In addition, the determination of the maximum hydrodynamic shear stress in Villiger et al.[11] is used to examine the stress generated by the gas jet occurring during bubble detachment from the sparger, stress induced during bubble rise, and burst, or stress generated by the turbulent flow due to impeller motion. However, in the present invention, it was found that the shear stresses induced by gassing or stirring in a conventional bioreactor have only a rather negligible influence on the hydrodynamic shear stress and never reach a level of >10 Pa, regardless of the conditions applied. Stirring and gassing are only present as a kind of background shear stress and play practically no role in determining the total shear stress (shear stress intensity) that occurs in cell cultivation processes. Only the shear stress parameters (maximum shear stress, duration of shear stress exposure, shear stress frequency) play a role in a cultivation system. The experimental results in Villiger et al.[11] are therefore not relevant to the present invention.

For the sake of completeness, US 2007/034014 A1[26] and US 2023/103671 A1[27] are also referred to. US 2007/034014 A1[26] discloses a wall shear stress sensor that measures the shear stress at a test surface. This is only a minimal section of a highly complex system, such as a bioreactor, and does not provide any reliable indication of the conditions in the bioreactor as a whole.

Finally, US 2023/103671 A1[27] describes a method of quantifying the impact of shear stress on cells, wherein the method comprises the steps of: (a) exposing immobilised cells to forces that cause shear stress; and (b) nanoindenting the cells from step (a) to determine their mechanical properties at different stress levels. Initially, a nanoindenter is used here on stationary cells to measure the shear stress, for example after shaking in a flask or stirring in a bioreactor. Nanoindentation is a method of materials testing for determining the hardness of materials on small length scales, in particular the hardness determination of thin layers. A nanoindenter is an indenter that is an optical probe that measures in situ. The type of nanoindenter used strongly influences the results obtained and can therefore falsify them. In US 2023/103671 A1[27] samples are taken for measurement, so the conditions in a dynamic system such as a bioreactor are not reflected. According to the invention, the determination is carried out directly in the process. In addition, the mechanical shear stress is measured here by the nanoindenter, which has nothing to do with the hydrodynamic shear stress according to the invention. The procedure described has nothing to do with practice.

The object of the present invention is to overcome deficiencies of the prior art and to find out the maximum tolerable shear stress for an organism or a cell line before important performance indicators of a cell culture, such as biomass/viable cell density or productivity as well as quality characteristics, are significantly affected. Furthermore, it was an object to investigate the equipment and/or parameters used in a cultivation process with respect to their contribution to shear stress.

SUMMARY

The object of the disclosure is solved by a method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during a cultivation process comprising a liquid culture medium, which comprises the following steps:

(1) providing a shear stress sensitivity device comprising

- a bioreactor,

- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;

(2) selecting cells, liquid culture medium, cultivation mode and cultivation conditions for the cultivation process to be performed;

(3) selecting one, two or three shear stress parameters to be investigated from the group consisting of:
- maximum shear stress represented by a rotational speed of the pump;
- duration of shear stress exposure represented by a residence time of the cell culture within the pump; and/or
- shear stress frequency represented by a number of passages of the cell culture comprising cell culture medium and cells through the loop system per time;

(4) characterizing the shear stress sensitivity device by determining the shear stress values that occur at varying maximum shear stress by using a calibration method;

(5) performing one, two, three or more cultivation runs of the same cultivation process of step (2) in the shear stress sensitivity device over the same selected period of time, by successively increasing or decreasing one, two or three shear stress parameters in each subsequent cultivation run, and measuring one or more performance characteristics of the cells during each run of the cultivation process and generating a curve for each performance characteristic in each run over time;

(6) performing a control cultivation process in the shear stress sensitivity device for each performance characteristic using the same cultivation process as in step (5), wherein a lower maximum shear stress than in step (5) is used and generating a control curve for each performance characteristic over time;

(7) selecting the curve from the set of curves in step (5) for a performance characteristic that is closest to the control curve for said performance characteristic of step (6) which represent the shear stress sensitivity of the cells and constitute the shear stress limit for the cells;

whereby it is ensured that the maximum shear stress occurring in the closed loop system is higher than the maximum shear stress occurring in the bioreactor.

