on the outputs of the scales and/or load cells, control the one or more of fluid transfer devices to transfer the culture medium between the two or more vessels such that a volume of the culture medium in the two or more vessels is controlled.

In certain embodiments, the fluidic paths include tubing and aseptic connectors attached to the two or more vessels. In some cases, the fluidic paths attach proximate to bottoms of the two or more vessels. In some cases, at least one of the fluid transfer devices includes a pump.

The control system may be configured to maintain particular parameter values within certain bounds. It may also be configured to maintain the parameter values at a consistent level from vessel-to-vessel. In some implementations, the control system is configured to adjust conditions in the two or more vessels to ensure that the mean pH of the culture medium in the two or more vessels does not vary from one vessel to another by more than about 0.1. In some implementations, the control system is configured to adjust conditions in the two or more vessels to ensure that shear forces experienced by cells in the two or more vessels are substantially equal from one vessel to another during the bioreaction. In certain embodiments, the control system is configured to adjust conditions in the two or more vessels to ensure that culture medium gas transfer rates in the two or more vessels are substantially equal from one vessel to another during the bioreaction.

The bioreactor system may also be designed such that the variance (or standard deviation) in a parameter value is minimized or at least kept within a defined bound in any of the vessels. For example, the two or more vessels and the control system may be configured such that the pH of the culture medium within any of the vessels has variance of at most about 0.1 pH units. In certain embodiments, the control system is a proportional-integral-derivative controller.

In certain bioreactor systems of this disclosure, the control system includes a single control loop that is configured to control a master vessel. In such cases, one of the two or more vessels is the master vessel and the others are satellite vessels. The master vessel may have a mixing system configured to agitate the culture medium.

In various embodiments, bioreactor system and/or one or more of its vessels is configured to operate in a perfusion mode. In accordance with such embodiments, at least one of the two or

more fluidically coupled vessels includes a fluidic inlet and a fluidic outlet, and the bioreactor system is configured to introduce the culture medium to the at least one vessel through the fluidic inlet, exchange the culture medium in the vessel while retaining the biological cells in that vessel, and flow the culture medium out the fluidic outlet. In some such perfusion embodiments, the fluidic outlet includes a filter configured to prevent the biological cells in the at least one vessel from leaving the at least one vessel and the fluidic outlet. In some embodiments, one of the two or more vessels is a master vessel configured to provide culture media to the at least one vessel through the fluidic inlet to provide perfusion. In some perfusion embodiments, the control system and master vessel are together configured to substantially maintain defined values of dissolved oxygen concentration, temperature, and pH in the culture medium in the master vessel and in the at least one vessel. As with some other embodiments providing master and satellite vessels, the master vessel may include a mixing system, while the at least one vessel does not include a mixing system. In some embodiments, a vessel configured to operate in perfusion mode includes one or more micro-carriers, hollow fibers, and/or adhesion plates. In some embodiments, two or more vessels are configured to operate in perfusion mode. For example, at least two of the two or more fluidically coupled vessels each include a fluidic inlet and a fluidic outlet and the bioreactor system is configured to introduce the culture medium to the at least two vessels through the fluidic inlet, exchange the culture medium in the vessel while retaining the biological cells in that vessel, and flow the culture medium out the fluidic outlet.

Another aspect of the disclosure provides methods of carrying out a bioreaction in a bioreactor system that may be characterized by the following: (i) two or more fluidically coupled vessels configured to collectively carry out a bioreaction; (ii) one or more fluidic paths coupling the two or more vessels to one another, where the one or more fluidic paths are configured to provide flow of culture medium between the two or more vessels during the bioreaction; and (iii) one or more fluid transfer devices along at least some of the one or more fluidic paths, where each of the fluidic paths is configured to permit bidirectional flow of the culture medium between two of the vessels, and/or the two or more vessels are arranged in closed loop. The methods may be characterized by the following operations: (a) reading or receiving values of one or more parameters characterizing the culture medium in one or more of the vessels during the bioreaction; (b) using the values to determine an adjusted flow rate in at least one of the fluidic paths to maintain substantially uniform values of the one or more

conditions in the two or more vessels to ensure that culture medium gas transfer rates in the two or more vessels are substantially equal from one vessel to another during the bioreaction.

