface of the reactor wall is provided using separators, the separators may be the rigid element. Furthermore, portions of the hollow conduit of the probe may be rigid and thus form the rigid element.

Preferably, the curvature of the first surface of the probe is also provided by a rigid element attached to the first surface. More preferably, it is the same rigid element as the one attached to the second surface of the probe, if the rigid element is attached to the second surface on the inside of the probe.

Furthermore, the hollow conduit of the probe may comprise an impermeable portion made from a material impermeable to the components of the fluids to be transported through the hollow conduit, wherein the impermeable portion extends from the inlet opening of the hollow conduit to at a position within the probe and wherein the semipermeable portion of the hollow conduit extends from the position at which the impermeable portion ends to the outlet opening of the hollow conduit, wherein the impermeable portion and the semipermeable portion are in fluid communication with each other at said position.

This means that a fluid introduced into the inlet opening will flow from the inlet opening through the impermeable portion of the hollow conduit to the position at which the impermeable portion ends, and further through the semipermeable portion of the hollow conduit to the outlet opening, where the fluid is released. The impermeable portion may be made from any material impermeable to the components of the fluids to be transported through the hollow conduit. Preferably, the impermeable portion is made from a polymer or a metal, more preferably a metal, more preferably stainless steel. Particularly preferably, the impermeable portion of the hollow conduit is the rigid element as described above.

Typically, the probe will have a length L , a width W , and a thickness D . The thickness D of the probe corresponds to the dimension of the probe perpendicular to the first surface and the second surface, i.e. the distance between the first surface and the second surface; the length L corresponds to the largest dimension of the probe along the second surface of the probe; and the width W corresponds to the dimension along the second surface of the probe that is perpendicular to the length L . Where the second surface of the probe deviates from being planar, the length L and width W are measured along the respective curvature of the second surface of the probe (i.e. not along a straight line between two end points).

Preferably, the second surface of the probe, more preferably the second and first surfaces of the probe, deviate from being planar at least along the largest dimension (i.e. length L) of the probe. For example, where the reactor is essentially cylindrical,

the second surface of the probe, preferably the second and first surfaces of the probe, preferably deviate from being planar along the length L of the probe, but not along the width W of the probe, whereas if the reactor is essentially ellipsoid, the second surface of the probe, preferably the second and first surfaces of the probe, preferably deviate from being planar along both the length L and the width W of the probe.

Preferably, the length L , width W and thickness D satisfy the conditions $L > W$ and $W \geq D$, more preferably $L > 2 W$ and $W \geq 1.5 D$, more preferably $L \geq 3 W$ and $2 D \leq W \leq 4 D$, more preferably $L \geq 4 W$ and $2.5 D \leq W \leq 3.5 D$.

The size of the probe is not particularly limited, and can be selected e.g. depending on the size of the reactor, the scale of the process to be carried out or the fluid medium to be measured and/or monitored. For example the volume of the probe may be 100 mL or less, such as 1 μ L to 20 mL, preferably 100 μ L to 10 mL, more preferably 1 mL to 5 mL. In the apparatus of the invention, the ratio of the internal volume of the probe to the internal volume of the reactor is preferably less than 1:50, more preferably between 1:250,000 and 1:100, more preferably between 1:100,000 and 1:1,000, more preferably between 1:50,000 to 1:2,500.

When such low volume ratios are used, the effects of diffusion of certain components between a perfusion fluid within a probe and a fluid medium in the reactor on the overall concentration thereof in the fluid medium are low, thus that the monitored process is not substantially affected. In contrast, the effects of said diffusion on the overall concentration of said components in the perfusion fluid within the probe are high, thus that the measurement sensitivity is improved.

Preferably, where the hollow conduit comprises an impermeable portion as described above, the probe has a first end and a second end along the largest dimension of the probe, and both the inlet opening and the outlet opening of the hollow conduit are located at a position proximate to the first end of the probe, and the position at which the impermeable portion of the hollow conduit ends, and transitions to the permeable portion, is located at a position proximate to the second end of the probe.

Preferably, the largest dimension of the probe is oriented horizontally in the reactor. With such orientation, e.g. in a stirred tank reactor, the forces exhibited by an agitated fluid medium onto the surface of the reactor (e.g. due to centrifugal acceleration) are more evenly distributed, thus that the risk of damaging the probe is reduced. Furthermore, with such orientation, a fluid medium rotated in the reactor and moving along the inner surface of the reactor walls in a horizontal direction,

encounters a short side of the probe defined by width W , thus that the area of the probe that may affect the fluid dynamics of the agitated fluid medium is reduced, which in turn reduces the risk of damaging the probe and potentially sensitive components of the fluid medium, e.g. cell tissue.

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Preferably, the reactor is essentially cylindrical, having a cylinder radius r at the inner surface of the wall, or essentially spherical, having a sphere radius r at the inner surface of the wall, and wherein the second surface of the probe has a curvature radius in the range of $0.9 r$ to $1 r$, preferably $0.95 r$ to $0.99 r$, wherein the largest
10 dimension of the probe runs horizontally along the circumference of the sphere or cylinder, and preferably wherein the largest dimension of the second surface of the probe has a radian measure of $0.25 \pi r$ to $1.75 \pi r$, more preferably of $0.3 \pi r$ to $1 \pi r$, more preferably of $0.5 \pi r$ to $0.95 \pi r$, more preferably of $0.6 \pi r$ to $0.8 \pi r$.

