MIDSCALE MODEL FOR ORGANIC GROWTH AND PHASING

Related Applications

[0001] The present application is based on and claims priority to U.S. Provisional Patent application Serial No. 62/679,076, filed on June 1, 2018, which is incorporated herein by reference.

BACKGROUND

[0002] The pharmaceutical industry has traditionally focused significant attention on so-called blockbuster drugs, which may demand significant investment in permanent facilities dedicated to a single process. Once established, the efficiency of a single process flow can provide strong return on the initial investment.

[0003] However, market factors demand industrial processes with increased flexibility at a reduced cost. In particular, an increasing need for specialized pharmaceuticals have highlighted the shortcomings of facilities designed around a single dominant product line. The pressures for quality control and extensive development and refinement may require multiple revisions or iterations of a particular process flow.

[0004] For example, various biologic cell culture processes may be used to extract useful organic components, such as proteins, enzymes, metabolites, or other biomass. Such processes are often time consuming and require significant capital investment to construct a single facility to execute the culturing and growth process. In many cases, existing facilities, previously configured for one product, may not be easily or quickly reconfigured to support iterations of a new product in a trial capacity or in a production capacity.

[0005] Furthermore, when the production stage is reached, existing equipment is not easily reconfigured to expand upon the initially small product yields. However, traditional high-yield facilities may not be suitable for the initial stages of development, becoming inefficient when subjected to rapidly changing product cycles.

[0006] In view of the above, a need exists for a flexible production line that may rapidly adapt and grow based on needs, allowing for fast reconfiguration, greater throughput, and greater product diversity. There is a further need for

flexibility in both the process and equipment design as well as the facility and building designed to contain the process. In summary, a need exists for an organic growth and phasing design for the industrial growth of biologics.

SUMMARY

[0007] In general, the present disclosure is directed to a system for the growth of biologics. The system includes a bioreactor subsystem comprising at least two bioreactors and at least two harvest subsystems. Each harvest subsystem is in fluid communication with each of the bioreactors in the bioreactor subsystem, alone or in combination.

[0008] In some embodiments, the system includes a flow control assembly positioned in between the at least two bioreactors and the at least two harvest subsystems. The flow control assembly may be configured to selectively control flow of a fluid from a first bioreactor to a first harvest subsystem and from the first bioreactor to a second harvest subsystem. The flow control assembly may also be configured to control flow of a fluid from a second bioreactor to the first harvest subsystem and from the second bioreactor to the second harvest subsystem.

[0009] In some embodiments, the system also includes at least one purification subsystem. Each purification subsystem is in fluid communication with each of the harvest subsystems, alone or in combination. In some embodiments, at least one of the purification subsystems is configured to produce a different biologic than at least one other purification subsystem.

[0010] In some embodiments, the system as disclosed herein may be configured to process more than 100 batches per year.

[0011] In some embodiments, at least one of the bioreactors is 6000 L or larger. In some embodiments, the bioreactor subsystem comprises a seed train of fed-batch bioreactors and/or at least one perfusion seed train. In some embodiments, a perfusion seed train may feed at least one fed-batch bioreactor.

[0012] The present disclosure is also generally directed to a system for buffer distribution including at least one buffer dilution device which accepts at least two inputs (i) and (ii), wherein (i) is a buffer concentrate source stream and (ii) is a water for injection source stream. The system also includes at least one buffer dilution device which delivers a buffer mixture comprising (i) and (ii) to a buffer mixture

distribution manifold. At least one buffer mixture distribution manifold may be used to control parameters of the buffer mixture delivered to each of a plurality of destinations independently, the parameters including at least one of the following: flow rate or concentration. The buffer mixture is not stored before delivery to any of the plurality of destinations.

[0013] In one embodiment, the system for buffer distribution also includes a first buffer dilution device. The buffer mixture output from the first buffer dilution device is delivered to at least one downstream buffer dilution device as input (i). The input (i) of any buffer dilution device downstream of the first buffer dilution device may be partially diverted to another buffer dilution device or to at least one buffer mixture distribution manifold. At least two buffer mixture distribution manifolds distribute buffer of a different concentration.

[0014] In some embodiments, at least two buffer dilution devices deliver buffer of about the same concentration to at least one buffer distribution manifold.

[0015] In some embodiments, at least one buffer destination comprises a media preparation subsystem, a cell culture subsystem, a bioreactor subsystem, a harvest subsystem, and/or a purification subsystem.

[0016] The present disclosure is also generally directed to a system for the growth of biologics including a perfusion seed train, a bioreactor subsystem containing at least one bioreactor fed from the perfusion seed train in whole or in part, and at least two harvest subsystems. Each harvest subsystem may be configured to receive the effluent of each of the bioreactors in the bioreactor subsystem, alone or in combination.

[0017] The present disclosure is also generally directed to a building containing any of the previously described systems. For example, in one embodiment, a building may contain at least two purification subsystems, where each purification subsystem includes three chromatography column stations and three chromatography skid stations in a first room, a chromatography column station and a chromatography skid station in a second adjacent room, and a maintenance area accessible from either the first room or the second room. The maintenance area is accessible from the first and second rooms by airlocks and is further accessible from the remainder of the building by another airlock.

[0018] In some embodiments, the second room further includes a filling device.

[0019] In some embodiments, the bioreactor subsystem includes a plurality of bioreactor stations in one room, wherein some or all of the stations are filled with a bioreactor. In some embodiments, at least one bioreactor station is not filled with a bioreactor. In some embodiments, at least one of the bioreactor stations not filled with a bioreactor and at least one of the bioreactor stations filled with a bioreactor are configured to maintain fluid communication with a flow control assembly. The flow control assembly may be configured to selectively control the flow of a fluid from any one of at least two bioreactor stations to any one of at least two harvest subsystems.

[0020] In some embodiments, a building prepared as herein also includes logistics corridors, personnel areas, packing areas, shipping areas, and/or receiving areas, and may be configured to meet Leadership in Energy and Environmental Design certification standards. The building may also include a plurality of motion, temperature, and/or moisture sensors distributed on, in, and/or around equipment in the building as well as a monitoring system configured to track the outputs of the plurality of sensors and generate records and/or alerts responsive to outputs departing from predetermined ranges.

[0021] The present disclosure is also generally directed to a process for the growth of biologics. The process includes growing a cell mass; harvesting material comprising (i) the cells or (ii) components of the cells; passing a first portion of the harvested material to a first purification subsystem; passing a second portion of the harvested material to a second purification subsystem; producing a first product via the first purification subsystem; and producing a second product via the second purification subsystem.

BRIEF DESCRIPTION OF THE FIGURES

[0022] A full and enabling disclosure of the present disclosure is set forth more particularly in the remainder of the specification, including reference to the accompanying figures, in which:

[0023] Figure 1 contains an example layout of a facility prepared as herein;

[0024] Figure 2 contains a schematic of a buffer distribution subsystem;

[0025] Figure 3 contains a schematic of a media preparation subsystem;

[0026] Figure 4 contains a schematic of a bioreactor subsystem;

[0027] Figure 5 contains a schematic of a harvest subsystem; and

[0028] Figure 6 contains a schematic of a purification subsystem.

[0029] Repeat use of reference characters in the present specification and drawings is intended to represent the same or analogous features or elements of the present invention.

DETAILED DESCRIPTION

[0030] It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only and is not intended as limiting the broader aspects of the present disclosure.

[0031] In general, the present disclosure is directed to a system for the growth of biologics. As used herein, the growth of biologics refers generally to the process of culturing, feeding, and processing biological material to achieve a particular purpose. In some cases, the purpose is the capture of the grown material itself. For example, a particular cell culture may be desired for its therapeutic use, and the growth process expands a given amount of the cell culture and harvests, washes, and perhaps packages the desired cells. In other cases, some byproduct of the biological mass is desired. For example, in some cases, a protein product of a particular cell culture is desired, and the growth process expands a given amount of the cell culture and extracts, purifies, and perhaps packages the protein product.

[0032] The system can be described as a collection of organized subsystems. These subsystems may be organized in a single building or facility. When required, the facility may be constructed to meet standards for controlled and/or classified spaces, as defined by ISO 14644-1, FED STD 209E, and/or EU Good Manufacturing Practice (GMP) standards. By way of example only, reference may be made to single-use equipment. It is to be understood that reusable equipment falls within the scope of the present disclosure.

[0033] A flexible process according to the present disclosed may be housed in a facility **000** shown in **Fig. 1**. The example facility may be divided into three subfacilities: an upstream subfacility **001**, a downstream subfacility **002**, and a staff subfacility **003**. Multiple of any of the subfacilities may be present. The subfacilities

may be constructed to be adjacent or stacked, such as on separate floors of a building. For example, the upstream subfacility **001** may reside on a second floor while a downstream subfacility **002** may reside on a first floor. Alternatively, the subfacilities may be housed in distinct but adjacent buildings. In some examples, the upstream and downstream subfacilities are located in distinct subdivisions of a building (*e.g.,* rooms or floors) while the staff subfacility **003** may be located in, around, between, below, and/or alongside the upstream and/or downstream subfacilities.