In certain method embodiments, each vessel of the bioreactor system has a working volume of not greater than about 500 liters or not greater than about 50 liters. In certain embodiments, the ratio of total working volume of the two or more vessels to the working volume of largest of the two or more vessels is at least about 3.

In certain method embodiments, one of the two or more vessels is a master vessel and the others are satellite vessels, where the master vessel includes a mixing system configured to agitate the culture medium. In some implementations, one or more of the satellite vessels does not include a mixing system. In some implementations, the master vessel has a larger working volume than any of the satellite vessels.

In some of the method aspects of this disclosure, the method additionally includes: reading or receiving at least one output of one or more scales and/or load cells, on which the two or more vessels are provided; and based on the outputs of the scales and/or load cells, controlling the one or more of fluid transfer devices to transfer the culture medium between the two or more vessels such that a volume of the culture medium in the two or more vessels is controlled. In certain embodiments, the fluidic paths include tubing and aseptic connectors attached to the two or more vessels. In some cases, the at least one of the fluid transfer devices includes a pump.

In some of the disclosed methods, at least one of the two or more fluidically coupled vessels includes a fluidic inlet and a fluidic outlet. In such cases, the method additionally includes introducing the culture media to the at least one vessel through the fluidic inlet, flowing the culture media over biological cells while they are retained in the at least one vessel, and flowing the culture media out the fluidic outlet, to thereby operate the at least one vessel in a perfusion mode. When operating in perfusion mode, a vessel may make use of a filter configured to prevent the biological cells in the at least one vessel from leaving the at least one vessel and the fluidic outlet. Alternatively or in addition, a vessel may additionally include micro-carriers, hollow fibers, and/or adhesion plates for retaining cells. In certain embodiments, one of the two or more vessels is a master vessel configured to provide culture media to the at least one vessel through the fluidic inlet to provide perfusion. In such cases, a method may

maintain values of dissolved oxygen concentration, temperature, and pH in the culture medium in the master vessel and in the perfusion vessel. The master vessel may include a mixing system, while, in some cases, the perfusion vessel does not include any mixing system.

The bioreactor system is not limited to embodiments having only a single perfusion vessel. For example, the system may include a second fluidically coupled perfusion vessel including its own fluidic inlet, fluidic outlet, and cell retention structure configured to prevent biological cells from leaving during the bioreaction. In some cases, the system employs a second perfusion vessel that is initially fluidically unconnected to the two or more fluidically connected vessels. However, the second perfusion vessel includes a supplemental fluidic connection for connecting to the bioreactor system after the bioreactor system has been operating in perfusion mode.

In some implementations, the bioreactor system is configured to at least partially fill the perfusion vessel with culture medium from one or more other vessels during the bioreaction. In some implementations, the perfusion vessel is configured to be removed from the bioreactor system, along with the perfusion vessel's culture medium and cells grown in the culture medium, before the bioreaction completes.

These and other features of the disclosure will be described in more detail below with reference to the associated drawings.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Prior art scale-up train of single-use bioreactors from a 2014 publication.

Figure 2. A simple tank introducing the differential equations that describe mixing.

Figure 3. A mathematical abstraction of a "star architecture" for multi-tank mixing.

Figure 4. Two additional bi-directional mathematical models of tank arrangements for potential mixing systems.

Figure 5 Two uni-directional mathematical models of tank arrangements for potential mixing systems.

Figure 6. Two slave (or satellite) vessels connected to a master vessel with pumps and aseptic connectors. This figure shows the basic configuration for multiple tanks used in a scale-up or expansion process.

Figure 7. This figure shows a typical animal cell growth process divided into phases. It also depicts the doubling time.

Figure 8. This figure shows a physical representation of the star architecture for n vessel expansion.

Figure 9. A multi-tank expansion system equipped to provide modalities for personalized medicine applications.