Where the largest dimension of the second surface of the probe has a radian
15 measure of less than $1 \pi r$, it is possible to include two or more probes of the same size at the same height within the reactor. Similarly, where said radian measure is $0.66 \pi r$ or less, it is possible to include three or more probes of the same size at the same height within the reactor, and where said radian measure is less than $0.5 \pi r$, it is possible to include four or more probes of the same size at the same height
20 within the reactor. Furthermore, since the probe has the shape of an interrupted ring (radian measure is less than $2 \pi r$) in the above cases, the introduction and removal of the probe into the reactor is facilitated.

Preferably, the apparatus of the present invention comprises at least two, preferably at least three, more preferably at least four probes as described above. More
25 preferably, at least two probes are positioned at the same height of a side wall of the reactor. It may be useful to provide probes at different heights of a reactor side wall, which enables e.g. control of the homogeneity of the chemical composition of a fluid medium. In addition, it may be useful to provide multiple probes with different semipermeable membranes (for example in terms of pore size and/or material) at
30 the same height of a reactor side wall, which allows to detect and/or eliminate measurement errors that may be due to the selection of a membrane that is, e.g., incompatible with one or more component of a fluid medium.

Apart from the reactor and the probe, the apparatus of the invention may comprise further components.

Preferably, the apparatus comprises at least one fluid source, connected to the inlet opening of the hollow conduit of the probe and in fluid communication therewith. Such fluid communication may be established by any suitable means known in the art, e.g. by using ports, such as quick-release ports, plug-in ports or screw-on ports, valves, such as solenoid valves, pneumatic valves or diaphragm valves, and/or conduits, such as hoses, pipes or tubes.

The connection of the inlet opening with the fluid source may for example pass through a wall of the reactor, or through the interior volume of the reactor, e.g. as along a wall and through the top of the reactor. The connection may also be established within the reactor, if e.g. the fluid source is integrated into the reactor.

Fluid sources useful for the present invention are not particularly limited and may be any means known in the art, capable of delivering a fluid to the inlet opening of the hollow conduit of the probe. For example, the fluid source may be a volumetric pump capable of delivering a predetermined volume of a fluid to the inlet opening, or an adjustable pump capable of delivering a continuous flow of fluid to the inlet opening at a predetermined rate, or a container, from which a flow of fluid to the inlet opening is delivered by pressure, e.g. by the hydrostatic pressure of the fluid in the container. Preferably, the fluid source is a syringe pump or a peristaltic pump. Particularly preferably, the apparatus of the invention comprises at least two fluid sources for delivering at least two different fluids to the inlet opening.

Where more than one fluid source is used, it is preferable to include means for selecting the fluid source from which fluid is delivered to the inlet opening. For example, a three-way valve or a combination of valves may be used for such selection.

Preferably, the apparatus of the invention comprises at least one instrument for collecting and/or analyzing fluids released from the outlet opening, which is connected to the outlet opening and is in fluid communication therewith. Such fluid communication may be established by any suitable means, e.g. as described above for the fluid source.

Instruments for collecting fluids are not particularly limited and may be vessels, such as bottles, flasks or vials, or automatic sampling apparatuses, where one or more samples may be stored. Instruments for analyzing fluids may be any analytical instruments suitable for determining at least one parameter of a fluid. For example, such instruments may be spectroscopic instruments, spectrometric instruments, chromatographic instruments and combinations thereof.

Preferably the apparatus of the invention comprises at least one instrument for analyzing the chemical composition of a fluid. More preferably, the apparatus of the invention comprises at least one instrument for liquid chromatography (LC), high performance liquid chromatography (HPLC), gas chromatography (GC), gel electrophoresis, gel permeation chromatography (GPC), anion exchange chromatography (AEC), high performance anion exchange chromatography (HPAEC), refractometry and/or mass spectrometry. More preferably, the apparatus of the invention comprises at least one instrument for high performance liquid chromatography (HPLC).

Further components and devices that may be included into the apparatus of the invention will be apparent to those skilled in the art, e.g. depending on the specific requirements of the process to be carried out with the apparatus.

The kit of components according to the present invention comprises the necessary components of the apparatus, i.e. the reactor and the probe described above, and optionally further components and devices, such as one or more fluid sources and/or one or more instruments for collecting and/or analyzing fluids. In the kit of components, the respective components are provided individually, i.e. the apparatus is not assembled, but the components are configured to interact with each other as described above. General and preferred features and/or embodiments described above for the apparatus are applicable for the kit of components accordingly.

The probe according to the present invention is the probe as described above for the apparatus, wherein the first surface and the second surface of the probe deviate from being planar, and the curvatures of the first surface and the second surface of the probe are essentially concentric. The probe is configured to be fixed permanently or non-permanently to a portion of an inner surface of a wall of a reactor with the second surface of the probe facing said portion. General and preferred features and/or embodiments described above for the probe as part of the apparatus are applicable accordingly for the probe of the invention.