In one aspect, the present disclosure provides a method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during a cultivation process comprising a liquid culture medium, which comprises the following steps:

(1) providing a shear stress sensitivity device comprising
- a bioreactor,
- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;

(2) selecting cells, liquid culture medium, cultivation mode and cultivation conditions for the cultivation process to be performed;

(3) selecting one, two or three shear stress parameters to be investigated from the group consisting of:
- maximum shear stress represented by a rotational speed of the pump;
- duration of shear stress exposure represented by a residence time of the cell culture within the pump; and/or
- shear stress frequency represented by a number of passages of the cell culture comprising the liquid culture medium and cells through the loop system per time;

(4) characterizing the shear stress sensitivity device by determining the shear stress values that occur at varying maximum shear stress in the shear stress sensitivity device by using a calibration method or by using computer calculations and simulations;

(5) performing one, two, three or more cultivation runs of the same cultivation process of step (2) in the shear stress sensitivity device over the same selected period of time, by successively increasing or decreasing one, two or three shear stress parameters in each subsequent cultivation run, and measuring one or more performance characteristics of the cells during each run of the cultivation process and generating a curve for each performance characteristic in each run over time;

(6) performing a control cultivation process in the shear stress sensitivity device for each performance characteristic using the same cultivation process as in step (5), wherein a lower maximum shear stress than in step (5) is used and generating a control curve for each performance characteristic over time;

(7) selecting the curve from the set of curves in step (5) for a performance characteristic that is closest to the control curve for said performance characteristic of step (6) which represents the shear stress sensitivity of the cells and constitute the shear stress limit for the cells;

whereby it is ensured that the maximum shear stress occurring in the closed loop system is higher than the maximum shear stress occurring in the bioreactor.

The core concept of the disclosure is that the cells are stressed, in particular by a pump connected to a bioreactor via an external loop. This allows the bioreactor to be operated in various cultivation modes such as batch, fed batch, continuous such as perfusion or other process control strategies, continuously applying shear stress in a defined manner by continuously pumping the cell culture comprising the liquid culture medium and cells in a loop. Through this external loop, the sole effect of shear stress on the culture can be studied without changing the conditions inside the bioreactor. Not only the maximum shear stress that can occur - which will be explained in more detail later - which is adjusted by the rotational speed of the pump, but also the shear stress frequency and duration of shear stress exposure can be controlled and thus studied.

The shear stress sensitivity device provides insights into cell behavior and hydrodynamic shear stress that allow for accurate conclusions about the level of shear stress applied, contributing to predictions for other cultivation procedures, of equal, larger or smaller scale using similar or different hardware components.

Because the shear stress of the cells is quantified by the calibration with a mechanic model the result is independent of the specifically used shear stress sensitivity device like the individually applied pump but reflects the conditions of the respective complete cultivation system.

The disclosure is also directed to a process for culturing cells in liquid cell culture, wherein the shear stress sensitivity determined according to the method of the present disclosure is used in the same cultivation process but performed on another scale as the method for determining the shear stress sensitivity.

In another aspect, the present disclosure provides a process for culturing cells in a liquid culture medium, wherein the shear stress sensitivity of the cells determined in the method as described herein is used in the same cultivation process but performed on a larger or smaller scale.

The disclosure is also directed to a process for culturing eukaryotic cells or prokaryotic cells in liquid cell culture in a bioreactor and performing a method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during the cultivation process according to the present disclosure.

In another aspect, the present disclosure provides a process for culturing eukaryotic or prokaryotic cells in a liquid culture medium in a bioreactor and performing a method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during the cultivation process as described herein.

The disclosure is also directed to a process for producing a recombinant protein, the process comprising the steps of:

step I) culturing eukaryotic or prokaryotic cells expressing a recombinant protein in cell culture in a bioreactor;

step II) harvesting the recombinant protein;

step III) purifying the recombinant protein;

wherein performing in step I) a method for determining the shear stress sensitivity of the cells in a shear stress sensitivity device during the cultivation process according to the present disclosure.