DESCRIPTION

Certain details of scale-up processes and the complications experienced by workers in this field are described in, for example, *Physiological Similarity and Bioreactor Scale-up*, Votruba et al., Folia Microbiology, vol 37 (5), p. 331, 1992, *Shake Flask, to Fermentor: What Have We Learned*, Humphrey, Biotechnology Progress, vol. 14, p. 3, 1998, and *Living With Heterogeneities in Bioreactors,* Lara et al., Molecular Biotechnology, vol. 24, p. 355, 2006, each incorporated herein by reference in its entirety. It has been suggested by many authors and summarized by Ju (*Improved scale-up strategies of bioreactors*, Bioprocess Engineering vol. 8, p. 49, 1992 all information incorporated herein) that in order to effectively scale-up a bioprocess the following process characteristics would be maintained as constant:

1. Reactor geometry;
2. The volumetric oxygen transfer coefficient k_La (m/s m^2/m^3);
3. Maximum shear experienced by cells (kg/m^2 s);
4. Power input per unit volume of liquid P(kW)/V (m^3);
5. Volumetric gas flow rate per unit volume of liquid Q (m^3/s)/V(m^3)

exact system under study. Item 9, apparently less frequently used in the literature, is the momentum factor and is related to both shear and mass transfer in the bioreactor.

The number of process parameters and combination of process parameters from the above list that are desired to be held constant during scaling changes based on a large number of considerations. These considerations include but are likely not limited to: the cell line chosen, the type of product being produced, the yield required from the product, the type of bioreactor used, the details of the process implemented, and the degree of scale-up desired. It can be gleaned from the brief review of the aforementioned literature that scale-up is not generally a simple process and has occupied the time and efforts of many skilled workers in the field of bioprocessing for many years.

For the process to maintain a consistent yield and be clinically and economically viable, the cells/process must minimize, or at worst maintain, bioreactor heterogeneity of the above parameters (and sometimes related parameters) during the scale-up process. As mentioned above, this is to date a significant challenge even for those skilled in this art. This often results in lower titer or cell density for the end of a seed train than for the small scale bioreactors in the laboratory. Ideally then, a system by which the volume can be scaled up without changing the bioreactor scale would yield optimal results and allow straightforward changes from R&D through production. This is in contrast with scale-up and scale-down today where most of the optimization work is performed to match the titer and productivity of small glass vessels (<15L) at the production volumes envisaged (biocompare.com/Editorial-Articles/165203-Scaling-Up-Your-Cell-cultures-to-Bioreactors/), and where scalability inconsistencies are still being observed in both titer and product quality as a result of the different bioreactor volumes [J. Li, G. Zhang, et al. "Challenges of scale down model for disposable bioreactors: case studies on growth & product quality impacts", Single-Use Technologies, Leesburg, VA, Oct 18-21, 2015 (incorporated herein by reference in its entirety) or infors-ht.com/index.php/en/applications/scale-up]. Similarly, the performance of new large-scale bio-reactors is always compared to running the same process in a glass vessel (Pall Application Note: USD2926, "Cultivation of CHO Cells in Allegro™ STR 200 Single-Use Stirred Tank Bioreactor System," incorporated herein by reference in its entirety or

eight or even more) can be coupled, where N is only limited by practical considerations (geometrical and space issue, distances, cost). As Figure 3 is a mathematical idealization of the physical problem, many considerations for applying the aforementioned mathematical machinery to bioreactors has not been specifically addressed.

Figure 4 shows two more mathematical representations of tank arrangements that can used for mixing. Reference numeral 4-100 is a linear chain with fluid moving bi-directionally in-between each tank, while 4-101 is a ring configuration that also employs bidirectional mixing. Note that for well characterized vessels that have been designed with appropriate scale-up/scale-down consistency in their operating parameters, any of these configurations could also contain vessels of different sizes.

Bidirectional flow between vessels should increase the speed with which the vessels reach equilibrium, but if real mixing can be ensured this directionality is not strictly necessary. This has also been mathematically modelled and two such unidirectional configurations are shown in Figure 5. Reference numeral 5-100 is the unidirectional analog of the ring 4-101 shown in Figure 4, while 5-101 is the unidirectional analog of the chain 4-100. Reference numeral 5-102 is the inlet and 5-103 is the outlet which will need to be tied together in a fluid conservative system.