The probe according to the present invention can be used for measuring and/or monitoring the chemical composition of a fluid medium in a reactor, when the probe is fixed permanently or non-permanently to a portion of an inner surface of a wall of such reactor with the second surface of the probe facing the portion to which the probe is fixed, thereby reducing disturbances in the fluid dynamics of the fluid medium in the reactor.

The method of the present invention for measuring and/or monitoring the chemical composition of a fluid medium is carried out in the apparatus of the invention as described above. The fluid medium is introduced into the reactor of the apparatus, thus that the first surface of the at least one probe of the apparatus exposed to the interior volume of the reactor is immersed in the fluid medium. The fluid medium may then optionally be agitated, preferably stirred. Preferably, the fluid medium is an aqueous solution, more preferably an aqueous cell culture medium or nutrient solution, more preferably an aqueous nutrient solution useful for cultivation of cell tissue.

To allow measurement and/or monitoring of the chemical composition of the fluid medium, a perfusion fluid is fed into the hollow conduit through the inlet opening at a predetermined, preferably constant, rate. For example, the perfusion fluid can be fed into the hollow conduit from a fluid source as described above. The feeding of the perfusion fluid into the hollow conduit may begin before the introduction of the fluid medium into the reactor. This may be beneficial to prevent collapse of the hollow conduit of the probe due to hydrostatic pressure of the fluid medium within the reactor, which may occur, if the probe does not contain a perfusion fluid.

The semipermeable membrane of the probe is configured to permit diffusion of at least one component of the fluid medium and/or the perfusion fluid between the fluid medium in the reactor and the perfusion fluid in the hollow conduit. In particular, the material and the pore size of the semipermeable membrane are selected to permit diffusion of the at least one component of the fluid medium and/or the perfusion fluid. Preferably, the material and pore size of the semipermeable membrane is selected to permit diffusion of proteins, enzymes, minerals and/or fatty acids.

When the first surface of the probe is immersed in the fluid medium in the reactor and the perfusion fluid is fed into the hollow conduit, diffusion of at least one component occurs between the fluid medium and the perfusion fluid through the semipermeable membrane. This is a dynamic process, thus that along the first surface of the probe, the diffusion will occur in both directions, until the concentration of the at least one component reaches an equilibrium between the fluid medium and the perfusion fluid. Accordingly, by the principles of microdialysis, if the concentration of a certain component in the fluid medium in the reactor is higher than in the perfusion fluid, said component will diffuse into the perfusion fluid, thus that its concentration in the perfusion fluid increases. If the concentration of a certain component in the fluid medium in the reactor is lower than in the perfusion fluid, said component will diffuse from the perfusion fluid, thus that its concentration in the perfusion fluid decreases. This increase or decrease of concentration leads to the

generation of a sample fluid from the perfusion fluid, which is released from the outlet opening of the hollow conduit.

The chemical composition of this sample fluid, and optionally other parameters thereof, are then analyzed at predetermined, preferably the same, intervals. For example, any of the analytical instruments described above may be used for this purpose.

Optionally, the sample fluid may be collected and stored, prior to analysis, or may be chemically processed, e.g. to allow detection of certain components that may otherwise be difficult to detect by one or more analytical instruments. For example, one or more of the instruments for collecting fluids described above may be used for storage.

The probe of the invention may be operated in any way, in which a common microdialysis probe can be operated. However, it is preferred that the chemical composition of the perfusion fluid fed into the hollow conduit corresponds essentially to the initial chemical composition of the fluid medium as introduced into the reactor. For example, the same fluid may be introduced into the reactor and be used as a perfusion fluid.

When a combination of perfusion fluid and fluid medium with essentially corresponding chemical compositions is used, no detectable concentration changes are detected in the sample fluid released from the outlet opening at the start of the process, and only changes in concentration need to be monitored. When in addition, the predetermined feeding rate of the perfusion fluid is constant and the predetermined intervals of analysis are the same, the size of the analyzed samples is kept at a constant value. With some analytic instruments, e.g. chromatographic means such as HPLC, this allows subtraction of the analytic results of the initial chemical composition of the perfusion fluid from the results of each individual sample of the sample fluid, for example using computer software, thus that only the changes of the chemical compositions are visualized.

The predetermined rate of feeding the perfusion fluid into the hollow conduit and the predetermined intervals for analyzing the sample fluid may be selected, for example, based on the size of the probe and the required measurement sensitivity, the viscosity of the perfusion fluid and/or the diffusion rate of certain components between the fluid medium and the perfusion fluid. For example the feeding rate may be selected such that the time between the perfusion fluid entering the inlet opening and exiting the outlet opening is in the range of from 0.1 s to 60 min, preferably from 1 s to 10 min, more preferably from 5 s to 1 min. For example, the intervals for

analyzing the sample fluid may be in the range of 0.1 s to 20 h, preferably from 1 s to 1 h, more preferably from 5 s to 10 min, more preferably from 10 s to 5 min.

Furthermore, it may be beneficial to calibrate one or more analytic instruments with unused perfusion fluid at regular intervals, to ensure that no sensitivity loss occurs
5 over time during the operation of the apparatus.

