SINGLE-USE CELL CULTURE CONTAINER WITH ONE OR MORE IN-SITU ONLINE SENSORS

The present invention is in the field of mammalian cell cultivation. In more detail, the current invention inter alia relates to a single-use small volume cell culture container, also termed single-use small volume bioreactor (SUSVB), comprising one or more, especially two or more, in-situ sensors, as well as its use in small volume cultivations of mammalian cells.

Background

The acceleration of bioprocess development for biologics and vaccines can be enabled by automated high throughput technologies, which can alleviate the significant resource burden from the multi-factorial statistical experimentation required for controlling product quality attributes of complex biologics (Bareither, R., et al., Biotechnol. Bioeng. 110 (2013) 3126-3138).

Recently, Bareither, R., et al., provided the proof of concept evaluations of an automated disposable small scale reactor for high throughput upstream process development by establishing a small scale stirred tank disposable 250 mL reactor as similar to those of lab and pilot scale with respect to process performance for industrial biologics processes for therapeutic protein and monoclonal antibody production using CHO cell culture, Pichia pastoris and E. coli (Biotechnol. Bioeng. 110 (2013) 3126-3138). This included similar growth, cell viability, product titer, and product quality. The technology was shown to be robust across multiple runs and met the requirements for the ability to run high cell density processes (>400 g/L wet cell weight) with exponential feeds and sophisticated event triggered processes.

Single use cell culture containers are well known in the art, such as, e.g., the ambr® 15 and 250 systems marketed by Sartorius Stedim Biotech.

US 2019/048305 reported a perfusion bioreactor and method for using same to perform a continuous cell culture, wherein the reactor comprises a single sensor port (240) with a single sensor (254) mounted thereto.

US 2019/153381 reported a perfusion bioreactor and related methods of use, wherein the reactor comprises as one sensing element a RAMAN sensor in the headspace, i.e. a cultivation-medium contactless sensor.

US 8,026,096 reported in vivo active erythropoietin produced in insect cells using bioreactors of two liters or more working volume.

Summary ~~of the Invention~~

Commercially available single-use small volume bioreactors (SUSVBs) provide at best for the control of temperature, dissolved oxygen and pH and only have capabilities for feed addition(s) and sampling. However, there is no option for a second in-situ, i.e. in direct contact with the cultivation medium, metabolite sensor. Thus, if, e.g., beside the pH control an additional monitoring of glucose is required, inevitably off-line analysis by sampling has to be done. This increases amongst other things the handling efforts as well as the risk of contamination of the cultivation.

It has now been found by the current inventors that by using a small volume bioreactor according to the current ~~invention~~disclosure timelines for analytics and for developing biological processes can be shortened. Additionally costs can be reduced. All that is achieved without losing the transferability with and to later large-scale processes.

One ~~aspect~~example of the current ~~invention~~disclosure is a small volume bioreactor comprising one or more in-situ sensors, wherein at least one in-situ sensor is for the determination of glucose.

One ~~aspect~~example of the current ~~invention~~disclosure is a small volume bioreactor comprising

- a cultivation vessel (105),
- a stirrer comprising a stirrer shaft (108) and one or more impellers (112; 109),
- an electrode or electrode-type sensor as first in-situ sensor,
- a gas sparger (127),
- a reactor head plate (104) comprising
    - a motor fitting (103) for connecting the drive axis of the motor to the stirrer shaft (108) (122),
    - at least one gas inlet (116) and a gas outlet (117),
    - a supply port area (133),

- an in-situ sensor port (130) for taking up an electrode or electrode-type sensor,

characterized in that the small volume bioreactor comprises a second in-situ sensor.

In another aspect, the present disclosure refers to a small volume bioreactor comprising a cultivation vessel (105) that

- has a working volume of from 20 ml to 350 ml,
- comprises a stirrer shaft (108) with at least one impeller (112) affixed thereto,
- comprises a feed pipe (107) comprising i) a sparging tube connected to a sparger (127) at its end, and ii) at least one feed line (118) with an opening at its end,
- comprises two or more baffles (114; 126) extending from the wall of the cultivation vessel (105) perpendicular in the direction to the center of the cultivation vessel (105), and
- a reactor head plate (104),

wherein the reactor head plate (104) comprises

- a fitting (122) for connecting the drive axis of a motor to the stirrer shaft (108),
- a sparger gas inlet (116) connected to the sparging tube in the feed pipe (107), optionally a gas inlet connected to the headspace (132),
- a gas outlet connected to the headspace of the cultivation vessel (117),
- at least one inlet for liquids of a feed line (118) being part of the feed pipe (107),
- one in-situ sensor port (130) with a pH electrode (101) mounted thereto, and
- a supply port area (133) comprising the sparger gas inlet and the inlet of the at least one feed line (118),

wherein the cultivation vessel (105) and the reactor head plate (104) are both substantially made of non-metal material,

characterized in that the small volume bioreactor comprises an in-situ glucose sensor.

The following are ~~embodiments~~examples of all ~~aspects~~examples as outlined before. It is expressly stated that the combination of each ~~embodiment~~example with each other ~~embodiment~~example as well as with each ~~aspect~~example is likewise encompassed, even if it is not written down.

In one preferred ~~embodiment~~example, the small volume bioreactor comprises two or more in-situ sensors, wherein one in-situ sensor is for the determination of glucose and one in-situ sensor is for the determination of the pH value. In one preferred ~~embodiment~~example, both sensors are submersed sensors, i.e. in direct contact with the cultivation medium.