Figure 6 presents a further example. As shown there, the central bioreactor 6-100 acts as the "master" or controlling bioreactor, while expansion or satellite bioreactors 6-106 and 6-108 are the "slave" bioreactors. As shown, a central bioreactor 6-100 is outfitted with sensors used for control of various analytes or physical levels including but not limited to sensors for: dissolved oxygen, pH, temperature, CO_2, cell density, conductivity, and cell viability. The master bioreactor is also equipped with a mixing system and impeller 6-102, and spargers 6-107 to control the dissolved oxygen level or strip CO_2. These features are typically used with a control loop (e.g. PID loop) to maintain optimal levels of any of the aforementioned quantities. The control in Figure 6 occurs through measurements in the master bioreactor, while a slave system is used for monitoring and feedback to both the master loops and the pumps 6-103 and 6-104. In certain embodiments such as those depicted in Figure 4, the capacity of the bioreactor can be expanded or scaled by attaching other bioreactors such as bioreactors 6-106 and 6-108 to

In some implementations, the individual vessels of the bioreactor system are similar to one another in working volume, geometry, materials of construction, and/or agitation/mixing system. For example, the vessels may have similar turndown ratios; e.g., no two vessels have turndown ratios varying from one another by more within about 20%. In some embodiments, all vessels are made from the same material; e.g., all vessels have polymeric vessel walls or vessels have stainless steel walls.

In certain embodiments, as explained, one of the multiple vessels is a master vessel and the others are satellite or slave vessels. Frequently, though not necessarily, the master vessel has a larger working volume than any of the satellite vessels. Typically, the master vessel is used to provide some measure of control over the conditions in the satellite vessels. For example, the control system may monitor and adjust conditions first in the master vessel and then use inter-vessel fluid transfer have conditions in the satellite vessels follow those in the master. In certain embodiments, the master vessel includes one or more sensors for the pH, temperature, a cell metabolite concentration, and dissolved oxygen in the culture medium. In certain embodiments, the control system receives values of such parameters from sensors on the master and/or satellite vessels and uses these values to first adjust conditions in the master vessel. In certain embodiments, the control system includes a single control loop configured to control the master vessel, and in some cases at least a mixing system configured to agitate the culture medium in the master vessel. In certain embodiments, the satellite vessels contain spargers set at a substantially constant rate. In such cases, sparging air or oxygen can occur in the satellite vessels, while the primary control point employs sensors in the master vessel.

In some cases, the master vessel may include mixing system configured to agitate the culture medium in the master. In some of these cases, none of the satellite vessels includes a mixing system. Examples of suitable mixing systems, whether implemented in a master or satellite vessel, include impellers, orbital shakers, wave rockers, and plungers.

As explained, the arrangement of vessels, as dictated by direct connections between individual vessels in the bioreactor system, may have many configurations. Examples include a star configuration, a linear configuration, closed loop configuration, and a combination of any two or more of these. For example, the arrangement may be a spoke a wheel configuration, or a

move liquid by mechanical action. Examples include rotodynamic pumps (e.g., centrifugal or axial) and positive displacement pumps (e.g., a syringe type, a gear type, diaphragm type, a piston type, a plunger type, a screw type, or vane type).

PERFUSION

As explained, one or more vessels of the bioreactor system may be configured to operate in a perfusion mode, whereby culture medium is distributed (flows) over the cells constrained to the vessel. Typically, in perfusion mode, conditions are maintained such that cells are retained in a vessel while fresh media is brought in. The rate that the media is brought in depends on the cell line and the phase of growth. A goal is to maintain a substantially optimal growth environment (in terms of nutrient concentrations, product titer, temperature, hydrodynamic conditions, etc.), and, as a consequence, the cells in a vessel maintain a relatively consistent biological state in terms of production rate, viability, etc. for a longer period of time compared to batch mode or fed batch modes of growth. In certain embodiments, a bioreactor system configured to operate in perfusion mode includes two or more fluidically coupled vessels configured to collectively carry out a bioreaction, where at least one of the vessels is a perfusion vessel having a fluidic inlet, a fluidic outlet, and a filter or other mechanism configured to prevent biological cells from leaving the perfusion vessel during the bioreaction. Additionally, as with some other systems described herein, a perfusion mode bioreactor system may include one or more fluidic connectors (or more generally fluidic paths) coupling the two or more vessels to one another, during the bioreaction. The system typically includes one or more fluid transfer devices (e.g., pumps) in at least one of the one or more fluidic connectors. Collectively, the fluidic connectors and the fluid transfer devices are configured to provide flow of culture medium between the two or more vessels.