Preferably, the method of the invention further includes injecting a separating fluid into the hollow conduit through the inlet opening at predetermined intervals corresponding to the predetermined intervals mentioned above for analyzing the sample fluid. For example, the separating fluid may be injected into the hollow
10 conduit from a second fluid source, such as those described above.

The separating fluid is essentially immiscible with the perfusion fluid under the conditions present in the reactor. "Essentially immiscible" in the present context means that a clear and discrete phase boundary is formed between the perfusion fluid and the separating fluid. For example, where the perfusion fluid is an aqueous
15 solution, the separating fluid may be an oil, preferably a vegetable oil.

The separating fluid serves for separating samples of the sample fluid released from the outlet opening, but may also serve as a marker for the instruments for analyzing fluids, identifying the starting point and the end point of a measurement. For example, it is possible to use computer software to identify signals derived from the
20 separating fluid, and to separate the analytic results of two consecutive samples before and after detection of said signals, and to attribute the separate results to a respective sample. This allows automated and continuous monitoring of the chemical composition of the sample fluids, and thus of the chemical composition of the fluid medium within the reactor.

Preferably, during the injection of the separating fluid, the feeding of the perfusion fluid is interrupted, e.g. using a three-way valve that is configured to select the fluid source from which a fluid is introduced into the hollow conduit. Also preferably, the injection of the separating fluid is carried out at essentially the same rate as the feeding of the perfusion fluid.

The duration of the method of the invention depends on the particular process, in which the chemical composition of the fluid medium is to be monitored. For example, the duration may be in the range of 10 min to 1 year, preferably from 10 h to 6 months, more preferably from 24 hours to 2 months, more preferably from 1 week to 30 days.

35 The invention will be further illustrated by the Figures and claims.

FIG. 1A shows a first embodiment of the apparatus of the invention; FIG. 1B shows an increased depiction of a portion of FIG. 1A, where the probe of the invention is enlarged; FIG. 1C shows the probe from FIG. 1A without the reactor.

FIG. 2A shows a horizontal cross-section of the reactor and probe according to a second embodiment of the apparatus of the invention. FIG. 2B shows a vertical cross-section of the reactor wall and probe according to said second embodiment.

FIG. 3A shows a horizontal cross-section of the reactor and probe according to a third embodiment of the apparatus of the invention. FIG. 3B shows a vertical cross-section of the reactor wall and probe according to said third embodiment.

FIG. 4A shows a horizontal cross-section of the reactor and probe according to a fourth embodiment of the apparatus of the invention. FIG. 4B shows a vertical cross-section of the reactor wall and probe according to said fourth embodiment.

FIG. 5 shows a horizontal cross-section of the reactor and probe according to a fifth embodiment of the apparatus of the invention.

FIG. 6 shows a horizontal cross-section of the reactor and probe according to a sixth embodiment of the apparatus of the invention.

FIG. 7 shows a schematic depiction of the apparatus of the invention used in a process of the invention.

FIG. 8 depicts some principles of the process of the invention, when carried out in the fifth embodiment of the apparatus of the invention depicted in FIG. 5.

The drawings are discussed in detail below. It will be understood by those skilled in the art that individual features of the disclosed embodiments may be combined in different ways. Moreover, it will be understood that the size and proportions of individual features in the drawings may vary.

FIG. 1A discloses an apparatus 300 of the invention comprising a reactor 200 and four probes 100. The reactor 200 is essentially cylindrical, with the cylinder's lateral surface forming the side wall of the reactor 200, the bottom wall of the reactor 200 being dome-shaped, and the top of the reactor 200 being open to the interior volume of the reactor 200. As shown in FIG. 1A, the reactor 200 may have further features, such as windows for visual inspection shown as circular cut-outs in the side walls, and an opening for fitting in an agitator such as a stirrer, shown as a circular cut-out in the bottom of the reactor 200. The probes 100 are positioned at two different heights of the side wall of the reactor 200 in pairs, and in an encircling manner. This enables sampling along a large area of the reactor wall. The dotted rectangle in FIG.

1A identifies the portion of FIG. 1A that is enlarged and shown in FIG. 1B. In FIG. 1C, the probe 100 is shown isolated from the reactor and further enlarged and without coloration.

As can be seen from FIGs. 1B and 1C, the probe 100 is fixed to a portion of the inner surface 220 of the wall 210 of the reactor 200, thus that a first surface 150 of the probe 100 is exposed to the interior volume of the reactor 200, and a second surface 160 of the probe 100 faces the portion of the inner surface 220 of the wall 210 of the reactor 200, to which the probe 100 is attached.

Moreover, the probe 100 comprises a hollow conduit 110 with an inlet opening 120 and two outlet openings 130. The flow path through the hollow conduit 110 of a fluid introduced through the inlet opening 120 is shown by the arrows in FIG. 1B. The first surface 150 and second surface 160 of the probe 100 deviate from being planar, in accordance with the portion of the inner surface 220 of the wall 210 of the reactor 200, to which the probe 100 is fixed, and the probe touches the inner surface 220 of the wall 210 along the entire second surface 160 of the probe 100. The second surface 160 deviates from being planar along the largest dimension of the probe 100, which is oriented horizontally within the reactor 200. The first surface 150 and the second surface 160 of the probe 100 have a curvature that is concentric with the curvature of the portion of the inner surface 220 of the wall 210 of the reactor 200, to which the probe 100 is fixed, and the curvature radius of the second surface 160 is hardly smaller than the curvature radius of said portion of the inner surface 220 of the wall 210, to ensure contact along the entire second surface 160.