In another aspect, the present disclosure provides a process for producing a recombinant protein, the process comprising the steps of:

step I) culturing eukaryotic or prokaryotic cells expressing a recombinant protein in a liquid culture medium in cell culture in a bioreactor;

step II) harvesting the recombinant protein;

step III) purifying the recombinant protein;

wherein performing in step I) a method for determining the shear stress sensitivity of the cells in a shear stress sensitivity device during the cultivation process as described herein.

The terms ‘method’ and ‘process’ both refer to a procedure in connection with the present disclosure and are synonymous and interchangeable. The two terms have been used to make it easier to understand and distinguish between the different procedures: ‘Method’ refers to the method according to the disclosure, while the term ‘process’ refers to other procedures used in connection with the method according to the disclosure or to procedures in which the method according to the disclosure is used.

The present disclosure is also directed to the use of a device comprising

- a bioreactor,
- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;

as a shear stress sensitivity determining device or a shear stress sensitivity device,
wherein the device has no nozzle,
for culturing cells in a liquid culture medium, wherein a defined shear stress is set during the culturing.

In another aspect, the present disclosure provides use of a device comprising

- a bioreactor,
- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;

as a shear stress sensitivity device,
wherein the device has no nozzle,
for culturing cells in a liquid culture medium, wherein a defined shear stress is set during the culturing.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the prior art and of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale so

Claims

1. A method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during a cultivation process comprising a liquid culture medium, which comprises the following steps:

(1) providing a shear stress sensitivity device comprising
- a bioreactor,
- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;

(2) selecting cells, liquid culture medium, cultivation mode and cultivation conditions for the cultivation process to be performed;

(3) selecting one, two or three shear stress parameters to be investigated from the group consisting of:
- maximum shear stress represented by a rotational speed of the pump;
- duration of shear stress exposure represented by a residence time of the cell culture within the pump; and/or
- shear stress frequency represented by a number of passages of the cell culture comprising the liquid culture medium and cells through the loop system per time;

(4) characterizing the shear stress sensitivity device by determining the shear stress values that occur at varying maximum shear stress in the shear stress sensitivity device by using a calibration method or by using computer calculations and simulations;

(5) performing one, two, three or more cultivation runs of the same cultivation process of step (2) in the shear stress sensitivity device over the same selected period of time, by successively increasing or decreasing one, two or three shear stress parameters in each subsequent cultivation run, and measuring one or more performance characteristics of the cells during each run of the cultivation process and generating a curve for each performance characteristic in each run over time;

(6) performing a control cultivation process in the shear stress sensitivity device for each performance characteristic using the same cultivation process as in step (5), wherein a lower maximum shear stress than in step (5) is used and generating a control curve for each performance characteristic over time;

(7) selecting the curve from the set of curves in step (5) for a performance characteristic that is closest to the control curve for said performance characteristic of step (6) which represents the shear stress sensitivity of the cells and constitute the shear stress limit for the cells;

whereby it is ensured that the maximum shear stress occurring in the closed loop system is higher than the maximum shear stress occurring in the bioreactor.

2. The method according to claim 1,
characterized in that

the cultivation mode in step (2) is selected from batch or fed-batch
or
the cultivation mode in step (2) is selected from continuous cultivation mode, particularly perfusion, whereby a cell retaining means is then additionally provided in the shear stress sensitivity device, the cell retaining means is particularly selected from a hollow fibre filter module.

3. The method according to claim 1 or 2,
characterized in that
the means to control the hydraulic resistance in the loop system are selected from a variable tube having a variable length and/or a variable diameter, reducers for the variable tube, valves, such as pinch valves, and variable winding of the variable tube.

4. The method according to any of the preceding claims,
characterized in that
the calibration method in step (4) is selected from a method wherein a shear stress-dependent quantity is determined and correlated with determined occurring maximum shear stress values, in particular the shear stress-dependent quantity is the aggregate size of shear sensitive aggregates.