[0034] In one embodiment, as shown in **Fig. 1**, the upstream subfacility **001** contains a buffer distribution subsystem **100**, a media preparation subsystem **200**, and a bioreactor subsystem **300**; the downstream subfacility **002** contains two harvest subsystems **400** and two purification subsystems **500**; and the staff subfacility **003** may contain logistics corridors, personnel areas, packing areas, shipping areas, and/or receiving areas, for example.

[0035] In some embodiments, the facility **000** is located in the same building as other facilities used for the production of other products. For example, multiple facilities **000** may be co-located for the production of a plurality of biologics.

[0036] Of particular advantage, a single facility **000** may also produce any number of biologics within its enclosed subsystems. For example, the subsystems within the facility may be highly interconnected. In one embodiment shown in **Fig. 1**, the effluent of the bioreactor subsystem **300** may be in fluid communication with both of the harvest subsystems **400** independently. Additionally, any one of the harvest streams from any one of the harvest subsystems **400** may be in fluid communication with any one or both of the purification subsystems **500**. The buffer mixture generated in the buffer distribution subsystem **100** may be directed to any one of the other subsystems. In this manner, a single facility **000** may provide the utmost flexibility for configuring one or more desired process flows.

[0037] Fluid communication, as used herein, indicates a direct and intentional connection via some variety of conduit or conduits whereby fluid may flow from one point to another. The fluid communication may be regulated by any number of valves, manifolds, or controllers.

[0038] For example, in one embodiment, a flow control assembly may provide a framework for interconnected fluid communication. In some cases, a flow control

assembly may be a system of pipes. Some embodiments may also employ any number of valves, diverters, throttles, meters, or manifolds, each optionally controllable manually and/or in an automated fashion. In this manner, a flow control assembly may facilitate the fluid communication between any number of subsystems or locations with subsystems of a facility **000**.

[0039] A flow control assembly may be selective in its flow control. For example, when a flow control assembly is used to provide fluid communication between a single bioreactor subsystem **300** having two or more bioreactors and two or more harvest subsystems **400**, the flow control assembly may selectively control the effluent from any subset of the bioreactors individually, directing each subset to one or more destination harvest subsystems individually chosen for the effluent with flow parameters similarly individually customized. In this manner, the flow control assembly may provide a highly flexible fluid communication network between all or any subset of the bioreactors in the bioreactor subsystem **300** to all or any subset of a plurality of harvest subsystems **400**. In some embodiments, a similar flow control assembly may enable the selective and individually controlled distribution of buffer throughout a facility **000** to any number of subsystems.

[0040] A flexible embodiment of a facility **000** may, in some examples, contain a fluid connection network between a plurality of subsystems. The subsystems may be arranged and rearranged at different locations within the facility and the fluid connection network infrastructure (*e.g.,* containing a flow control assembly) may permit rapid reconnection of the equipment.

[0041] Advantageously, a fluid connection network is created to service more subsystems than initially constructed. For example, a facility **000** as in **Fig. 1** may include a fluid connection network that is fully configured to provide fluid connections between one bioreactor subsystem **300** and two harvest subsystems **400**, but is only initially operated with one harvest subsystem **400**.

[0042] Such an interconnected facility **000** may operate in parallel, in whole or in part; for example, the total product train may or may not be completely parallelized. In one embodiment, a number of parallel bioreactor subsystems **300** may feed a single harvest subsystem **400** which feeds a number of parallel purification subsystems **500**. In another example, a single bioreactor subsystem **300** feeds a plurality of harvest subsystems **400** which feed a plurality of purification

subsystems **500**. In this manner, a single facility **000** may produce a number of different products or a number of grades of the same product; for example, a single harvest subsystem **400** may provide the same harvested culture to two purification subsystems **500**, one optimized for quality and one optimized for yield. Similarly, various test scenarios may be run in parallel to observe different process effects on the exact same source batch.

[0043] Although a facility prepared according to the present disclosure has the capability being optimized to maximize output, the subsystems may be configured independently to achieve any desired output requirements. For example, different purification trains may be adjusted with different operating outputs to minimize surplus product. Additionally, some embodiments may intentionally operate at less than total capacity in order to temporarily lower operating costs when maximum output is not required.

[0044] In some embodiments, the total output of the facility **000** may be greater than about 50 batches per year, such as greater than about 75 batches per year, such as greater than about 100 batches per year, such as greater than about 150 batches per year, such as greater than about 300 batches per year. Generally, however, facilities **000** may be scaled to produce less than about 1000 batches per year.

[0045] The facility **000** may require an operating staff, perhaps in some cases a large operating staff. In some examples, however, the facility **000** may be partially or completely automated. For example, predictive analytics technology (PAT) may enable streamlined maintenance and monitoring procedures. For example, a number of sensors, such as vibration sensors, noise sensors, moisture sensors, temperature sensors, or power draw sensors may provide a monitoring system with a picture of the operational health of the facility **000**. For example, vibration and temperature sensors may be mounted on equipment casings. If any output of any of the sensors departs from a predetermined range, or alternatively a range interpreted by the monitoring system to be ordinary or stable, the monitoring system may generate alerts to indicate a need for attention in a particular area of the facility **000**, such as in a particular subsystem. In this manner, the total operational staff required may be reduced.

[0046] Advantageously, the flexibility of a facility **000** prepared as herein enables quick recovery from unplanned equipment downtime. For example, if one of two harvest subsystems **400** were to go offline, the interconnected fluid communication network would allow the effluent of the bioreactor subsystem **300** to be quickly and easily redirected to a second harvest subsystem **400**.

[0047] Due to the highly flexible nature of facilities **000** prepared according to the present disclosure, the facilities may be constructed to be energy efficient. For example, by selectively operating various subsystems only as needed to meet demand, the total utilities consumption may be optimized to be efficient. In some embodiments, the buildings enclosing the facility may be constructed to meet various standards, such as Leadership in Energy and Environmental Design certification standards.

[0048] In one embodiment, the system contains a buffer distribution subsystem **100** shown in **Fig. 2**. A buffer solution may be, for example, a salt solution used to alter or maintain a given pH level in a target environment, such as a bicarbonate solution. For example, buffer may be added to bioreactor feed media to attain and/or maintain a particular pH. The area in which buffer is prepared may be a controlled airspace, such as ISO 8/Grade C/Class 100,000.

[0049] Buffer may be mixed and subsequently stored in large tanks before delivery to a target environment. However, in some embodiments, a buffer concentrate may be mixed, for example, in a small single use mixer (SUM) **102** or a large SUM **104**. If used, the small and/or large SUM may be various sizes. For example, the small SUM may be larger than about 100 L, such as larger than about 500 L, such as larger than about 1000 L. Generally, however, the small SUM may be smaller than about 3000 L, such as smaller than about 1000 L. A large SUM for buffer concentrate preparation may be larger than about 500 L, such as larger than about 1000 L, such as larger than about 2000 L, such as larger than about 3000 L. Generally, however, the large SUM may be smaller than about 4000 L, such as smaller than about 3000 L.

[0050] The ingredients of the buffer may be selected appropriately for a given application. In some embodiments, the processing solution is a buffer such as Tris, Tricine, HEPES, MOPS, PIPES, TAPS, bicine, BES, TES, cacodylate, MES, acetate, MKP, ADA, ACES, glycinamide and acetamidoglycine or acetic acid.

[0051] In some embodiments, a bicarbonate buffer may be desired. For example, sodium carbonate and sodium bicarbonate may be provided in dry form and mixed with water for injection. Dry ingredients may be deposited by automated equipment or manually.

[0052] The buffer may be stored in a concentrated form in a holding tank **106**. The holding tank may be of any useful configuration. For example, the holding tank may be a stainless steel vessel. In other embodiments, the holding tank may contain one or more single-use bags.

[0053] A stored concentrate may then be pumped to a buffer dilution device **112**. A buffer dilution device **112** may accept the buffer concentrate stream **108** and a sterile water for injection stream **110**. In some embodiments, a buffer mixture stream **114** proceeds directly to a buffer mixture distribution manifold **116** after a single dilution stage.

[0054] In some embodiments, a buffer mixture stream is directed into a second buffer dilution device **112** which dilutes the buffer mixture stream into a second buffer mixture stream **118**. The second buffer mixture stream **118** may be distributed by a second buffer mixture distribution manifold **120**. In this manner, the buffer distribution subsystem **100** may produce and distribute buffer solutions having any number of different concentrations.

[0055] In some embodiments, more than one buffer dilution device **112** produces buffer of the same concentration. Such embodiments are resistant to downtime due to system malfunction or maintenance, and may also enable increase flexibility to fluctuating buffer demand.

[0056] In one embodiment, the buffer mixture distribution manifold **116** controls the flow rate of the buffer mixture to any number of destinations. In some embodiments, the buffer mixture distribution manifold controls the flow of buffer mixture to a plurality of destinations individually, optionally automated to be responsive to sensor feedback indicating target parameters, such as the pH of the destination environment.

[0057] The buffer mixture distribution manifold may optionally be centrally located to enable compact and efficient routing of buffer distribution lines to any number of destinations. In some embodiments, some destinations may also be manifolds intended for localized distribution. For example, a central manifold may

pump buffer to a particular room of a processing facility and a smaller manifold within the room may distribute the buffer to devices in the room requiring a buffered solution.