The hollow conduit 110 comprises an impermeable portion 113, formed from a rigid element 170, which is a hollow profile with rectangular cross section in the present case, and may be made e.g. from a metal. The rigid element 170 provides the curvature of the second surface 160 and the first surface 150 of the probe and is attached to both surfaces. The impermeable portion 113 ends at position 114 and is in fluid communication with a semipermeable portion 111 of the hollow conduit 110, a wall of which is formed from a semipermeable membrane exposed to the interior volume of the reactor 200. The first surface 150 of the probe 100 in this embodiment is formed partially from the semipermeable membrane forming a wall of the semipermeable portion 111, and partially from the material of the impermeable portion 113 of the hollow conduit 110. When a perfusion fluid is introduced into the hollow conduit 110, it flows from the inlet opening 120 through the impermeable portion 113 up to the position 114 where the impermeable portion 113 ends, and further flows through the semipermeable portion 111 to the outlet opening 130. Diffusion of components between the perfusion fluid and a fluid

medium in the reactor 200 occurs while the perfusion fluid flows through the semipermeable portion 111.

In the present embodiment, the semipermeable portion 111 of the conduit 110 is divided into two lines ending in two outlet openings 130, which allows e.g.
5 connection of the probe to two instruments for analyzing and/or collecting fluids.

FIGs. 2A and 2B show a horizontal and a vertical cross-section, respectively, through the reactor 200 and probes 100 at the position where the probe 100 is attached to the reactor 200, according to a second embodiment of the apparatus of the invention. The shape of the reactor 200 may be as shown in FIG. 1A, and the
10 probes 100 may be attached at positions within the reactor 200, as shown in FIG. 1A. The thick, dotted lines in FIG. 2A and FIG. 2B represent the portion 230 of the inner surface 220 of the wall 210 of the reactor 200 to which the probe 100 is attached.

The second embodiment depicted in FIGs. 2A and 2B differs from the first
15 embodiment depicted in FIGs. 1A, 1B and 1C in that the impermeable portion 113 of the hollow conduit 110, which is also the rigid element 170, is only attached to and forms the second surface 160 of the probe 100 facing the portion 230 of the inner surface 220 of the reactor wall 210, to which the probe 100 is fixed. The first
20 surface 150 of the probe 100 in the present embodiment is entirely made from the semipermeable membrane 112 forming a wall of the semipermeable portion 111. In this embodiment, the semipermeable portion 111 of the conduit 110 is not divided into two lines as in the first embodiment. As can be seen from FIG. 2A, the probe has a first end 180 proximate to the inlet opening 120 and the outlet opening 130,
25 and a second end 190 distant from the inlet opening 120 and the outlet opening 130, but proximate to the position 114 at which the impermeable portion 113 ends. These elements are also found in the first embodiment, but are not identified therein. In the second embodiment, a fluid source 310 and an instrument 320 for collecting and/or analyzing fluids are also included and shown in the FIG. 2A.

FIGs. 3A and 3B show a horizontal and a vertical cross-section, respectively,
30 through the reactor 200 and a probe 100 at the position where the probe 100 is attached to the reactor 200, according to a third embodiment of the apparatus of the invention. In FIG. 3A, one of two probes 100 is not shown, exposing the portion 230 of the inner surface 220 of the wall 210 of the reactor on one side. Instead of the probe 100, a place holder may be introduced into said portion, e.g. an arc formed
35 from the same material as the reactor wall, in order to, e.g. cover gaps in the reactor

wall. On the side where the probe 100 is present, the portion 230 to which it is fixed is not shown as a dotted line in FIG. 3A.

The third embodiment differs from the first embodiment in that the rigid element 170, forming the impermeable portion 113 of the hollow conduit 110, provides the curvature of the second surface 160 and the first surface 150 of the probe and is attached to both surfaces, but is not exposed to the interior volume of the reactor 200. Instead, the impermeable portion 113 of the hollow conduit 110 is covered by the semipermeable membrane 112 of the first surface 150 of the probe 100, as can be seen in FIG. 3B, thus that the first surface 150 is made entirely from the semipermeable membrane 112.

The third embodiment also differs from the first embodiment in that the wall 210 of the reactor 200 has a structural feature, e.g. a groove, in the inner surface 220 at the portion 230 to which the probe 100 is fixed, thus that the probe 100 can be embedded or integrated into said structural feature. Preferably the depth of said structural feature corresponds essentially to the thickness of the probe 100, thus that after embedding the portions of the inner surface 220 of the wall 210 to which the probe 100 is not fixed have a smooth transition to the first surface 150 of the probe. While such configuration of the reactor wall 210 is more complex than in the first and second embodiments, it further improves the effects of the invention in terms of the effects on fluid dynamics of a fluid medium introduced into the reactor.