Still further a perfusion mode bioreactor system may contain a control system configured to (i) read or receive values of two or more parameters characterizing the culture medium in one or more of the vessels during the bioreaction, (ii) use the values to determine process conditions to maintain substantially uniform values of the two or more parameters in the culture medium from vessel-to-vessel among the two or more vessels, and (iii) introduce the culture media to the

vessel includes a mixing system, while the at least one vessel does not include any mixing system.

METHODS OF SCALING A BIOPROCESS

As explained, the disclosed concepts facilitate scaling a bioprocess working volume from that of a small-scale bioreactor to that of a large-scale bioreactor. In certain embodiments, a scaling process initially involves determining appropriate process conditions for performing the bioprocess in a test vessel having a relative small working volume (e.g., no greater than about 700 liters). Examples of process conditions that may be designed for the test vessel include values of two or more parameters characterizing culture medium in the vessel during the bioprocess. Relevant parameters discussed above including temperature, pH, dissolved oxygen concentration, etc. may be employed. After determining process conditions for the test vessel, the scaling process involves designing a bioreactor system having a control system and two or more fluidically connected production vessels. Each of production vessels has a working volume that is similar to that of the test vessel (e.g., within the range of the turndown ratio for the test vessel. In some embodiments, each production vessel has a working volume that is between about 0.7 and 1.5 times the working volume of the test vessel. In certain embodiments, the sum of the working volumes of the two or more production vessels is at least about 2 times larger than the working volume of the test vessel.

In certain embodiments, the design process involves designing the control system to maintain substantially uniform values of one or more parameters, from production vessel to production vessel, during the intended bioreaction. As discussed above, a suitable control system may (i) read or receive production values of the two or more parameters characterizing the culture medium in one or more of the two or more vessels during the bioprocess, (ii) use the read or received production values to determine an adjusted flow rate of the culture medium between the two or more production vessels to maintain substantially uniform production values of the two or more parameters in the culture medium from vessel-to-vessel among the two or more production vessels during the bioprocess, and (iii) control one of the fluid transfer devices disposed between the two or more production vessels to adjust the flow rate as determined in (ii).

It should be noted that such control loop keeps conditions substantially the same from vessel-to-vessel in the bioreactor system, as opposed to a different control loop, that may be implemented in a master vessel that keeps the parameter values within the ranges required (absolute ranges).

Often the process of designing the control system for large-scale bioreactor system involves designing the control system to maintain, during the bioprocess, the production values of the two or more parameters to be substantially equal to the test values of the two or more parameters.

After designing the production vessels and the control system, the process involves actually constructing and/or arranging the two or more production vessels and the control system to as specified to produce the large-scale bioreactor. Finally, the bioprocess may be performed in the large-scale bioreactor. In some implementations, the large-scale bioreactor system is configured to permit bidirectional flow of the culture medium between two of the production vessels, and/or the two or more production vessels are arranged in closed loop.

During the bioprocess, the total amount of culture medium in the bioreactor system may increase. For example, the bioprocess may be conducted at a first total working volume of culture medium in the bioreactor, and then the bioprocess may be conducted at a second total working volume of culture medium that is greater than the first total working volume but still uses only the two or more production vessels of the large-scale bioreactor. This may be accomplished by adding culture medium to the bioreactor system before the bioprocess completes. In another approach, at least a portion of the bioprocess may be conducted in the bioreactor, and then, one more additional production vessels are added to or activated in the bioreactor system. Thereafter, the bioreactor system may be operated to at least partially fill the one or more additional production vessels with culture medium from one or more other production vessels of the bioreactor system. Yet another mode of operating involves (i) conducting the bioprocess in the bioreactor system, and then (ii) removing one or more of the two or more production vessels containing culture medium and cells grown in the culture medium. This approach may be appropriate when the cells in a vessel are cultivated for treating a patient.

