

A METHOD OF PROVIDING A HOMOGENEOUS FEED STREAM WITHIN A PLUG FLOW REACTOR

[0001] FIELD OF THE INVENTION

The present disclosure generally relates to the field of biotechnological production, more specifically to continuous bioprocessing and to a device and a method for an in-line homogenization of a feed stream within a plug flow reactor (PFR).

[0002] BACKGROUND OF THE INVENTION

In continuous bioprocessing, at least one unit operating within the manufacturing cascade receives a continuous input of a material of interest without interruption. An essential element for continuous bioprocessing is simultaneous or continuous viral inactivation (CVI), a necessary step after the appropriate chromatographic purification steps that may include bind-and-elute column chromatography such as Protein A (ProA) chromatography. For example, the product or material of interest of a variable concentration may be eluted from a ProA column in a manner represented by an asymmetric Gaussian curve primarily based on the inherent properties of the specific bind-and-elute column (Fig. 1). In the next step of the bioprocess after bind-and-elute column chromatography, attempts to homogenize the product feed stream before further analysis, either use a surge vessel such as a tank or bag; direct-transfer tubing; or alternative means such as recycling the feed stream. The following prior art publications are examples of the state of the art, which contains inherent deficiencies and drawbacks.

[0003] WO 2014/004103 teaches a system design and method for the in-line addition of a viral-inactivation reagent, such as an acid or a detergent, to a composition including a biological

product continuously. This system design and method uses one or more in-line static mixers with mixing elements to combine the biological product with a viral-inactivation reagent during flow from a first unit operation to a second unit operation.

[0004] WO 2015/158776 teaches a system design and method that uses a surge vessel to combine a viral-inactivation reagent, such as an acid or a detergent, to a composition including a biological product continuously, to inactivate viruses that may be present in the composition. In particular, blending multiple column elution cycles makes troubleshooting difficult because if a deviation from the normal operation occurred in one of the cycles, the blending process obscures the location of the deviant product. Also, surge vessels or an open circuit homogenization loops make system sanitization difficult because product retention in the system is not easily addressed.

[0005] US 2018/0117495 A1, teaches the process of continuous purification of a target biological molecule in which viruses are inactivated in-line within the continuous purification system by a method of in-line blending ProA elution peaks within an open circuit flow path where previously eluted peaks are blended backward with currently eluting peaks. This technology utilizes an open circuit homogenization loop to direct product flow through an inlet and an outlet during the mixing. This methodology minimally dampens the ProA peaks as they move through the system and aims to redistribute the acid used to lower the pH more evenly. The relative flow rates utilized in this system design and method are not ideal for scaling up the process; the flow rate during the redistribution or recycle step is 90 times faster than the incubation step.

[0006] Additional attempts to avoid surge vessels implement a tank-less hold that captures one elution cycle for a subsequent transfer of the non-uniform feedstream to the next step in the

process to a unit in which the non-uniform feed stream (with a protein or product of interest) is replaced with a non-product containing fluid thereby voiding the product to prepare for the cleaning/sanitization process required before the next cycle is captured. This strategy, however, is typically unable to produce homogenization because after repeated circulations, the feed stream remains non-uniform (Figure 11). It is well-known in the art, that a non-uniform feed stream composition results in mutable viscosity and a variable protein concentration, both of which negatively affect the downstream CVI step. Therefore, there is a need for a device or method to reduce or eliminate these complexities.

SUMMARY OF THE INVENTION

[0007] The present invention solves the above problems with the methods and devices disclosed herein, which results in faster and better homogenization of a non-uniform feed stream (e.g., from a ProA column) than the existing art.

[0008] In one or more aspects, embodiments, the invention is directed to a device for an in-line homogenization of a non-uniform feed stream. In our system, the eluted product, which will be defined as the feed stream which contains at least the product of interest, is first stored within a plug flow reactor (PFR) as a non-uniform feed stream in which the concentration of the stream is variable in that it has a maximum and minimum concentration. The contained non-uniform feed stream is then circulated until the difference between the maximum and the minimum concentration is reduced to a sufficient range, the maximum is sufficiently reduced, or the minimum is sufficiently increased.