5. The method according to claim 4,
characterized in that
the same shear stress-dependent quantity is used in the calibration method and in the characterization of the shear stress sensitivity device, particularly the shear stress-dependent quantity is the aggregate size, more particularly the radius of gyration of particle aggregates, especially the aggregates are selected from poly(methyl methacrylate) nanoparticle aggregates or Blauton polymer floc system.

6. The method according to any of the preceding claims,
characterized in that
the calibration method in step (4) is performed as follows:
- the aggregates size of shear sensitive aggregates is selected as shear stress-dependent quantity;
- a calibration method using a calibration device is selected which uses the aggregate sizes and correlates the aggregate sizes with determined maximum shear stress values;
- reworking the selected calibration method and measuring the aggregates sizes of the aggregates;
- generating a calibration curve in which the measured aggregates size values are plotted against the determined maximum shear stress values,

particularly the aggregate size is selected to be the radius of gyration of particle aggregates and the aggregates are selected from poly(methyl methyacrylate) nanoparticle aggregates or Blauton polymer floc system.

7. The method according to claim 6,
characterized in that
the characterization in step (4) is performed as follows:
- selecting the same calibration method as used in claim 6;
- reworking the selected calibration method using the shear stress sensitivity device instead of the calibration device, in particular with the same aggregates as used in the calibration method;

- performing continuous recirculating runs of the aggregates in the shear stress sensitivity device by the pump until the aggregates no longer change their size by increasing or decreasing the rotational speed of the pump in each subsequent run, and measuring the aggregate size after each run;
- generating a curve, wherein the measured aggregate size is plotted against the rotational speed of the pump and
- converting the measured aggregates size to maximum shear stress values using the calibration curve of the calibration method or using the calibration curve of claim 6;

in particular the aggregate size is selected to be the radius of gyration of particle aggregates and the aggregates are selected from poly(methyl methyacrylate) nanoparticle aggregates or Blauton polymer floc system.

8. The method according to any of the preceding claims,
characterized in that
the cultivation process in step (5) is performed as follows:
performing a 1st cultivation process in the form of a 1st cultivation run over a 1st period of time,
performing a 2nd cultivation process in the form of a 2nd cultivation run over a 2nd period of time,
performing a 3rd cultivation process in the form of a 3rd cultivation run over a 3rd period of time,
optionally performing further cultivation processes in the form of further runs over further periods of time,
wherein all periods of time are selected to be of equal length,
whereby in each cultivation run the cell culture comprising the liquid culture medium and the cells are continuously circulated in the loop system of the shear stress sensitivity device,
whereby the selected shear stress parameter(s) is(are) set to a predetermined value in the 1st cultivation run and kept constant during the 1st cultivation run, and for each subsequent cultivation run each selected shear stress parameter is set to a higher or lower value from cultivation run to cultivation run.

9. The method according to any of the preceding claims,
characterized in that
the control cultivation process in step (6) is performed
- in case the cultivation mode is batch or fed-batch in the same shear stress sensitivity device as used in step (5) but without loop system and without pump;
- in case the cultivation mode is continuous mode, especially perfusion, in the same shear stress sensitivity device as used in step (5), wherein the maximum shear stress is adjusted lower as used in step (5), particularly as low as possible.

10. The method according to any of the preceding claims,
characterized in that one, two, three or more of the following features are selected:
- the kinetic pump is selected from a centrifugal pump, in particular a magnetically levitating centrifugal pump;
- the loop system connects an outlet, particularly located in the lower part, of the bioreactor with an inlet, particularly located in the head part, of the bioreactor;
- the means to control the hydraulic resistance is placed in the loop system downstream of the kinetic pump;
- the pump is placed in the loop system downstream of the outlet from the bioreactor;
- the cell retaining means is arranged in the loop system between the means to control the hydraulic resistance and the bioreactor;