In one ~~embodiment~~example, the pH sensor is a pH electrode.

In one ~~embodiment~~example, the glucose sensor is an electrochemical and/or enzyme-based sensor.

In one preferred ~~embodiment~~example the small volume bioreactor comprises two or more in-situ sensors, wherein one in-situ sensor is for the determination of glucose and one in-situ sensor is for the determination of the pH value, wherein the pH sensor is a pH electrode and the glucose sensor is an electrochemical and/or enzyme-based sensor. In one preferred ~~embodiment~~example, both sensors are submersed sensors, i.e. in direct contact with the cultivation medium.

In one ~~embodiment~~example, the small volume bioreactor has a working volume of from 20 ml to 350 ml. In one ~~embodiment~~example, the working volume is from 25 ml to 300 ml. In one ~~embodiment~~example, the working volume is from 50 ml to 280 ml. In one ~~embodiment~~example, the working volume is from 55 ml to 270 ml.

In one ~~embodiment~~example, the small volume bioreactor is operated at a volume of from 60 ml to 260 ml. In one ~~embodiment~~example, the small volume bioreactor is operated at a volume of from 90 ml to 250 ml.

In one ~~embodiment~~example, the small volume bioreactor has a total volume of 500 ml or less. In one ~~embodiment~~example, the small volume bioreactor has a total volume of 450 ml or less. In one ~~embodiment~~example, the small volume bioreactor has a total volume of 400 ml or less.

In one ~~embodiment~~example, the small volume bioreactor comprises a cultivation vessel (105) and a reactor head plate (104).

In one ~~embodiment~~example, the at least two in-situ sensors comprise at least a glucose or a lactate sensor and a pH sensor.

In one ~~embodiment~~example, the supply port area comprises one to four inlets for liquids connected to individual feed lines (118, 119, 120, 121) and optionally the sparger gas inlet (116).

In one ~~embodiment~~example two impeller (109, 112) are connected to the stirrer shaft

In one ~~embodiment~~example, the small volume bioreactor comprises at least (i) one sparger gas inlet (116) and (ii) one headspace gas inlet (132).

In one ~~embodiment~~example, the glucose sensor determines the glucose concentration every 20 seconds and/or the glucose sensor provides a signal if there is a change in glucose concentration. In one ~~embodiment~~example, the determined glucose concentration value is transmitted by wire or wireless to the computer. In one ~~embodiment~~example, the transmittal of the glucose concentration is by WI-FI, RFID or Bluetooth. In one ~~embodiment~~example, the glucose sensor has a working range of up to 8 g/l glucose or up to 3 g/l glucose. In one ~~embodiment~~example, the glucose sensor is an electrochemical and/or enzyme-based sensor. In one ~~embodiment~~example, the glucose sensor is a screen-printed electrode coated with an immobilized enzyme. In one ~~embodiment~~example, the glucose sensor base material is polymer USP class VI.

In one ~~embodiment~~example, the small volume bioreactor is a single-use bioreactor.

In one ~~embodiment~~example, the cultivation vessel (105) and the reactor head plate (104) are made of non-metal material. In one ~~embodiment~~example the cultivation vessel (105) and the reactor head plate (104) are (made of) plastic.

In one ~~embodiment~~example, the reactor head plate (104) further comprises a sampling port (102).

In one ~~embodiment~~example, the small volume bioreactor is sterilizable.

In one ~~embodiment~~example, the small volume bioreactor is a radiation sterilized small volume bioreactor. In one ~~embodiment~~example the small volume bioreactor is a twice

radiation sterilized small volume bioreactor. In one ~~embodiment~~example, the radiation is beta and/or gamma radiation.

In one aspect, the present disclosure refers to a method for cultivating a mammalian cell using a small volume bioreactor as described herein. In another aspect, the present disclosure refers to a method for determining cultivation conditions using a small volume bioreactor as described herein.

Detailed Description of the Invention

In contrast to the early years of biotherapeutic production it has been well accepted in recent years that for the development of therapeutic proteins, such as antibodies, the decisive parameter is not only product titer but also specific product quality attributes such as, without being limiting, the by-product profile or the glycosylation profile/pattern (see, e.g., Bareither, R. and Pollard, D., Biotech. Prog. 155 (2011) 217-224).

Thus, there is the need to assess and engineer these properties as early as possible, best already during clone selection and process development. As this is, in addition, no none-time process, consecutive rounds of experimentation using multiple parallel reactors are typically required. Thus, the method needs to be high-throughput suitable.

In view of ecological and economical constrains no at-scale cultivations can be used for process development. Thus, a suitable scale-down system that is proven to reliably mirror the later commercial (large) scale process and process performance is employed.

One independent aspect according to the current invention is a small volume bioreactor comprising

a cultivation vessel (105) that

- has a working volume of from 20 ml to 350 ml,
- comprises a stirrer shaft (108) with at least one impeller (112) affixed thereto,
- comprises a feed pipe (107) comprising i) a sparging tube connected to a sparger (127) at its end, and ii) at least one feed line (118) with an opening at its end,
- comprises two or more baffles (114; 126) extending from the wall of the cultivation vessel (105) in direction to the center of the cultivation vessel (105) (perpendicular to the wall of the cultivation vessel), and
- a reactor head plate (104),

wherein the reactor head plate (104) comprises

- a fitting (122) for connecting the drive axis of a motor to the stirrer shaft (108),