[0009] In one embodiment, the device for in-line homogenization of a non-uniform feed stream comprises: (a) a plug flow reactor (PFR); (b) bypass line for diverting a percentage of the non-

uniform feed stream from a first location inside the PFR to a second location within the flow path; (c) a pump for circulating the non-uniform feed stream through the flow path. In a preferred embodiment the non-uniform feed stream comprises a protein or product of interest.

[0010] In another embodiment, the device is in-line placed between a first and a second process step. In an aspect, the first process step is a bind and elute column (Protein A) chromatography. In one aspect, the second process step is a virus inactivation step. In another aspect of the device, the flow path is a closed-circuit flow path. In yet another aspect, the device is tank-less

[0011] In one embodiment, the PFR comprises a tubing assembly packed in at least one chamber. The device may also include a bypass line as an extension of the flow path that fluidically connects to locations within the flow path. In one aspect, the second location is downstream from the first location. In another aspect, the second location is upstream from the first location. Alternatively, the second location is placed inside the PFR downstream or upstream from the first location. In one aspect of the device, the pump is selected from a group consisting of a centrifugal pump and /or a positive displacement pump. The device may further comprise a second pump in the flow path, wherein the second pump modulates the non-uniform feedstream flow in the bypass line. In another aspect, the second pump is a pump selected from the group consisting of a centrifugal pump and/or a positive displacement pump. In one aspect, the non-uniform feed stream flow in the bypass line is co-current or counter-current to the non-uniform feed stream flow in the flow path.

[0012] In another embodiment, a method for in-line homogenization of a non-uniform feed stream is provided, the method includes: (a) providing a non-uniform feed stream, (b) flowing the non-uniform feed stream through a flow path comprising a PFR, a bypass line, and a pump; (c) diverting a percentage of the non-uniform feed stream from a first location inside the PFR to

a second location within the flow path; (d) circulating the non-uniform feed stream through the flow path; and (e) homogenizing the non-uniform feed stream. In a preferred embodiment this method comprises a protein or product of interest. In one aspect of the method, the flow path is in-line between a first and a second process step. In an alternative aspect, the first process step is a Protein A (ProA) column chromatography. The second process step in the method can be a virus inactivation step. In an embodiment, the method is directed to virus inactivation.

[0013] In one embodiment, the flow path is a closed-circuit flow path, wherein the PFR comprises a tubing assembly packed in at least one chamber. In another aspect, the bypass line is an extension of the flow path that fluidically connects to the first and second locations within the flow path. The second location in the method can be positioned downstream from the first location, or the second location can be positioned upstream from the first location, or the second location can be positioned inside the PFR - downstream or upstream from the first location.

[0014] In another embodiment of the method, the pump is selected from a group consisting of a centrifugal pump and/or a positive displacement pump. In one aspect, the method further comprises a second pump in the flow path, wherein the second pump modulates the non-uniform feed stream flow in the bypass line. Alternatively, the method includes a second pump selected from the group consisting of: a centrifugal pump and/or a positive displacement pump.

[0015] In one embodiment, the non-uniform feed stream flow within the bypass line is co-current or counter-current to the non-uniform feed stream flow in the flow path. In one aspect, the bypass line diverts the non-uniform feed stream with a flow rate that is about 1% to about 99% of the fastest flow rate in the flow path.

[0016] In one or more embodiments the invention is directed to a method of manufacturing a product of interest by a device as defined above. The invention is also directed to a method of

manufacturing a protein by a device as defined above. In a preferred mode this protein is a therapeutic protein.

[0017] In one or more embodiments the invention is directed to a method of manufacturing a product of interest by a method for in-line homogenizing a non-uniform feed stream as defined above. The invention is also directed to a method of manufacturing a protein by a non-uniform feed stream as defined above. In a preferred mode this protein is a therapeutic protein.

[0018] Additional features and advantages of various embodiments, aspects, will be set forth, in the description that follows, and will, be apparent from the description, or may be learned by the practice of various embodiments. The objectives and other advantages of various embodiments will be realized and attained using the elements and combinations particularly pointed out in the description herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The present disclosure, its several aspects, and embodiments can be more fully understood from the detailed description, claims, and the accompanying drawings.

[0020] Fig. 1 is a graph illustrating the relative concentration of protein eluate resulting from Protein A column chromatography as represented by an asymmetric Gaussian curve based on the inherent properties of the bind and elute process. The X-axis shows the Column Volume of the elution stream, and the Y-axis shows the Relative Concentration (%) of the eluate.