[0058] In some embodiments, a system prepared according to the present disclosure contains a media preparation subsystem **200**, as shown in **Fig. 3**. In general, a media preparation subsystem **200** prepares a substrate on which the inoculated biologic material feeds. For example, media may include sugars, yeasts, sulfates, chlorides, or other suitable feed materials. The media may be prepared in a controlled airspace, such as under ISO 8/Grade C/Class 100,000 conditions.

[0059] In some embodiments, the media preparation subsystem **200** involves manual preparation of components. In some embodiments, components may be refrigerated in the media preparation subsystem **200**. Prepared media may be refrigerated after mixing for a longer shelf life.

[0060] Media may be prepared according to the requirements of any given application. The media may be defined, synthetic, undefined, or basal. The media may be enriched or highly enriched. The media may contain various amino acids, such as non-essential amino acids and essential amino acids, such as glutamine and branched-chain amino acids. The concentration of the media may be varied to manipulate the response of the intended cell culture.

[0061] Media components include, for example, buffer, amino acid content, vitamin content, salt content, mineral content, serum content, carbon source content, lipid content, nucleic acid content, hormone content, trace element content, ammonia content, co-factor content, indicator content, small molecule content, hydrolysate content and enzyme modulator content.

[0062] Minerals that are optionally present include bismuth, boron, calcium, chlorine, chromium, cobalt, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, rubidium, selenium, silicon, sodium, strontium, sulfur, tellurium, titanium, tungsten, vanadium, and zinc. Exemplary salts and minerals include CaCl2 (anhydrous), CuSO4 5H2O, Fe(NO3).9H2O, KCl, KNO3, KH2PO4, MgSO4 (anhydrous), NaCl, NaH2PO4H2O, NaHCO3, Na2SE3 (anhydrous), ZnSO4.7H2O; linoleic acid, lipoic acid, D-glucose, hypoxanthine 2Na, phenol red, putrescine 2HCl, sodium pyruvate, thymidine, pyruvic acid, sodium succinate, succinic acid, succinic acid.Na.hexahydrate, glutathione (reduced), para-

aminobenzoic acid (PABA), methyl linoleate, bacto peptone G, adenosine, cytidine, guanosine, 2′-deoxyadenosine HCl, 2′-deoxycytidine HCl, 2′-deoxyguanosine and uridine.

[0063] For example, suitable media and culture methods for mammalian cell lines are well-known in the art, as described in U.S. Pat. No. 5,633,162 for instance. Examples of standard cell culture media for laboratory flask or low density cell culture and being adapted to the needs of particular cell types are for instance: Roswell Park Memorial Institute (RPMI) 1640 medium (Morre, G., The Journal of the American Medical Association, 199, p.519 f. 1967), L-15 medium (Leibovitz, A. et al., Amer. J. of Hygiene, 78, 1p.173 ff, 1963), Dulbecco's modified Eagle's medium (DMEM), Eagle's minimal essential medium (MEM), Ham's F12 medium (Ham, R. et al., Proc. Natl. Acad. Sc.53, p288 ff. 1965) or Iscoves' modified DMEM lacking albumin, transferrin and lecithin (Iscoves et al., J. Exp. med. 1, p. 923 ff., 1978). It is known that such culture media can be supplemented with fetal bovine serum (FBS, also called FCS), the latter providing a natural source of a plethora of hormones and growth factors. Cell culture of vertebrate and mammalian cells, respectively, has become a routine matter and is covered in detail, for example, in R. Ian Fresney, Culture of Animal cells, a manual, 4th edition, Wiley-Liss/New York, 2000.

[0064] In some embodiments, a high-density growth culture medium may be employed. Such high-density growth media can usually be supplemented with nutrients such as all amino acids, energy sources such as glucose, inorganic salts, vitamins, trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), buffers, the four nucleosides or their corresponding nucleotides, antioxidants such as Glutathione (reduced), Vitamine C and other components such as important membrane lipids, *e.g.,* cholesterol or phosphatidylcholine or lipid precursors, *e.g.,* choline or inositol. A high-density medium will be enriched in most or all of these compounds, and will, except for the inorganic salts based on which the osmolarity of the essentially isotonic medium is regulated, comprise them in higher amounts (fortified) than the afore mentioned standard media as can be incurred from EP-435 911 A or GB-2251249 in comparison with RPMI 1640. GB-2251249 gives examples of suitable high-density growth media. In some embodiments, a high density cell culture medium comprises acetate in the amounts stated above, possibly in the

absence of a butyrate. Additionally, the culture medium may be balanced and fortified in that a majority of amino acids except for Tryptophane are in excess of 75 mg/L in the culture medium. In conjunction with the general amino acid requirement, the joint amounts of Glutamine and Asparagine may, in some examples, be in total in excess of 1 g/L, such as in excess of 2 g/L of high-density culture medium. It goes without saying that the latter more preferred embodiment is less suitable in case of a recombinant cell line transfected with a Glutamine synthetase (GS) vector, in particular after rounds of amplification of the GS gene sequence have taken place. In those cells, an excess of, for example, glutamine jointly from exogenous and endogenous source would lead to production of ammonia, which is to be avoided.

[0065] Small amounts of media may be prepared in a biosafety cabinet. A biosafety cabinet, controlled to ISO 5/Grade A/Class 100, offers isolation for preparation of media for small inoculations.

[0066] Generally, however, large amounts of media may be prepared continuously or batch-wise in SUMs. For example, batches may be prepared in a number of SUMs in the subsystem, such as in small SUMs **204** or large SUMs **206**, or both, as shown in **Fig. 3**. SUMs used for preparation of media may be larger than about 100 L, such as larger than about 500 L, such as larger than about 1000 L, such as larger than about 2000 L. Generally, however, the SUMs will be smaller than about 4000 L, such as smaller than about 3000 L, such as smaller than about 2000 L.

[0067] Buffer solution used in the media preparation subsystem **200** might be directly pumped into the SUMs from the buffer mixture distribution manifold(s). Alternatively, buffer solution may be pumped into a temporary holding tank (*e.g.,* a tote **202**) from which the buffer mixture may be fed as needed into the SUMs. Depending on the volume of the SUM and/or the demands of the process, different concentrations of buffer as received from the first and second buffer mixture distribution manifolds may be fed to different SUMs.

[0068] Prepared media may be stored in at least one holding tank **208**. In some embodiments, prepared media may be stored directly in single-use bags. If needed, the bags may be stored in a cold room to increase the shelf life of the media. In some embodiments, the holding tanks are refrigerated directly.

[0069] In some embodiments, a system prepared according to the present disclosure contains a bioreactor subsystem **300**, as shown in **Fig. 4**. The bioreactor subsystem may be housed in a single room, optionally designated as a Controlled Non-Classified area.

[0070] The bioreactor subsystem **300** generally cultures biologics through at least one seed train before placement in at least one production bioreactor. For example, some embodiments start the seed train from a small source, such as a flask or vial prepared by hand in a glove box **302**. For example, a flask containing a volume of inoculated media may be hand shaken to start the culturing process in an isolated incubator. In other examples, the process is automated by pumping a small amount of media into a flask pre-filled with the inoculation material.

[0071] After inoculation of a small sample, some embodiments transfer the growing cell mass through a number of incubation stages in the seed train. For example, the seed train may progress from a wave incubator **304** (*e.g.,* a perfusion-based wave incubator), to a first single-use bioreactor (SUB) **306** (*e.g.,* 20 L to 500 L), to a second SUB **308** (*e.g.* 100 L to 1000 L), and to a third SUB **310** (*e.g., 5*00 L to 4000 L) before reaching the production bioreactor **312**.

[0072] The seed train may progress by manually transferring the active cell mass from one incubation stage to the next. In some embodiments, the transfers may be automated, controlled by feedback from sensors measuring cell mass, cell concentration, glucose levels, or other such progress indicators.

[0073] In one embodiment, the seed train contains a perfusion-based reactor system **316** which feeds at least one production bioreactor directly. For example, a perfusion reactor may reduce the time required to grow a cell culture to the size or mass required by the production bioreactors. A perfusion seed train continuously provides fresh media to a cell culture while removing any spent media, retaining the cell culture either in a filtered area or in a holding vessel (*e.g.,* a bioreactor). In this manner, the culture is provided the optimum growing conditions at all times, enabling very high culture concentrations. A perfusion based reactor system **316** may replace one or more intermediate SUB stages in the seed train. For example, in some cases, a perfusion-based reactor system may feed at least one production bioreactor directly, such as two bioreactors, such as three bioreactors, such as five bioreactors. In some cases, all the bioreactors in the bioreactor subsystem **300** may be fed by a

perfusion-based reactor system **316**. Media and/or buffer may be directly pumped from the media preparation subsystem **200** or the buffer distribution subsystem **100** to the perfusion reaction system **316**.

[0074] If used in conjunction with a perfusion reactor, bioreactors **312** may or may not be especially designed for perfusion feeding. For example, with regard to the harvest port and tubing design of bioreactors used for perfusion modes of operation, the dip tube needs to be appropriately sized to permit unobstructed flow of culture out of the bioreactor and into a coupled cell retention device and to allow return from the cell retention device back into the bioreactor without foaming and shearing of the cells or cell aggregates. The unimpeded flow of cell culture during perfusion mode or during harvest phase of a fed-batch process is to ensure the cells are not mechanically damaged whilst passing through such tubings, as mechanical damage can result in release of cellular factors (*e.g.,* enzymes such as glutathione reductase, thioredoxin, and thioredoxin reductase or metabolites such as NADPH) which can adversely affect the performance of the process and quality of the product made. Secondly, unimpeded flow of cell culture can result in the culture not becoming hypoxic or anoxic while passing through such tubing and thereby prevent activation of the released cellular factors which can adversely affect the performance process and quality of the product during further processing.