FIGs. 4A and 4B show a horizontal and a vertical cross-section, respectively, through the reactor 200 and a probe 100 at the position where the probe 100 is attached to the reactor 200, according to a fourth embodiment of the apparatus of the invention. In FIG. 4A, one of two probes 100 is not shown, exposing the portion 230 of the inner surface 220 of the wall 210 of the reactor on one side. Instead of the probe 100, a place holder may be introduced into said portion, e.g. an arc formed from the same material as the reactor wall, in order to, e.g. cover gaps in the reactor wall. On the side where the probe 100 is present, the portion 230 to which it is fixed is not shown as a dotted line in FIG.4.

The fourth embodiment differs from the third embodiment shown in FIGs. 3A and 3B in that the hollow conduit 110 of the probe 100 does not contain an impermeable portion 113. Furthermore, the inlet opening 120 and the outlet opening 130 of the hollow conduit 110 are located at two different ends of the probe 100 along the largest dimension of the probe. This configuration of the probe is simpler than the other configurations, since no impermeable portion or rigid element is required, which is beneficial for reducing the manufacturing costs.

In addition, the probe 100 is fixed to the portion 230 of the inner surface 220 of the wall 210 of the reactor by integrating the probe 100 into the wall 210 of the reactor, thus that the second surface 160 of the probe corresponds to the interface formed by the interior volume of the probe 100 and the portion 230 of the inner surface 220 of the wall 210 of the reactor 200, to which the probe 100 is fixed.

This means that in the fourth embodiment, the conduit 110, the inlet opening 120 and the outlet opening 130 are formed as features within the wall 210 of the reactor 200. For example, the hollow conduit may be defined by a groove within the inner surface 220 of the wall 210, the inlet opening 120 and the outlet opening 130 may be defined by through holes in the wall 210, formed at two distant ends of the groove, and the second surface 160 of the probe 100 and the rigid element (not shown) is defined by the portion 230 of the inner surface 220 of the wall 210, to which the probe 100 is attached. To obtain the probe 100 within the reactor, the groove defining the hollow conduit 110 merely has to be covered by a semipermeable membrane 112, thus forming a wall of the semipermeable portion 111 of the hollow conduit 110, which corresponds to the first surface 150 of the probe 100.

FIG. 5 shows a horizontal cross-section through the reactor 200 and a probe 100 at the position where the probe 100 is attached to the reactor 200, according to a fifth embodiment of the apparatus of the invention. The apparatus according to the fifth embodiment differs from the apparatus of the fourth embodiment shown in FIGs. 4A and 4B in that only one probe 100 and one portion 230 to which the probe 100 is fixed is provided. Instead, said probe 100 and portion 230 extend along a major portion of the circumference of the inner surface 220 of the wall 210 of the reactor 200, forming an interrupted ring. This configuration of the apparatus allows sampling of a fluid medium within the reactor 200 along a larger area, thus that measurement inaccuracies due to inhomogeneity of the fluid medium may be reduced.

FIG. 6 shows a horizontal cross-section through the reactor 200 and a probe 100 at the position where the probe 100 is attached to the reactor 200, according to a sixth embodiment of the apparatus of the invention. The apparatus according to the sixth embodiment differs from the apparatus of the second embodiment shown in FIGs. 2A and 2B in that only one probe 100 and one portion 230 to which the probe 100 is fixed is provided. Instead, said probe 100 and portion 230 extend along a major portion of the circumference of the inner surface 220 of the wall 210 of the reactor 200, forming an interrupted ring. This configuration of the apparatus allows sampling of a fluid medium within the reactor 200 along a larger area, thus that measurement inaccuracies due to inhomogeneity of the fluid medium may be reduced, similarly as in the fifth embodiment.

FIG. 7 show a schematic depiction of an apparatus 300 of the invention used in a process of the invention. The apparatus comprises a reactor 200 with a probe 100 fixed to a portion of an inner surface of a wall of the reactor 200. For the probe 100, only the probe 100 itself, the inlet opening 120 and the outlet opening 130 are shown. The reactor 200 comprises an agitator 240, such as a stirrer, which agitates a fluid medium M in the reactor 200. Two fluid sources 310 are provided that are connected to and in fluid communication with the inlet opening 120 of the probe 100. These fluid sources 310 are configured to deliver and feed, alternately, a perfusion fluid P and a separating fluid S to the inlet opening 120 of the probe 100. Moreover, an instrument 320 for collecting and/or analyzing a sample fluid $P \pm C$ (C = Component) released from the outlet opening 130 of the probe 100 is connected to said outlet opening 130 and in fluid communication therewith.

FIG. 8 further illustrates an embodiment of a process of the invention in an apparatus of the invention as shown in FIG. 5. In particular, the agitator 240, preferably stirrer, agitates the fluid medium M, which is pressed in the direction of the walls of the reactor by e.g. centrifugal forces, and thus toward the semipermeable membrane 112 of the probe. A perfusion fluid P is introduced through the inlet opening 120 and flows through the hollow conduit in the direction of the outlet opening 130 along the semipermeable membrane 112. Both the perfusion fluid P and the fluid medium M comprise at least one component C which is capable of passing through the semipermeable membrane 112.