[0021] Fig. 2 illustrates an exemplary plurality of plug flow reactors.

[0022] Fig. 3 illustrates the flow pattern in each of the plug flow reactors shown in Fig. 2, according to an example of the present disclosure.

[0023] Fig. 4 illustrates a relationship between Protein A chromatography columns and the flow circulation device according to an example of the present disclosure;

[0024] Figs. 5(a) and 5(b) illustrate an example of a co-current flow circulation device with two plug flow reactors for homogenizing a non-uniform concentrated feed stream composition, according to an example of the present disclosure;

[0025] Fig. 6 illustrates an alternative co-current flow circulation device with six plug flow reactors for homogenizing a non-uniform concentrated feed stream composition, according to an example of the present disclosure;

[0026] Fig. 7 illustrates a counter-current flow circulation device with six plug flow reactors for homogenizing a non-uniform concentrated feed stream composition, according to an example of the present disclosure;

[0027] Fig. 8 illustrates the experimental results of circulating material around a simple non-PFR length of tubing to homogenize a composition;

[0028] Fig. 9 illustrates the washout volume of the simple non-PFR length of tubing, for which results are shown in Fig. 8;

[0029] Fig. 10 illustrates an experimental setup for a conventional in-line circulation;

[0030] Fig. 11 illustrates the results of the experimental setup of Fig. 10;

[0031] Fig. 12 illustrates the results of the experimental setup of Figs. 6, according to an example of the present disclosure;

[0032] Fig. 13 illustrates the pH and conductivity and UV per volume relating to the experimental setup shown in Figs. 5(a) and 5(b), according to an example of the present disclosure;

[0033] Fig. 14 illustrates the protein concentration after 30 minutes of circulation using the experimental setup shown in Figs. 5(a) and 5(b), according to an example of the present disclosure; and

[0034] Fig. 15 illustrates the washout volume required to wash out the Protein using the

experimental setup shown in Figs. 5(a) and 5(b), according to an example of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

[0035] The following description is exemplary and intended to be nonlimiting to explain various embodiments of the present invention.

[0036] This disclosure is directed to a device and process for providing a thorough mixture, i.e., homogenization of a solution comprising a biotechnological product after one or more chromatographic purification steps.

[0037] So that the present invention may be more readily understood, the following terms are defined as follows. Additional definitions are also set forth throughout the detailed description.

DEFINITIONS

[0038] As used herein, the term “plug flow reactor (PFR),” also referred to as a “jig in a box (JIB)” or “circulation loop,” refers to a tubing system or assembly (that forms a flow path) compressed in a 3-D space or chamber. An embodiment of a PFR can act as an elution stream chamber (ESC) or a tank-less hold, which is used between process steps wherein the output from a process that flows through the PFR to the next process step in a purification process. An embodiment of a PFR may include two or more chambers, as shown in Fig. 2. For example, in biopharmaceutical processing, different volumes of the elution stream flowing from at least one bind and elute (Protein A (ProA)) columns a PFR may have one more chambers fluidically connected to extend the flow path of a portion or the entire volume of the feed stream. A PFR may be used as a hold in between two process steps, e.g., between the bind and elute or ProA step and Virus Inactivation step. A PFR may also be a vessel used to provide residence time for the viral inaction step. A PFR also includes a bypass line. A PFR of the present invention also

has a bypass line.

[0039] A “pump” is a device that moves fluids by mechanical action from one location, a first location, along the flow path to another location; a second location, along the flow path positionally different from the first location

[0040] As used herein, the term “Chamber” or “PFR chamber” refers to a section of a PFR’s tubing assembly. A PFR chamber may include one or several loops or rows of tubing arranged in a continuous flow path, line, conduit, or tube of predetermined certain length and volume.