[0075] Production bioreactors **312** may include stirred, shaken, airlift, or other bioreactor types. In some embodiments, production bioreactors are continuous, fed-batch, and/or perfusion-fed SUBs. Additionally or alternatively, one or more production bioreactors **312** may be perfusion reactors. In some aspects, the bioreactors can be variable diameter such as those described in U.S. Patent Publication No. 2017/0369828A1, which is hereby incorporated by reference in its entirety.

[0076] When more than one production bioreactor **312** is present in the bioreactor subsystem **300**, the production bioreactors **312** may be the same or different sizes, such as larger than about 500 L, such as larger than about 1000 L, such as larger than about 2000 L, such as larger than about 5000 L, such as larger than about 8000 L, such as larger than about 12000 L, such as larger than about 18000 L. In one embodiment, the production bioreactors **312** are smaller than about 20000 L, such as smaller than about 15000 L, such as smaller than about 10000 L.

[0077] A bioreactor unit can perform one or more, or all, of the following: feeding of nutrients and/or carbon sources, injection of suitable gas (*e.g.,* oxygen), flow of fermentation or cell culture medium, separation of gas and liquid phases, maintenance of growth temperature, maintenance of pH level, agitation (*e.g.,* stirring), and/or cleaning/sterilizing. In one embodiment, the bioreactor is suitable for culturing suspension cells or anchorage-dependent (adherent) cells.

[0078] In one embodiment, the bioreactor subsystem **300** is suitable for cell therapy and/or viral therapy operations. In one embodiment, the bioreactor subsystem **300** is suitable for culturing prokaryotic cells or eukaryotic cells. Examples of cells include, but are not limited to, bacterial cells (*e.g.,* E. coli. P. pastoris), yeast cells (*e.g.,* S. cerevisae, T. reesei), plant cells, insect cells (*e.g.,* Sf9), Chinese hamster ovary cells (CHO, and any genetically modified or derived CHO cell line), mouse cells (*e.g.,* mouse embryonic fibroblasts, cells derived from mouse cancer models), human cells (*e.g.,* cells from any tissue or organ, cells from a cancer or other diseased cell line, stem cell), hybridoma cells, or other genetically modified or hybrid cells. In one embodiment, the cells express or produce a product, such as a recombinant therapeutic or diagnostic product. Examples of products produced by cells include, but are not limited to, antibody molecules (*e.g.,* monoclonal antibodies, bispecific antibodies), fusion proteins (*e.g.,* Fc fusion proteins, chimeric cytokines), other recombinant proteins (*e.g.,* glycosylated proteins, enzymes, hormones), or lipid-encapsulated particles (*e.g.,* exosomes, virus-like particles).

[0079] The production bioreactors **312** may optionally be located in a bioreactor station **314**. For example, a bioreactor station **314** may provide the physical floor, wall, or hanging rack space in a room for placement of a bioreactor, as well as connections for any electrical, mechanical, heated/cooled water, media, buffer, sterile WFI, and/or cell culture conduits required by a bioreactor. For example, each bioreactor station **314** may be in communication with at least one buffer distribution manifold **116** or **120** to provide buffer of the required concentration to the bioreactor.

[0080] Additionally or alternatively, a bioreactor station **314** may provide connections for the output of any effluent from a bioreactor. For example, a bioreactor station **314** may be in fluid communication with a flow control assembly. When a station **314** is not filled with a bioreactor, the fluid communication may be

controlled by a closed valve, for example. However, any existing fluid communication conduit aids the quick expansion of bioreactor capacity by enabling new bioreactors installed in stations **314** to be easily connected to the flow control assembly without the need to install additional conduits or other pipes with the addition of each new bioreactor.

[0081] In some examples, a bioreactor station **314** is uniquely configured to a particular size, type, or configuration of bioreactor. In other examples, each bioreactor station **314** may support a wide variety of bioreactors with little to no customization. In this manner, bioreactor reconfiguration is streamlined. For example, a bioreactor subsystem **300** may be quickly scaled in response to a growing product demand.

[0082] In some examples, a production bioreactor **312** is placed in each bioreactor station **314**. In other examples, such as shown in **Fig. 4**, some bioreactor stations **314** do not contain a production bioreactor **312**. In such examples, the empty stations stand ready to receive production bioreactors if the added output is required.

[0083] The stations **314** may be filled all at one time or may be filled in phases as desired. For example, the bioreactor subsystem may be operated at one time with a first portion of stations filled. At a second time, a second portion of stations may be filled, in addition to or in substitution of the first portion. That is, the first and second portions need not overlap with any number of stations. A bioreactor subsystem **300** may alternate between operating with the first portion and operating with the second portion in order to minimize downtime between bioreactor batches.

[0084] In some examples, a second portion of stations may be filled entirely in addition to the first portion, the second portion being equal to or less than the amount of previously unused bioreactor stations **314**. For example, a bioreactor subsystem **300** with six bioreactor stations **314** operating with a first portion of four bioreactors **312** initially may add a second portion of two bioreactors **312** if needed. In another example, when titers are below a design threshold, additional bioreactors may be quickly brought online to increase the total bioreactor volume. In this manner, a high-output bioreactor subsystem **300** may be constructed and implemented in phases as the demand ramps up from small scale to large scale.

[0085] A bioreactor subsystem **300** may be equipped with more than 1 bioreactor station, such as more than 2, such as more than 5, such as more than 10. In one embodiment, the bioreactor subsystem **300** is equipped with fewer than 30 stations, such as fewer than 20, such as fewer than 10. In some embodiments, the bioreactor subsystem **300** may be operated with fewer than 80% of stations filled, such as fewer than 60%, such as fewer than 40%, such as fewer than 20%. In additional examples, the bioreactor subsystem may be operated with more than 20% of stations filled, such as more than 40%, such as more than 60%, such as more than 80%, even up to 100%. Stations may be filled or deactivated in any number of phases or steps, such as one, two, three, four, or more.

[0086] In some embodiments, the production bioreactors may receive media directly from the media holding tanks **208**. For example, the media may be distributed to all bioreactor stations **314** containing a production bioreactor **312** via a main line to the bioreactor subsystem **300** subsequently distributed via manifold to each station or via direct lines to each station. The flow in each line may be monitored and/or controlled to ensure optimum operation.

[0087] In other examples, media may be manually distributed to each bioreactor station **314** in mobile vessels. For example, a tote or a bag may be filled directly in the media preparation subsystem **200** and transported to the bioreactor subsystem **300**. In some embodiments, the media preparation subsystem **200** and the bioreactor subsystem **300** are adjacent, and a pass-through airlock may enable easy transfer. The bioreactor stations may include bins or receptacles for the media-containing tote or bag. In such an example, once the bin or receptacle has been filled, the inflow of media to the bioreactor may be monitored and/or controlled to ensure optimum operation.

[0088] For example, the control may follow an open loop (*e.g.,* a timer) or a closed loop (*e.g.,* responsive to feedback from sensors reading temperature, cell density, glucose levels, etc.). The control system may also, in some examples, notify operational staff of the need to refill the media receptacle of the bioreactor station **314**.

[0089] A system prepared according to the present disclosure may, in some embodiments, contain at least one harvest subsystem **400**. For example, as shown in **Fig. 5**, a system may contain two harvest subsystems **400**, each having a

centrifuge **402** which may receive the effluent of the bioreactor subsystem **300**. For example, a centrifuge **402** may remove cells and large cell solids from the bioreactor effluent. The output of the centrifuge **402** may be passed to a series of one or more filtration steps. For example, a bank of depth filters **404** may further clarify the harvest stream and lower the turbidity to a target level. Additionally, depth filters **404** may be electrically charged to reduce other impurities, such as removing endotoxins, virus particles, and/or protein impurities.

[0090] After at least one centrifugal, clarification, and/or filtration step, the harvest stream may be directed to at least one harvest tank **406**. In some examples, each harvest subsystem **400** contains at least one harvest tank **406**. In other examples, two or more harvest subsystems may share at least one harvest tank **406**. For example, multiple centrifuges **402** and multiple depth filters **404** may optionally pass effluent to one tank **406**. The harvest tank **406** may be connected to at least one buffer mixture distribution manifold **116** or **120** for controlling the pH of the harvest solution. In some examples, multiple harvest subsystems **400**, each independently configured, may operate in parallel and be arranged adjacent or removed from each other.

[0091] In some embodiments, a system prepared according to the present disclosure contains a purification subsystem **500**, as shown in an example embodiment in **Fig. 5**. In some embodiments, the purification subsystem **500** may be divided into a pre-viral train **500-1** and a post-viral train **500-2**.

[0092] The pre-viral train **500-1** may include at least one chromatography, filtration, or mixing device.