- a flowmeter is placed in the loop system, preferably between the bioreactor and the variable tube or between the bioreactor and the hollow fibre filter module;
- one or more pressure gauges are provided in the loop system;
- the bioreactor comprises a stirrer;
- in step (5), in each cultivation run the performance characteristic(s) for the cell culture are measured on a regularly basis;
- in step (6), the performance characteristic(s) for the control cell culture are measured on a regularly basis;
- in step (7), an analysis of the shear stress behavior of the cells is performed with the obtained curve(s) for one or more performance characteristics, in particular in comparison to the control curve or without a control curve;
- a kinetic pump is used that has a replaceable pump head that may be replaced after all cultivation runs of a cell cultivation process have been performed;
- in step (5) and/or step (6) the period of time is at least 1 day, in particular up to 30 days;
- the characterization of the shear stress sensitivity device in step (4) is performed by reworking a calibration method, in particular by reconstructing the device of the calibration method and thus reworking the calibration method with the reconstructed device;
- if the calibration method in step (4) is selected from a method wherein a shear stress-dependent quantity is determined and the aggregate size is selected as shear stress-dependent quantity, the same batch of aggregates is used for both the calibration and the characterization methods;
- only one, two or three shear stress parameters are varied in step (5) while all other parameters of the shear stress sensitivity device are kept constant; and
- only one, two or three shear stress parameters and in addition the bypass flow are varied in step (5) while all other parameters of the shear stress sensitivity device are kept constant.

11. The method according to any of the preceding claims,
characterized in that
the one or more performance characteristics for the cultivation process or control cultivation process are selected from parameters which change over time during cell cultivation, in particular from the group consisting of the viable cell density, the viability, the glucose concentration, the lactate concentration, the LDH concentration, the titer, growth, productivity of the cell culture, and product quality.

12. The method according to any of the preceding claims,
characterized in that
one of the following modifications according to case 1 to case 4 is selected for the variation of one, two or three shear stress parameters in the shear stress sensitivity device over all runs of the cultivation process:

case 1 increasing the maximum shear stress from cultivation run to cultivation run while keeping constant the duration of shear stress exposure, and shear stress frequency in all cultivation runs;

or

case 2 increasing the duration of shear stress exposure from cultivation run to cultivation run while keeping constant the maximum shear stress and shear stress frequency in all cultivation runs;

or

case 3 increasing the shear stress frequency from cultivation run to cultivation run while keeping constant the maximum shear stress and the duration of shear stress exposure in all cultivation runs;

or

case 4 increasing the duration of shear stress exposure from cultivation run to cultivation run while keeping the maximum shear stress constant and decreasing the shear stress frequency from cultivation run to cultivation;

whereby particularly one or more of the following conditions are selected to control one, two or three shear stress parameters:

- Keeping the maximum shear stress constant in the form of the rotational speed by keeping the rotational speed of the pump at the same value or
- Increasing or decreasing the maximum shear stress in the form of the rotational speed by setting the rotational speed of the pump higher or lower;
- Keeping the duration of shear stress exposure constant if the rotational speed of the pump is increased or decreased by using the means to control hydrodynamic resistance, particularly by varying the length and/or diameter of the variable tube, in the loop system of the shear stress sensitivity device;
- Increasing the duration of shear stress exposure if the rotational speed of the pump is kept constant by adding additional pumps with the same rotational speed in series in the loop system;
- Increasing or decreasing the duration of stress exposure if the rotational speed of the pump is kept constant by using the means to control hydrodynamic resistance, particularly by varying the length and/or diameter of the variable tube, in the loop system of the shear stress sensitivity device;
- Keeping the shear stress frequency constant by using the means to control hydrodynamic resistance, particularly by varying the tube length and/or the diameter of the variable tube, in the loop system of the shear stress sensitivity device;
- Increasing the shear stress frequency if the rotational speed of the pump is kept constant by adding one or more external loops to the bioreactor or by adjusting the working volume of the bioreactor; and/or
- Increasing the shear stress frequency if the rotational speed of the pump is kept constant by providing one or more additional pumps, which are connected in parallel and all pumps operate with the same value of the rotational speed.