While the perfusion fluid P and the fluid medium M are in contact with the semipermeable membrane 112, diffusion of the at least one component C occurs along a concentration gradient of said component C between the fluid medium M and the perfusion fluid P. If the concentration of a component C in the perfusion fluid P introduced through the inlet opening 120 differs from the concentration of said component C in the fluid medium M within the reactor, said concentration in the perfusion fluid P changes, thus generating a sample fluid $P \pm C$ which is released through the outlet opening 130. By measuring the change of concentration of the component C in the sample fluid $P \pm C$ with respect to the perfusion fluid P, the change of the chemical composition of the fluid medium M within the reactor can be determined. In this process, the fluid dynamics of the fluid medium M generated by the agitator 240 are not affected by the probe, thus that damage of the probe and of sensitive components of the fluid medium M due to undesired turbulences is kept at a minimum.

Reference List

- 100 Probe
- 110 hollow conduit
- 111 semipermeable portion of hollow conduit
- 5 112 semipermeable membrane
- 113 impermeable portion of hollow conduit
- 114 position at which impermeable portion ends
- 120 inlet opening
- 130 outlet opening
- 10 150 first surface of the probe
- 160 second surface of the probe
- 170 rigid element
- 180 first end of the probe
- 190 second end of the probe
- 15 200 reactor
- 210 wall of the reactor
- 220 inner surface of the wall
- 230 portion of inner surface to which the probe is fixed
- 240 agitator
- 20 300 apparatus
- 310 fluid source
- 320 instrument for collecting and/or analyzing fluids.

Claims

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1. An apparatus (300) comprising:

a reactor (200), preferably a bioreactor; and

at least one probe (100) comprising a first surface (150) and a second surface (160) and at least one hollow conduit (110) for transporting fluids, formed between
10 the first surface (150) and the second surface (160), running along the first surface (150) and the second surface (160), wherein the hollow conduit (110) has an inlet opening (120) for receiving fluids, and an outlet opening (130) for releasing fluids received through the inlet opening (120) and transported through the hollow conduit (110),

15 wherein the first surface (150) is made at least partially from a semipermeable membrane (112), and the semipermeable membrane (112) forms a wall of a semipermeable portion (111) of the hollow conduit (110), and wherein the first surface (150) is exposed to the interior volume of the reactor (200), and

wherein the probe (100) is fixed permanently or non-permanently to a portion
20 (230) of an inner surface (220) of a wall (210) of the reactor (200) with the second surface (160) of the probe (100) facing the portion (230) to which the probe (100) is fixed.

2. The apparatus (300) according to claim 1, wherein the second surface (160)
25 of the probe (100) and the portion (230) of the inner surface (220) of the wall (210) of the reactor (200) to which the probe (100) is fixed, deviate from being planar, and a curvature of the second surface (160) of the probe (100) is essentially concentric with a curvature of the portion (230) to which the probe (100) is fixed, and the curvature radius of the second surface (160) deviates from the curvature
30 radius of the portion (230) to which the probe (100) is fixed by not more than 10%, preferably by not more than 5%, and preferably wherein the first surface (150) deviates from being planar and a curvature of the first surface (150) is essentially concentric with the curvature of the second surface (160).

3. The apparatus (300) according to claim 2, wherein the curvature of the second surface (160), and preferably of the first surface (150) of the probe (100), is provided by a rigid element (170) attached to the second surface (160) and preferably to the first surface (150).

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4. The apparatus (300) according to claim 2 or 3, wherein the hollow conduit (110) comprises an impermeable portion (113) made from a material impermeable to the components of the fluids to be transported through the hollow conduit (110), preferably made from a metal, wherein the impermeable portion (113) extends from the inlet opening (120) to at a position (114) within the probe (100) and wherein the semipermeable portion (111) extends from the position (114) at which the impermeable portion (113) ends to the outlet opening (130), wherein the impermeable portion (113) and the semipermeable portion (111) are in fluid communication with each other at the position (114), and preferably wherein the impermeable portion (113) of the hollow conduit (110) is a rigid element (170) as defined in claim 3.

5. The apparatus (300) according to claim 4, wherein in the probe (100), the first surface (150) and the second surface (160) deviate from being planar along the largest dimension of the probe (100), and wherein the probe has a first end (180) and a second end (190) along the largest dimension of the probe (100), wherein the inlet opening (120) and the outlet opening (130) of the hollow conduit (110) are located at a position proximate to the first end (180) of the probe (100), and the position (114) at which the impermeable portion (113) of the hollow conduit (110) ends is located at a position proximate to the second end (190) of the probe (100).

6. The apparatus (300) according to any one of claims 1 to 5, wherein the reactor (200) comprises an agitator (240), preferably a stirrer, and preferably wherein the largest dimension of the probe (100) is oriented horizontally.

7. The apparatus (300) according to any one of claims 1 to 6, wherein the reactor (200) is essentially cylindrical, having a cylinder radius r at the inner surface (220) of the wall (210), or essentially spherical, having a sphere radius r at the inner surface (220) of the wall (210), and wherein the second surface (160) of

the probe (100) has a curvature radius in the range of $0.9 r$ to $1 r$, preferably $0.95 r$ to $0.99 r$, wherein the largest dimension of the probe (100) runs horizontally along the circumference of the sphere or cylinder, and preferably wherein the largest dimension of the second surface (160) of the probe (100) has a radian measure of
5 $0.25 \pi r$ to $1.75 \pi r$, more preferably of $0.3 \pi r$ to $1 \pi r$, more preferably of $0.5 \pi r$ to $0.95 \pi r$, more preferably of $0.6 \pi r$ to $0.8 \pi r$.