[0093] Methods of 1-dimensional (1D) chromatography suitable for use in the methods described here are known to one of skill in the art and include, for example, affinity chromatography, gel filtration chromatography, ion exchange chromatography, reversed phase chromatography, hydrophobic interaction chromatography. In some embodiments, the one-dimensional chromatography method is HPLC reversed phase chromatography. Chromatography can include high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis, ion mobility. See also, for example, Process Scale Purification of Antibodies, Uwe Gottschalk 2011 John Wiley & Sons ISBN: 1118210743; Antibodies Vol 1 Production and Purification, G. Subramanian 2013 Springer Science &

Business Media; Basic Methods in Antibody Production and Characterization, Gary C. Howard 2000 CRC Press.

[0094] Additional exemplary chromatographic methods include, but are not limited to, Strong Anion Exchange chromatography (SAX), liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), thin layer chromatography (TLC), amide column chromatography, and combinations thereof. Exemplary mass spectrometry (MS) include, but are not limited to, tandem MS, LC-MS, LC-MS/MS, matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS), Fourier transform mass spectrometry (FTMS), ion mobility separation with mass spectrometry (IMS-MS), electron transfer dissociation (ETD-MS), and combinations thereof. Exemplary electrophoretic methods include, but are not limited to, capillary electrophoresis (CE), CE-MS, gel electrophoresis, agarose gel electrophoresis, acrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using antibodies that recognize specific glycan structures, and combinations thereof. Exemplary nuclear magnetic resonance (NMR) include, but are not limited to, one-dimensional NMR (1D-NMR), two-dimensional NMR (2D-NMR), correlation spectroscopy magnetic-angle spinning NMR (COSY-NMR), total correlated spectroscopy NMR (TOCSY-NMR), heteronuclear single-quantum coherence NMR (HSQC-NMR), heteronuclear multiple quantum coherence (HMQC-NMR), rotational nuclear overhauser effect spectroscopy NMR (ROESY-NMR), nuclear overhauser effect spectroscopy (NOESY-NMR), and combinations thereof.

[0095] Suitable spectroscopic techniques include, for example, Raman spectroscopy. Raman spectroscopy is a technique that is increasing in popularity among the different disciplines of forensic science. Some examples of its use today involve the identification of drugs (Hodges et al., "The Use of Fourier Transform Raman Spectroscopy in the Forensic Identification of Illicit Drugs and Explosives," Molecular Spectroscopy 46:303-307 (1990)), lipsticks (Rodger et al., "The In-Situ Analysis of Lipsticks by Surface Enhanced Resonance Raman Scattering," Analyst 1823-1826 (1998)), and fibers (Thomas et al., "Raman Spectroscopy and the Forensic Analysis of Black/Grey and Blue Cotton Fibres Part 1: Investigation of the Effects of Varying Laser Wavelength," Forensic Sci. Int. 152:189-197 (2005)), as well as paint (Suzuki et al., "In Situ Identification and Analysis of Automotive Paint

Pigments Using Line Segment Excitation Raman Spectroscopy: I. Inorganic Topcoat Pigments," J. Forensic Sci. 46:1053-1069 (2001)) and ink (Mazzella et al., "Raman Spectroscopy of Blue Gel Pen Inks," Forensic Sci. Int. 152:241-247 (2005)) analysis. The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, nondestructive laser light by a solid, liquid or gas sample. Very little or no sample preparation is needed, and the required amount of tested material could be as low as several picograms or femtoliters (10^{-12} gram or 10^{-15} liter, respectively). A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material (Grasselli et al., "Chemical Applications of Raman Spectroscopy," New York: John Wiley & Sons (1981)). Unlike infrared (IR) absorption spectroscopy, another type of vibrational spectroscopy, Raman spectroscopy shows very little interference from water (Grasselli et al., "Chemical Applications of Raman Spectroscopy," New York: John Wiley & Sons (1981)), and that makes it a great technique for analyzing body fluids and their traces. Proper Raman spectroscopic measurements do not damage the sample. The stain or swab could be tested on the field and still be available for further use in the lab for DNA analysis, and that is very important to forensic application. The design of a portable Raman spectrometer is a reality now (Yan et al., "Surface-Enhanced Raman Scattering Detection of Chemical and Biological Agents Using a Portable Raman Integrated Tunable Sensor," Sensors and Actuators B. 6 (2007); Eckenrode et al., "Portable Raman Spectroscopy Systems for Field Analysis," Forensic Science Communications 3:(2001)) which would lead to the ability to make identifications at the crime scene.

[0096] The types of Raman spectroscopy suitable for use in conjunction with the present disclosure include, but are not limited to conventional Raman spectroscopy, Raman microspectroscopy, near-field Raman spectroscopy, including but not limited to the tip-enhanced Raman spectroscopy, surface enhanced Raman spectroscopy (SERS) and surface enhanced resonance Raman spectroscopy (SERRS), coherent anti-Stokes Raman spectroscopy (CARS), etc. Both Stokes and anti-Stokes Raman spectroscopy can be used.

[0097] In some embodiments, a rapid non-invasive test is performed on one or more bioprocessing intermediates using the chromatographic or spectroscopic

methods described herein. In some embodiments, the rapid test is performed using Ramon spectroscopy.

[0098] In one embodiment shown in **Fig. 6**, a harvest stream from a harvest tank **406** is delivered to a chromatography skid **504** and its accompanying chromatography column **502**. After a first chromatography step, the product stream may be passed to a SUM **506**, such as for virus inactivation, before proceeding to a filtration device **508**.

[0099] A filtration device **508** may include a variety of filtration mechanisms. In some examples, a tangential flow filtration (TFF) device is used. For example, a TFF device may enable the diafiltration of the product stream. In such an example, the TFF device may optionally be connected to at least one buffer mixture distribution manifold **116** or **120** for a buffer exchange or concentration operation. The TFF may optionally work in conjunction with a SUM **506** for recirculation and/or holding of the retentate.

[0100] In some embodiments, a TFF process consists of two stages: volume reduction and diafiltration. During the volume reduction step the bulk volume (*e.g.,* cell culture media) is filtered out through the permeate side of the filter until a desired product concentration is reached in the processing bag or holding tank. In a diafiltration stage following the volume reduction stage, the concentrated product is washed with a fluid, such as a buffer, to remove cell culture or harvest media components that are undesired or unacceptable. Further volume reduction may also be carried out after diafiltration to reach a desired product density.

[0101] The pre-viral train **500-1** may include any number of chromatography, filtration, mixing, and/or other desired devices. For example, **Fig. 6** shows a pre-viral train with three chromatography stages. However, chromatography, filtration, and/or mixing devices may each be present in any quantity, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, such as 20 or 30, each device being present in an amount independently selected.

[0102] The post-viral train **500-2** may include any number of desired finishing devices. For example, the post-viral train may include one or more final polishing chromatography and/or filtration stages in addition to or in substitute of the chromatography stages in the pre-viral train **500-1**. The critical viral reduction step is considered to be the appropriate point for spatial segregation with the post-viral

separation sub-unit to be considered essentially virus free. The post-viral separation sub-unit houses equipment and utilities suitable for any one of the following: ultrafiltration (tangential filtration), normal filtration, chromatography, formulation, titration, mixing, concentration, buffer exchange, bulk drug substance container filling and freezing.

[0103] In some examples, the post-viral train may include empty equipment stations, such as a chromatography column station **512**, a chromatography skid station **514**, a SUM station **516**, and/or a TFF device station **518**, as shown in **Fig. 6**. Similar to the bioreactor stations **314**, the various stations **512**, **514**, and **516** may provide the respective devices with the required electrical and mechanical connections for operation, as well as any necessary heated/cooled water, sterile WFI, product stream, buffer, media, filtration media, adsorbent, eluent, or any other input required by the respective devices. For example, a chromatography column station **512** may provide the necessary electrical, adsorbent, eluent, and/or product stream connections while also providing the physical floor, wall, or hanging rack space for mounting.

[0104] Similar to as discussed regarding bioreactor stations **314**, chromatography column and skid stations may be present in a filled, empty, or deactivated capacity, in any of the exemplary quantities described above, or in other quantities as desired.

[0105] Optionally, both the pre-viral and post-viral trains may include equipment installed in an associated station. For example, each chromatography column and skid in the pre-viral train **500-1** may be installed in a column station **512** and a skid station **514**, respectively. If the post-viral train also has at least one column station **512** and skid station **514**, then any of the chromatography columns or skids in the previral train **500-1** may be easily uninstalled from the pre-viral train **500-1** and quickly installed in the post-viral train **500-2**. Optionally, the pre-viral and the post-viral trains may both contain at least one empty station to enable easy and rapid insertion of additional equipment if the need arose. For example, all stations required to support a parallel pre-viral train may be housed in the pre-viral train **500-1** in preparation for future throughput requirements. In this manner, the purification subsystem may quickly and flexibly adapt to growth in phases.

[0106] The post-viral train **500-2** may also include some variety of filling device **520**. For example, a filling device **520** may be a bottle filling device, or alternatively a bag filling device, or a device filling containers generally. The device may be, in some examples, a self-contained unit, optionally with its own controlled airspace, such as an airspace controlled to ISO 5/Grade A/Class 100.

[0107] In some embodiments, a maintenance area **500-3** may be adjacent to one or both of the pre-viral and post-viral trains. When adjacent to both, airlocks **510** permit access from each train as well as from outside the purification subsystem **500**. For example the maintenance area **500-3** may be used for equipment cleaning, repair storage, or staging. For example, chromatography columns may be packed and prepared for quick insertion into either the pre-viral or post-viral trains. In some embodiments, the maintenance area **500-3** has both a chromatography column station **512** and a chromatography skid station **514**.