In certain embodiments, the large-scale bioreactor system includes more than two production vessels—e.g., as explained above, three, four, five, six, or more production vessels—configured to collectively carry out the bioprocess. In certain embodiments, each production vessel has a working volume of no greater than about 500 liters. In some implementations, the ratio of total working volume of the two or more production vessels to the working volume of largest of the two or more production vessels is at least about 3. In certain embodiments, the two or more production vessels are connected in a star configuration or in a closed loop configuration.

In general, the large-scale bioreactor system may have any one or more of the features described elsewhere herein for such systems. For example, each of the two or more production vessels and the test vessel may have a turndown ratio, and the turndown ratios of any one of the two or more production vessels and the test vessel may be limited vary by no more than about 20%. Additionally, the control system may be configured to adjust conditions in the two or more production vessels to ensure that the mean pH of the culture medium in the two or more production vessels does not vary from one vessel to another by more than about 0.1 pH units.

In many embodiments, one of the two or more production vessels is a master vessel and the others are satellite vessels, where the master vessel includes a mixing system configured to agitate the culture medium. Any one of the satellite vessels may or may not include a mixing system. In some such embodiments, the master vessel has a larger working volume than any of the satellite vessels. In some implementations, the master vessel includes one or more sensors for the pH, temperature, a cell metabolite concentration, and dissolved oxygen in the culture medium.

12. The bioreactor system any one of claims 1-10, wherein the two or more vessels and the one or more fluidic paths provide the bioreactor system in a closed loop configuration.

13. The bioreactor system any one of claims 1-12, wherein the two or more vessels are provided on one or more scales and/or load cells to monitor their masses during the bioreaction.

14. The bioreactor system of claim 13, wherein the control system is further configured to:

read or receive at least one output of the one or more scales and/or load cells, as at least one of the values of one or more parameters; and

based on the outputs of the scales and/or load cells, control the one or more of fluid transfer devices to transfer the culture medium between the two or more vessels such that a volume of the culture medium in the two or more vessels is controlled.

15. The bioreactor system any one of claims 1-14, wherein the fluidic paths attach proximate to bottoms of the two or more vessels.

16. The bioreactor system any one of claims 1-15, wherein the control system is further configured to adjust conditions in the two or more vessels to ensure that the mean pH of the culture medium in the two or more vessels does not vary from one vessel to another by more than about 0.1.

17. The bioreactor system of claim 16, wherein the two or more vessels and the control system are configured such that the pH of the culture medium within any of the vessels has variance of at most about 0.1 pH units.

18. The bioreactor system any one of claims 1-17, wherein the control system is further configured to adjust conditions in the two or more vessels to ensure that shear forces experienced

24. The bioreactor system of claim 23, wherein the control system and master vessel are together configured to substantially maintain defined values of dissolved oxygen concentration, temperature, and pH in the culture medium in the master vessel and in the at least one vessel.

25. The bioreactor system of claim 23, wherein the master vessel comprises a mixing system, while the at least one vessel does not include any mixing system.

26. The bioreactor system of claim 21, wherein the at least one vessel further comprises one or more micro-carriers, hollow fibers, and/or adhesion plates.

27. The bioreactor of any one of claims 1-26, wherein the control system is further configured to control the one or more fluid transfer devices to transfer the culture medium between the two or more vessels, via the one or more fluidic paths, such that the time required to exchange the culture medium in the two or more vessels is at most about one-tenth the time required for cells in the vessels to double under conditions in the two or more vessels.

28. A method of carrying out a bioreaction in a bioreactor system comprising: (i) two or more fluidically coupled vessels configured to collectively carry out a bioreaction; (ii) one or more fluidic paths coupling the two or more vessels to one another, wherein the one or more fluidic paths are configured to provide flow of culture medium between the two or more vessels during the bioreaction; and (iii) one or more fluid transfer devices along at least some of the one or more fluidic paths, wherein each of the fluidic paths is configured to permit bidirectional flow of the culture medium between two of the vessels; and/or the two or more vessels are arranged in closed loop, the method comprising:

(a) reading or receiving values of one or more parameters characterizing the culture medium in one or more of the vessels during the bioreaction;