8. The apparatus (300) according to any one of claims 1 to 7, comprising at least two, preferably at least three, more preferably at least four probes (100) and
10 preferably wherein at least two probes (100) are positioned at the same height of a side wall of the reactor.

9. The apparatus (300) according to any one of claims 1 to 8, wherein volume of the reactor (200) is at least 0.25 L , preferably at least 5 L , more preferably at
15 least 20 L , more preferably 25 to 250 L , more preferably 30 to 100 L , and wherein the volume of the probe (100) is 100 mL or less, preferably $1 \mu\text{L}$ to 20 mL , more preferably $100 \mu\text{L}$ to 10 mL , more preferably 1 mL to 5 mL .

10. The apparatus (300) according to any one of claims 1 to 9, further
20 comprising at least one fluid source (310), preferably at least two fluid sources (310), wherein the at least one fluid source (310) is connected to the inlet opening (120) and is in fluid communication therewith, and/or further comprising at least one instrument (320) for collecting and/or analyzing fluids released from the outlet opening (130), wherein the at least one instrument (320) is connected to the outlet
25 opening (120) and is in fluid communication therewith.

11. A kit of components comprising the reactor (200) and probe (100) as defined in any one of claims 1 to 9, and optionally at least one fluid source (310) and/or at least one instrument (320) for collecting and/or analyzing fluids, wherein
30 the probe (100) is configured to be fixed permanently or non-permanently to a portion (230) of an inner surface (220) of a wall (210) of the reactor (200) with the second surface (160) of the probe (100) facing the portion (230) to which the probe (100) is fixed.

12. A probe (100) comprising a first surface (150) and a second surface (160) and a hollow conduit (110) for transporting fluids, formed between the first surface (150) and the second surface (160), running along the first surface (150) and the second surface (160), wherein the hollow conduit (110) has an inlet opening (120) for receiving fluids, and an outlet opening (130) for releasing fluids received through the inlet opening (120) and transported through the hollow conduit (110), wherein the first surface (150) is made at least partially from a semipermeable membrane (112), and the semipermeable membrane (112) forms a wall of a semipermeable portion (111) of the hollow conduit (110), and
- 10 wherein the first surface (150) and the second surface (160) of the probe deviate from being planar, and the curvatures of the first surface (150) and the second surface (160) of the probe (100) are essentially concentric,
- wherein the probe (100) is configured to be fixed permanently or non-permanently to a portion (230) of an inner surface (220) of a wall (210) of a reactor (200) with the second surface (160) of the probe (100) facing the portion (230), and preferably wherein the probe (100) is defined as in any one of claims 1 to 9.

13. The use of the probe (100) according to claim 12 for measuring and/or monitoring the chemical composition of a fluid medium (M) in a reactor (200), wherein the probe (100) is fixed permanently or non-permanently to a portion (230) of an inner surface (220) of a wall (210) of a reactor (200) with the second surface (160) of the probe (100) facing the portion (230) to which the probe (100) is fixed, thereby reducing disturbances in the fluid dynamics of the fluid medium (M) in the reactor (200).

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14. A method for measuring and/or monitoring the chemical composition of a fluid medium (M) comprising the steps:
- a) providing an apparatus (300) according to any one of claims 1 to 10;
 - b) introducing a fluid medium (M) into the reactor (200) of the apparatus (300), thus that the first surface (150) of the at least one probe (100) exposed to the interior volume of the reactor (200) is immersed in the fluid medium (M),
 - c) optionally agitating, preferably stirring the fluid medium (M);
 - d) feeding a perfusion fluid (P) into the hollow conduit (110) through the inlet opening (120) at a predetermined rate;

e) analyzing the chemical composition of the sample fluid ($P \pm C$) released from the outlet opening (130) at predetermined intervals, preferably by chromatographic means, more preferably by high performance liquid chromatography,

wherein the semipermeable membrane (112) is configured to permit diffusion of at least one component (C), preferably selected from proteins, enzymes, minerals and fatty acids, of the fluid medium (M) and/or the perfusion fluid (P) between the fluid medium (M) in the reactor (200) and the perfusion fluid (P) in the hollow conduit (110), thus generating the sample fluid ($P \pm C$) in the hollow conduit (110), and

10 wherein preferably the chemical composition of the perfusion fluid (P) fed into the hollow conduit (110) through the inlet opening (120) corresponds essentially to the initial chemical composition of the fluid medium (M) as introduced into the reactor (200).

15 15. The method according to claim 14, wherein a separating fluid (S) that is essentially immiscible with the perfusion fluid (P) under the conditions present in the reactor (200), preferably an oil, more preferably a vegetable oil, is injected into the hollow conduit (110) through the inlet opening (120) at predetermined intervals corresponding to the predetermined intervals of step e), thereby separating
20 consecutive samples of the sample fluid ($P \pm C$).