[0108] The descriptions of the various embodiments of the present disclosure can be utilized in the production of pharmaceuticals and biopharmaceutical products. The devices, facilities and methods described herein are suitable for culturing any desired cell line including prokaryotic and/or eukaryotic cell lines. Further, in embodiments, the devices, facilities and methods are suitable for culturing suspension cells or anchorage-dependent (adherent) cells and are suitable for production operations configured for production of pharmaceutical and biopharmaceutical products—such as polypeptide products, nucleic acid products (for example DNA or RNA), or cells and/or viruses such as those used in cellular and/or viral therapies.

[0109] In embodiments, the cells express or produce a product, such as a recombinant therapeutic or diagnostic product. As described in more detail below, examples of products produced by cells include, but are not limited to, antibody molecules (*e.g.,* monoclonal antibodies, bispecific antibodies), antibody mimetics (polypeptide molecules that bind specifically to antigens but that are not structurally related to antibodies such as DARPins, affibodies, adnectins, or IgNARs), fusion proteins (*e.g.,* Fc fusion proteins, chimeric cytokines), other recombinant proteins (*e.g.,* glycosylated proteins, enzymes, hormones), viral therapeutics (*e.g.,* anti-cancer oncolytic viruses, viral vectors for gene therapy and viral immunotherapy), cell therapeutics (*e.g.,* pluripotent stem cells, mesenchymal stem cells and adult

stem cells), vaccines or lipid-encapsulated particles (*e.g.,* exosomes, virus-like particles), RNA (*e.g.,* siRNA) or DNA (*e.g.,* plasmid DNA), antibiotics or amino acids. In embodiments, the devices, facilities and methods can be used for producing biosimilars.

[0110] As mentioned, in embodiments, devices, facilities and methods allow for the production of eukaryotic cells, for example, mammalian cells or lower eukaryotic cells such as for example yeast cells or filamentous fungi cells, or prokaryotic cells such as Gram-positive or Gram-negative cells and/or products of the eukaryotic or prokaryotic cells, for example, proteins, peptides, antibiotics, amino acids, nucleic acids (such as DNA or RNA), synthesized by the eukaryotic cells in a large-scale manner. Unless stated otherwise herein, the devices, facilities, and methods can include any desired volume or production capacity including but not limited to bench-scale, pilot-scale, and full production scale capacities.

[0111] In embodiments, the cells are eukaryotic cells (*e.g.,* mammalian cells). The mammalian cells can be for example human or rodent or bovine cell lines or cell strains. Examples of such cells, cell lines or cell strains are, for example, mouse myeloma (NSO)-cell lines, Chinese hamster ovary (CHO)-cell lines, HT1080, H9, HepG2, MCF7, MDBK Jurkat, NIH3T3, PC12, BHK (baby hamster kidney cell), VERO, SP2/0, YB2/0, Y0, C127, L cell, COS, *e.g.,* COS1 and COS7, QC1-3, HEK-293, VERO, PER.C6, HeLA, EB1, EB2, EB3, oncolytic or hybridoma-cell lines. Preferably the mammalian cells are CHO-cell lines. In one embodiment, the cell is a CHO cell. In one embodiment, the cellis a CHO-K1 cell, a CHO-K1 SV cell, a DG44 CHO cell, a DUXB11 CHO cell, a CHOS, a CHO GS knock-out cell, a CHO FUT8 GS knock-out cell, a CHOZN, or a CHO-derived cell. The CHO GS knock-out cell (*e.g.,* GSKO cell) is, for example, a CHO-K1 SV GS knockout cell. The CHO FUT8 knockout cell is, for example, the Potelligent® CHOK1 SV (Lonza Biologics, Inc.). Eukaryotic cells can also be avian cells, cell lines or cell strains, such as for example, EBx® cells, EB14, EB24, EB26, EB66, or EBv13.

[0112] In one embodiment, the eukaryotic cells are stem cells. The stem cells can be, for example, pluripotent stem cells, including embryonic stem cells (ESCs), adult stem cells, induced pluripotent stem cells (iPSCs), tissue specific stem cells (*e.g.,* hematopoietic stem cells) and mesenchymal stem cells (MSCs).

[0113] In one embodiment, the cell is a differentiated form of any of the cells described herein. In one embodiment, the cell is a cell derived from any primary cell in culture.

[0114] In embodiments, the cell is a hepatocyte such as a human hepatocyte, animal hepatocyte, or a non-parenchymal cell. For example, the cell can be a plateable metabolism qualified human hepatocyte, a plateable induction qualified human hepatocyte, plateable Qualyst Transporter Certified™ human hepatocyte, suspension qualified human hepatocyte (including 10-donor and 20-donor pooled hepatocytes), human hepatic kupffer cells, human hepatic stellate cells, dog hepatocytes (including single and pooled Beagle hepatocytes), mouse hepatocytes (including CD-1 and C57BI/6 hepatocytes), rat hepatocytes (including Sprague-Dawley, Wistar Han, and Wistar hepatocytes), monkey hepatocytes (including Cynomolgus or Rhesus monkey hepatocytes), cat hepatocytes (including Domestic Shorthair hepatocytes), and rabbit hepatocytes (including New Zealand White hepatocytes). Example hepatocytes are commercially available from Triangle Research Labs, LLC, 6 Davis Drive Research Triangle Park, N.C., USA 27709.

[0115] In one embodiment, the eukaryotic cell is a lower eukaryotic cell such as a yeast cell (*e.g.,* Pichia genus (*e.g.,* Pichia pastoris, Pichia methanolica, Pichia kluyveri, and Pichia angusta), Komagataella genus (*e.g.,* Komagataella pastoris, Komagataella pseudopastoris or Komagataella phaffii), Saccharomyces genus (*e.g.,* Saccharomyces cerevisae, cerevisiae, Saccharomyces kluyveri, Saccharomyces uvarum), Kluyveromyces genus (*e.g.,* Kluyveromyces lactis, Kluyveromyces marxianus), the Candida genus (*e.g.,* Candida utilis, Candida cacaoi, Candida boidinii), the Geotrichum genus (*e.g.,* Geotrichum fermentans), Hansenula polymorpha, Yarrowia lipolytica, or Schizosaccharomyces pombe. Preferred is the species Pichia pastoris. Examples for Pichia pastoris strains are X33, GS115, KM71, KM71H; and CBS7435.

[0116] In one embodiment, the eukaryotic cell is a fungal cell (*e.g.,* Aspergillus (such as A. niger, A. fumigatus, A. orzyae, A. nidula), Acremonium (such as A. thermophilum), Chaetomium (such as C. thermophilum), Chrysosporium (such as C. thermophile), Cordyceps (such as C. militaris), Corynascus, Ctenomyces, Fusarium (such as F. oxysporum), Glomerella (such as G. graminicola), Hypocrea (such as H. jecorina), Magnaporthe (such as M. orzyae), Myceliophthora (such as M.

thermophile), Nectria (such as N. heamatococca), Neurospora (such as N. crassa), Penicillium, Sporotrichum (such as S. thermophile), Thielavia (such as T. terrestris, T. heterothallica), Trichoderma (such as T. reesei), or Verticillium (such as V. dahlia)).

[0117] In one embodiment, the eukaryotic cell is an insect cell (*e.g.,* Sf9, Mimic™ Sf9, Sf21, High Five™ (BT1-TN-5B1-4), or BT1-Ea88 cells), an algae cell (*e.g.,* of the genus Amphora, Bacillariophyceae, Dunaliella, Chlorella, Chlamydomonas, Cyanophyta (cyanobacteria), Nannochloropsis, Spirulina, or Ochromonas), or a plant cell (*e.g.,* cells from monocotyledonous plants (*e.g.,* maize, rice, wheat, or Setaria), or from a dicotyledonous plants (*e.g.,* cassava, potato, soybean, tomato, tobacco, alfalfa, Physcomitrella patens or Arabidopsis).

[0118] In one embodiment, the cell is a bacterial or prokaryotic cell.

[0119] In embodiments, the prokaryotic cell is a Gram-positive cells such as Bacillus, Streptomyces Streptococcus, Staphylococcus or Lactobacillus. Bacillus that can be used is, *e.g.,* the B. subtilis, B. amyloliquefaciens, B. licheniformis, B. natto, or B. megaterium. In embodiments, the cell is B. subtilis, such as B. subtilis 3NA and B. subtilis 168. Bacillus is obtainable from, for example, the Bacillus Genetic Stock Center, Biological Sciences 556, 484 West 12th Avenue, Columbus Ohio 43210-1214.

[0120] In one embodiment, the prokaryotic cell is a Gram-negative cell, such as Salmonella spp. or Escherichia coli, such as, for example, TG1, TG2, W3110, DH1, DHB4, DH5a, HMS 174, HMS174 (DE3), NM533, C600, HB101, JM109, MC4100, XL1-Blue and Origami, as well as those derived from E. coli B-strains, such as for example BL-21 or BL21 (DE3), all of which are commercially available.

[0121] Suitable host cells are commercially available, for example, from culture collections such as the DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Braunschweig, Germany) or the American Type CultureCollection (ATCC).