[0041] In an embodiment, the PFR holds or collects the entire volume of output from a process step and enables a continuous flow of output from one process step to the next (e.g., bind and elute or ProA step and Virus Inactivation step). In another embodiment, the volume of a PFR is about six times (6x, 6-fold, or 600%) the entire product containing volume of the output from a previous process step. In another embodiment, the volume of a PFR is about 500%, about 400%, about 300%, about 200%, about 150%, about 130%, about 120%, or about 110% of the entire product containing volume of the output from a previous process step. In alternative embodiments, the volume of a PFR is about 100% of the entire product-containing volume of the output from a previous process step. In another embodiment, the volume of a PFR is no more than 95%, 85%, 75%, 65%, 55%, 50% (half), 45%, 35%, 25%, 15%, 10% or 5% of the entire product containing volume of the output from a prior process step

[0042] As used herein, the term “bypass line” in the context of a PFR refers to a tube or conduit that can divert about 1% to about 99% of the fastest fluid rate from a first location to a second location in a homogenization device of the present invention. See, for example, line **152** in Fig. 5(a) and 5 (b). The first location and the second location may be inside or outside of the PFR provided that one of the locations is inside the PFR. For example, the bypass line may divert

25% of the flow rate from a first location inside the PFR to a second location outside of the PFR within the flow path or tubing system that circulates the feed stream through the PFR.

Alternatively, the bypass line may divert from about 1% to about 99%, or from about 10% to about 90%, or about 20% to 80%, or about 25% to 75 of a flow rate in the PFR from a first location to a second location. In an example, the bypass line is an extension of the PFR flow path that short-circuits the path between the first and second location by fluidically connecting two locations within the flow path. In an embodiment, the non-uniform feed stream flow in the bypass line operates in a co-current or counter-current direction to the non-uniform feed stream flow in the flow path. In another embodiment, the bypass line includes a pump that modulates the non-uniform feed stream flow into or through the bypass line in a co-current or counter-current direction to the non-uniform feed stream flow in the flow path or PFR.

[0043] As used herein, the term “substantially homogenized” term refers to a feed stream (e.g., bind and elute or ProA eluent stream) whose concentration profile has changed from a bell-shaped curve (e.g., Fig. 1) to an almost straight line. For example, a substantially homogenized feed stream may be a feed stream in which the feed stream concentration difference between the maximum and the minimum concentration contained within the PFR is reduced to a sufficient range. In another example, a substantially homogenized feed stream may be a feed stream whose peak maximum concentration (e.g., the non-uniform ProA elution) is dampened by from about 50% to about 99%. In an embodiment, a substantially homogenized feed stream has a concentration peak max dampened by about 50%, 60%, 70%, or about 80%, or about 90%, or about 95%, or about 99% compared to the concentration peak max of the same feed stream before the homogenization.

[0044] The term “in-line” or “in-line operation” refers to a process of moving a liquid sample

through a tube or some other conduit without storage in a vessel, tank, or bag. Accordingly, in some embodiments, according to the present invention, a PFR is used in an “in-line operation” through which a liquid sample containing a target protein is moved from one process step to another.

[0045] The term “virus inactivation” or “VI” refers to the treatment of a sample potentially containing one or more viruses in a manner such one or more viruses are no longer able to replicate or are rendered inactive. Virus inactivation may be achieved by physical means, e.g., heat, ultraviolet light, ultrasonic vibration, or using chemical means, e.g., pH change or addition of a chemical virus inactivation reagent, and is a typical process step which is used during most protein purification processes, especially in case of purification of therapeutic proteins. In methods described herein, VI is performed using one or more in-line PFRs.

[0046] The term “virus inactivating agent” or “virus inactivation agent,” refers to any physical or chemical means capable of rendering one or more viruses inactive or unable to replicate, A virus inactivating agent, as used by the methods described herein may include a solution condition change (e.g., pH, conductivity, temperature, etc.) or the addition of a solvent/detergent, a salt, a polymer, a small molecule, a drug molecule or any other suitable entity, for example, which interacts with one or more viruses in a sample or a physical means (e.g., exposure to UV light, vibration, etc.), such that exposure to the virus inactivating agent renders one or more viruses inactive or incapable of replicating. In a particular embodiment, a virus inactivation agent is a pH change, where the virus inactivating agent is mixed with a sample containing a target molecule (e.g., an eluate from a Protein A bind and elute chromatography step) using a PFR.

[0047] To reach the desired product-stream pH target as the feed stream elutes from the ProA

column (given the product's inherent buffering capacity), two approaches could be implemented: (1) either the quantity of acid addition would have to change as a function of protein concentration, or (2) the fixed volumetric addition of acid would require to have enough strength to make the worst-case condition (i.e., peak maximum, where protein concentration is highest) reach the pH set point.