13. The method according to any of the preceding claims,
characterized in that

step (2) is replaced by step (2'): performing a cultivation process or using a cultivation process already performed and providing one or more cultivation process curves of the cultivation process, wherein one or more performance characteristics of the cells are plotted over a selected period of time;

step (4) is replaced by step (4'): performing a control cultivation process or using a control cultivation process already performed and providing one or more cultivation process curves of the control cultivation process, wherein one or more performance characteristics of the cells are plotted over a selected period of time, wherein the control cultivation process is the same cultivation process as in step (2'), but wherein a lower shear stress intensity is used than in the cultivation process and the same performance characteristics of the cells and the same period of time is used as in step (2');

step (5) is replaced by step (5’): performing one, two, three or more control cultivation runs of the control cultivation process of step (4’) in the shear stress sensitivity device over the same selected period of time, by successively increasing or decreasing one, two or three shear stress parameters in each subsequent control cultivation run, wherein each subsequent control cultivation run is modified by varying the shear stress parameter(s) toward the selected cultivation process of step (2’) and measuring one or more performance characteristics of the cells during each run of the control cultivation process and generating a curve for each performance characteristic in each run over time;

step (6) is replaced by step (6'): selecting the curve from the set of curves in step (5’) for a performance characteristic that is closest to the cultivation process curve for said performance characteristic;

step (7) is replaced by step (7'): determining how the shear stress intensity needs to be changed to move from the control cultivation process to the cultivation process by comparing the obtained curves;

whereby it is ensured that the shear stress intensity occurring in the cultivation process is higher than the shear stress intensity occurring in the control cultivation process.

14. The method according to claim 13,
characterized in that
the cultivation process in step (2’) is selected to be carried out on a larger scale than the control cultivation process in step (4’).

15. A process for culturing cells in a liquid culture medium, wherein the shear stress sensitivity of the cells determined in the method of any one of claims 1 to 12 is used in the same cultivation process but performed on a larger or smaller scale.

16. A process for culturing eukaryotic or prokaryotic cells in a liquid culture medium in a bioreactor and performing a method for determining the shear stress sensitivity of cells in a shear stress sensitivity device during the cultivation process according to any one of claims 1 to 14.

17. A process for producing a recombinant protein, the process comprising the steps of:
step I) culturing eukaryotic or prokaryotic cells expressing a recombinant protein in a liquid culture medium in cell culture in a bioreactor;
step II) harvesting the recombinant protein;
step III) purifying the recombinant protein;
wherein performing in step I) a method for determining the shear stress sensitivity of the cells in a shear stress sensitivity device during the cultivation process according to any one of claims 1 to 14.

18. Use of a device comprising
- a bioreactor,
- a closed loop system connected with and located outside the bioreactor;
- a pump placed in the loop system, the pump being a kinetic pump and
- means to control the hydraulic resistance in the loop system;
as a shear stress sensitivity device,
wherein the device has no nozzle,

for culturing cells in a liquid culture medium, wherein a defined shear stress is set during the culturing.

19. Use of the device according to claim 18,
characterized in that

- as the defined shear stress, a maximum shear stress value is used which is obtained by varying shear stress parameter(s) in the device, the maximum shear stress value being determined on the basis of a shear stress-dependent physical quantity, in particular the shear stress-dependent physical quantity being the aggregate size of shear-sensitive aggregates;

or

- as the defined shear stress, relative shear stress levels are used which result from two runs of a cultivation process in which different shear stress levels are present, one of the runs is used as a control run and the control run is repeated and modified by varying the shear stress parameter(s) until it runs like the other run.

20. Use of the device according to claim 19,
characterized in that
the maximum shear stress value being determined on the basis of a shear stress-dependent physical quantity, in that

- the shear stress dependent physical quantity is exposed to defined, predetermined conditions, whereby the physical quantity varies according to the magnitude of the maximum shear stress;
- the shear stress dependent physical quantity is exposed to unknown conditions in the device, whereby the physical quantity varies according to the magnitude of the maximum shear stress;
- the magnitude of the maximum shear stress value in the device can be inferred from the physical quantity obtained under the defined, predetermined conditions, whereby the same magnitude for the physical quantity means that the same maximum shear stress value is present.