[0122] In embodiments, the cultured cells are used to produce proteins, for example, antibodies, for example, monoclonal antibodies, and/or recombinant proteins, for therapeutic use. In embodiments, the cultured cells produce peptides, amino acids, fatty acids or other useful biochemical intermediates or metabolites. For example, in embodiments, molecules having a molecular weight of about 4000

daltons to greater than about 140,000 daltons can be produced. In embodiments, these molecules can have a range of complexity and can include posttranslational modifications including glycosylation.

[0123] In embodiments, the protein is, for example, BOTOX, Myobloc, Neurobloc, Dysport (or other serotypes of botulinum neurotoxins), alglucosidase alpha, daptomycin, YH-16, choriogonadotropin alpha, filgrastim, cetrorelix, interleukin-2, aldesleukin, teceleulin, denileukin diftitox, interferon alpha-n3 (injection), interferon alpha-n1, DL-8234, interferon, Suntory (gamma-1a), interferon gamma, thymosin alpha 1, tasonermin, DigiFab, ViperaTAb, EchiTAb, CroFab, nesiritide, abatacept, alefacept, Rebif, eptoterminalfa, teriparatide (osteoporosis), calcitonin injectable (bone disease), calcitonin (nasal, osteoporosis), etanercept, hemoglobin glutamer 250 (bovine), drotrecogin alpha, collagenase, carperitide, recombinant human epidermal growth factor (topical gel, wound healing), DWP401, darbepoetin alpha, epoetin omega, epoetin beta, epoetin alpha, desirudin, lepirudin, bivalirudin, nonacog alpha, Mononine, eptacog alpha (activated), recombinant Factor VIII+VWF, Recombinate, recombinant Factor VIII, Factor VIII (recombinant), Alphnmate, octocog alpha, Factor VIII, palifermin, Indikinase, tenecteplase, alteplase, pamiteplase, reteplase, nateplase, monteplase, follitropin alpha, rFSH, hpFSH, micafungin, pegfilgrastim, lenograstim, nartograstim, sermorelin, glucagon, exenatide, pramlintide, iniglucerase, galsulfase, Leucotropin, molgramostim, triptorelin acetate, histrelin (subcutaneous implant, Hydron), deslorelin, histrelin, nafarelin, leuprolide sustained release depot (ATRIGEL), leuprolide implant (DUROS), goserelin, Eutropin, KP-102 program, somatropin, mecasermin (growth failure), enlfavirtide, Org-33408, insulin glargine, insulin glulisine, insulin (inhaled), insulin lispro, insulin deternir, insulin (buccal, RapidMist), mecasermin rinfabate, anakinra, celmoleukin, 99 mTc-apcitide injection, myelopid, Betaseron, glatiramer acetate, Gepon, sargramostim, oprelvekin, human leukocyte-derived alpha interferons, Bilive, insulin (recombinant), recombinant human insulin, insulin aspart, mecasenin, Roferon-A, interferon-alpha 2, Alfaferone, interferon alfacon-1, interferon alpha, Avonex′ recombinant human luteinizing hormone, dornase alpha, trafermin, ziconotide, taltirelin, diboterminalfa, atosiban, becaplermin, eptifibatide, Zemaira, CTC-111, Shanvac-B, HPV vaccine (quadrivalent), octreotide, lanreotide, ancestirn, agalsidase beta, agalsidase alpha, laronidase, prezatide copper acetate (topical gel),

rasburicase, ranibizumab, Actimmune, PEG-Intron, Tricomin, recombinant house dust mite allergy desensitization injection, recombinant human parathyroid hormone (PTH) 1-84 (sc, osteoporosis), epoetin delta, transgenic antithrombin III, Granditropin, Vitrase, recombinant insulin, interferon-alpha (oral lozenge), GEM-21S, vapreotide, idursulfase, omnapatrilat, recombinant serum albumin, certolizumab pegol, glucarpidase, human recombinant C1 esterase inhibitor (angioedema), lanoteplase, recombinant human growth hormone, enfuvirtide (needle-free injection, Biojector 2000), VGV-1, interferon (alpha), lucinactant, aviptadil (inhaled, pulmonary disease), icatibant, ecallantide, omiganan, Aurograb, pexigananacetate, ADI-PEG-20, LDI-200, degarelix, cintredelinbesudotox, FavId, MDX-1379, ISAtx-247, liraglutide, teriparatide (osteoporosis), tifacogin, AA4500, T4N5 liposome lotion, catumaxomab, DWP413, ART-123, Chrysalin, desmoteplase, amediplase, corifollitropinalpha, TH-9507, teduglutide, Diamyd, DWP-412, growth hormone (sustained release injection), recombinant G-CSF, insulin (inhaled, AIR), insulin (inhaled, Technosphere), insulin (inhaled, AERx), RGN-303, DiaPep277, interferon beta (hepatitis C viral infection (HCV)), interferon alpha-n3 (oral), belatacept, transdermal insulin patches, AMG-531, MBP-8298, Xerecept, opebacan, AIDSVAX, GV-1001, LymphoScan, ranpirnase, Lipoxysan, lusupultide, MP52 (beta-tricalciumphosphate carrier, bone regeneration), melanoma vaccine, sipuleucel-T, CTP-37, Insegia, vitespen, human thrombin (frozen, surgical bleeding), thrombin, TransMID, alfimeprase, Puricase, terlipressin (intravenous, hepatorenal syndrome), EUR-1008M, recombinant FGF-I (injectable, vascular disease), BDM-E, rotigaptide, ETC-216, P-113, MBI-594AN, duramycin (inhaled, cystic fibrosis), SCV-07, OPI-45, Endostatin, Angiostatin, ABT-510, Bowman Birk Inhibitor Concentrate, XMP-629, 99 mTc-Hynic-Annexin V, kahalalide F, CTCE-9908, teverelix (extended release), ozarelix, rornidepsin, BAY-504798, interleukin4, PRX-321, Pepscan, iboctadekin, rhlactoferrin, TRU-015, IL-21, ATN-161, cilengitide, Albuferon, Biphasix, IRX-2, omega interferon, PCK-3145, CAP-232, pasireotide, huN901-DMI, ovarian cancer immunotherapeutic vaccine, SB-249553, Oncovax-CL, OncoVax-P, BLP-25, CerVax-16, multi-epitope peptide melanoma vaccine (MART-1, gp100, tyrosinase), nemifitide, rAAT (inhaled), rAAT (dermatological), CGRP (inhaled, asthma), pegsunercept, thymosinbeta4, plitidepsin, GTP-200, ramoplanin, GRASPA, OBI-1, AC-100, salmon calcitonin (oral, eligen), calcitonin (oral, osteoporosis), examorelin,

capromorelin, Cardeva, velafermin, 131I-TM-601, KK-220, T-10, ularitide, depelestat, hematide, Chrysalin (topical), rNAPc2, recombinant Factor V111 (PEGylated liposomal), bFGF, PEGylated recombinant staphylokinase variant, V-10153, SonoLysis Prolyse, NeuroVax, CZEN-002, islet cell neogenesis therapy, rGLP-1, BIM-51077, LY-548806, exenatide (controlled release, Medisorb), AVE-0010, GA-GCB, avorelin, ACM-9604, linaclotid eacetate, CETi-1, Hemospan, VAL (injectable), fast-acting insulin (injectable, Viadel), intranasal insulin, insulin (inhaled), insulin (oral, eligen), recombinant methionyl human leptin, pitrakinra subcutancous injection, eczema), pitrakinra (inhaled dry powder, asthma), Multikine, RG-1068, MM-093, NBI-6024, AT-001, PI-0824, Org-39141, Cpn10 (autoimmune diseases/inflammation), talactoferrin (topical), rEV-131 (ophthalmic), rEV-131 (respiratory disease), oral recombinant human insulin (diabetes), RPI-78M, oprelvekin (oral), CYT-99007 CTLA4-Ig, DTY-001, valategrast, interferon alpha-n3 (topical), IRX-3, RDP-58, Tauferon, bile salt stimulated lipase, Merispase, alaline phosphatase, EP-2104R, Melanotan-II, bremelanotide, ATL-104, recombinant human microplasmin, AX-200, SEMAX, ACV-1, Xen-2174, CJC-1008, dynorphin A, SI-6603, LAB GHRH, AER-002, BGC-728, malaria vaccine (virosomes, PeviPRO), ALTU-135, parvovirus B19 vaccine, influenza vaccine (recombinant neuraminidase), malaria/HBV vaccine, anthrax vaccine, Vacc-5q, Vacc-4x, HIV vaccine (oral), HPV vaccine, Tat Toxoid, YSPSL, CHS-13340, PTH(1-34) liposomal cream (Novasome), Ostabolin-C, PTH analog (topical, psoriasis), MBRI-93.02, MTB72F vaccine (tuberculosis), MVA-Ag85A vaccine (tuberculosis), FARA04, BA-210, recombinant plague FIV vaccine, AG-702, OxSODrol, rBetV1, Der-p1/Der-p2/Der-p7 allergen-targeting vaccine (dust mite allergy), PR1 peptide antigen (leukemia), mutant ras vaccine, HPV-16 E7 lipopeptide vaccine, labyrinthin vaccine (adenocarcinoma), CML vaccine, WT1-peptide vaccine (cancer), IDD-5, CDX-110, Pentrys, Norelin, CytoFab, P-9808, VT-111, icrocaptide, telbermin (dermatological, diabetic foot ulcer), rupintrivir, reticulose, rGRF, HA, alpha-galactosidase A, ACE-011, ALTU-140, CGX-1160, angiotensin therapeutic vaccine, D-4F, ETC-642, APP-018, rhMBL, SCV-07 (oral, tuberculosis), DRF-7295, ABT-828, ErbB2-specific immunotoxin (anticancer), DT3SSIL-3, TST-10088, PRO-1762, Combotox, cholecystokinin-B/gastrin-receptor binding peptides, 111In-hEGF, AE-37, trasnizumab-DM1, Antagonist G, IL-12 (recombinant), PM-02734, IMP-321, rhIGF-BP3, BLX-883, CUV-1647 (topical), L-19

based radioimmunotherapeutics (cancer), Re-188-P-2045, AMG-386, DC/1540/KLH vaccine (cancer), VX-001, AVE-9633, AC-9301, NY-ESO-1 vaccine (peptides), NA17.A2 peptides, melanoma vaccine (pulsed antigen therapeutic), prostate cancer vaccine, CBP-501, recombinant human lactoferrin (dry eye), FX-06, AP-214, WAP-8294A (injectable), ACP-HIP, SUN-11031, peptide YY [3-36] (obesity, intranasal), FGLL, atacicept, BR3-Fc, BN-003, BA-058, human parathyroid hormone 1-34 (nasal, osteoporosis), F-18-CCR1, AT-1100 (celiac disease/diabetes), JPD-003, PTH(7-34) liposomal cream (Novasome), duramycin (ophthalmic, dry eye), CAB-2, CTCE-0214, GlycoPEGylated erythropoietin, EPO-Fc, CNTO-528, AMG-114, JR-013, Factor XIII, aminocandin, PN-951, 716155, SUN-E7001, TH-0318, BAY-73-7977, teverelix (immediate release), EP-51216, hGH (controlled release, Biosphere), OGP-I, sifuvirtide, TV4710, ALG-889, Org-41259, rhCC10, F-991, thymopentin (pulmonary diseases), r(m)CRP, hepatoselective insulin, subalin, L19-IL-2 fusion protein, elafin, NMK-150, ALTU-139, EN-122004, rhTPO, thrombopoietin receptor agonist (thrombocytopenic disorders), AL-108, AL-208, nerve growth factor antagonists (pain), SLV-317, CGX-1007, INNO-105, oral teriparatide (eligen), GEM-OS1, AC-162352, PRX-302, LFn-p24 fusion vaccine (Therapore), EP-1043, S pneumoniae pediatric vaccine, malaria vaccine, Neisseria meningitidis Group B vaccine, neonatal group B streptococcal vaccine, anthrax vaccine, HCV vaccine (gpE1+gpE2+MF-59), otitis media therapy, HCV vaccine (core antigen+ISCOMATRIX), hPTH(1-34) (transdermal, ViaDerm), 768974, SYN-101, PGN-0052, aviscumnine, BIM-23190, tuberculosis vaccine, multi-epitope tyrosinase peptide, cancer vaccine, enkastim, APC-8024, GI-5005, ACC-001, TTS-CD3, vascular-targeted TNF (solid tumors), desmopressin (buccal controlled-release), onercept, and TP-9201.

[0124] In some embodiments, the polypeptide is adalimumab (HUMIRA), infliximab (REMICADE™), rituximab (RITUXAN™/MAB THERA™) etanercept (ENBREL™) bevacizumab (AVASTIN™), trastuzumab (HERCEPTIN™), pegrilgrastim (NEULASTA™), or any other suitable polypeptide including biosimilars and biobetters.

[0125] Other suitable polypeptides are those listed in Table 1 of US2016/0097074 and in Tables 1-3 of US2017/0260763.

[0126] These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the spirit

and scope of the present invention, which is more particularly set forth in the appended claims. In addition, it should be understood that aspects of the various embodiments may be interchanged either in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention so further described in such appended claims.

CLAIMS

1. A system for the growth of biologics comprising:

   a perfusion seed train or a fed-batch bioreactor seed train;

   a bioreactor subsystem comprising at least two bioreactors fed from the perfusion seed train or the fed-batch bioreactor seed train;

   at least two harvest subsystems, wherein each harvest subsystem is configured to be in fluid communication with each of the bioreactors in the bioreactor subsystem, individually or in combination; and

   at least one purification subsystem, wherein the at least one purification subsystem is configured to be in fluid communication with each of the at least two harvest subsystems individually or in combination, wherein the at least one purification subsystem is downstream from the at least two harvest subsystems.

2. A system as defined in claim 1, further comprising a flow control assembly positioned in between the at least two bioreactors and the at least two harvest subsystems, the flow control assembly being configured to selectively control flow of a fluid from a first bioreactor to a first harvest subsystem and from the first bioreactor to a second harvest subsystem, the flow control assembly also being configured to control flow of a fluid from a second bioreactor to the first harvest subsystem and from the second bioreactor to the second harvest subsystem.

3. A system as defined in claim 1, wherein the system comprises at least two purification subsystems, wherein at least one purification subsystem is configured to produce a different biologic than at least one other purification subsystem.

4. A system as defined in claim 1, configured to process more than 100 batches per year.

5. A system as defined in claim 1, wherein at least one of the bioreactors is 6000 L or larger.

6. A system as defined in claim 1, wherein the bioreactor subsystem comprises at least one fed-batch bioreactor.

7. The system as defined in claim 1, wherein the at least two harvest subsystems feed at least two purification subsystems, wherein the at least two purification subsystems are operated in parallel.

8. The system as defined in claim 1, wherein the at least two bioreactors feed the at least two harvest subsystems, wherein the at least two harvest subsystems are operated in parallel.

9. The system as defined in claim 1, wherein the at least one purification subsystem comprises at least one chromatography column station.

10. The system as defined in claim 9, wherein the at least one purification subsystem comprises three chromatography column stations.

11. The system as defined in claim 1, wherein the at least one purification subsystem comprises a pre-viral train.

12. The system as defined in claim 11, wherein the pre-viral train comprises at least one chromatography column station.

13. The system as defined in claim 1, wherein the at least one purification subsystem comprises a post-viral train.

14. The system as defined in claim 13, wherein the post-viral train comprises at least one chromatography column station.

15. The system as defined in claim 1, wherein the at least one purification subsystem comprises a pre-viral train and a post-viral train.

16. The system as defined in claim 15, wherein the pre-viral train and the post-viral train are configured such that one of the pre-viral train and the post-viral train comprises at least one chromatography column station, wherein the at least one chromatography column station comprises at least one chromatography column, wherein the at least one chromatography column is configured such that it can be transferred from the pre-viral train to the post-viral train or from the post-viral train to the pre-viral train.

17. A system for the growth of biologics comprising:

    a perfusion seed train or a fed-batch bioreactor seed train;

    a bioreactor subsystem comprising at least two bioreactors fed from the perfusion seed train or the fed-batch bioreactor seed train;

    at least two harvest subsystems, wherein each harvest subsystem is configured to be in fluid communication with each of the bioreactors in the bioreactor subsystem individually or in combination, wherein each harvest subsystem is

    downstream from the at least two bioreactors, wherein each harvest subsystem receives the effluent of the at least two bioreactors;

    at least one purification subsystem, wherein the at least one purification subsystem is configured to be in fluid communication with each of the at least two

    harvest subsystems individually or in combination, wherein the at least one purification subsystem is downstream from the at least two harvest subsystems, wherein the at least one purification subsystem is downstream from the at least two harvest subsystems, wherein the at least one purification subsystem comprises at least one chromatography column station;

    a flow control assembly positioned in between the at least two bioreactors and the at least two harvest subsystems, the flow control assembly being configured to selectively control flow of a fluid from a first bioreactor to a first harvest subsystem and from the first bioreactor to a second harvest subsystem, the flow control assembly also being configured to control flow of a fluid from a second bioreactor to the first harvest subsystem and from the second bioreactor to the second harvest subsystem; and

    wherein the bioreactor subsystem, the at least two harvest subsystems, and the at least one purification subsystem are selectively operable such that one or more subsystems are operable while one or more subsystems are not operating.

18. The system as defined in claim 17, wherein the at least one purification subsystem comprises three chromatography column stations.

19. The system as defined in claim 17, wherein the at least one purification subsystem comprises a pre-viral train.

20. The system as defined in claim 19, wherein the pre-viral train comprises the at least one chromatography column station.

21. The system as defined in claim 17, wherein the at least one purification subsystem comprises a post-viral train.

22. The system as defined in claim 21, wherein the post-viral train comprises the at least one chromatography column station.

23. The system as defined in claim 17, wherein the at least one purification subsystem comprises a pre-viral train and a post-viral train.

24. The system as defined in claim 23, wherein the pre-viral train and the post-viral train are configured such that one of the pre-viral train and the post-viral train comprises the at least one chromatography column station; wherein the at least one chromatography column station comprises at least one chromatography column, wherein the at least one chromatography column is configured such that it can be transferred from the pre-viral train to the post-viral train or from the post-viral train to the pre-viral train.