[0048] Alternatively or in addition, to avoid complexities associated with the product elution peak, such as variable viscosity and acid addition (i.e., the variable quantity of acid required to attain the pH set point as a function of protein concentration or fixed volume addition as a function of worst-case protein concentration), the peak concentration may be mixed such that the entire feed stream is of similar concentration and/or composition. Moreover, the feed stream should not be mixed in containers such as tanks or bags, as these vessels can't be easily sanitized, can't be completely emptied of protein elution from cycle to cycle, and have a probability of leaking the feed stream through a breach, a rupture from over pressurization, or any manner that potentially exposes the feed stream to the environment. Tanks and bags also have the complexity of containing air-liquid interfaces.

[0049] Accordingly, the device and method described herein avoids the complexities described above and homogenizes the feed stream significantly faster and more efficiently than existing systems: it can be easily sanitized, it has no air-liquid interfaces, and it is less likely to leak due to its rigid structure and design. Without wishing to be bound by any particular theory, the present invention is based, in part, on the unexpected finding that a non-uniform ProA elution feed stream (shown in Fig. 1) is effectively homogenized in-line and in a continuous and tank-less system by applying co-current and/or counter-current flows to the feed stream as it travels through one or more PFRs.

[0050] In an example, a plug flow reactor (PFR) **100** is shown in Fig. 2. The example PFR **100** shown in the figure includes six chambers **100a-100f**.

[0051] As shown in Fig. 3, each PFR **100** or a chamber thereof includes a tubular flow path **112** that includes turns or curves **110**. Details of similar PFRs are described in detail in PCT Application No. PCT/US2019/054959, the entire content of which is incorporated herein by reference. As stated earlier, although the flow path is designed such that the direction of curvature from one turn to the next changes and/or shifts to enhance radial mixing and reduce axial dispersion, complete mixing is not achieved even after the feed stream is circuited through the PFR more than 17 times. See Fig. 11.

[0052] Referring to Fig. 4, in an example, the non-uniform feed stream composition is a ProA elution stream from ProA chromatography column(s) **130**. Generally, the total volume of the PFR **100** can be from about 1 to about 6 times the volume of the ProA chromatography column(s) **130**, excluding the volume inside the stream lines, such as fluidic lines **151**, **152**, **155** and **156** (also shown in Fig. 5). The volume of elution inside the fluidic lines **151**, **152**, **155** and **156** relative to the PFR **100** can be from about 10% to 0.5%, or about 7% to 3%, or a volume selected from the group consisting of about 20%, about 15%, about 10%, about 7%, about 5%, about 3%, about 1% and about 0.5%. In an embodiment, the volume of fluidic lines **151**, **152**, **155**, and **156** is about 3%, or about 2.2%, or about 1.6% of the total volume of the PFR **100**, depending on the length of the fluidic lines.

[0053] Referring to Figs. 5(a) and 5(b), in an example, the device may also include at least a first pump **134**, an optional second pump **136** (which modulate the non-uniform feed stream flow in the bypass line **152**), a plurality of optional valves **141-148**, and a plurality of fluidic lines consecutively numbered **151-157**. In an embodiment, valves **141**, **144**, **145**, **146** and/or **147** may

be optional. In an example, each pair of valves **141** and **142**, **143** and **144** or **147** and **148** may be combined and replaced with a three-way valve. One of ordinary skill in the art appreciates that the position of valves or pumps, or their number or types is not limiting as depicted and may be arranged differently to achieve a similar result of this disclosure. Initially, to load an example, PFR **100** with a non-uniform feed stream, e.g., the ProA elution stream, from column chromatography **130**, the first set of valves **142** and **143** are switched to an open position and the second set of valves **141**, **144**, **145**, **146**, **147**, and **148** are switched to a closed position. As shown in Fig. 5(a), the ProA elution stream can then travel via feed stream line **154** and fluidic line **155** into the PFR **100** and displacing the internally held fluid out of fluidic lines **156** and **157**. [0054] Referring to Fig. 5(b), once the ProA elution stream from column chromatography **130** is contained within the PFR **100**, the first set of valves **142** and **143** are switched to a closed position and a second set of valves **141**, **144**, **145**, **146**, **147** and **148** is switched to an open position to create a closed circuit loop or flow path such that there is no inlet and outlet to the flow path. Each of the first and second pumps **134** and **136** may then circulate the ProA elution stream several times via fluidic lines **151**, **152**, **155**, and **156** such that the ProA elution stream is homogenized (*i.e.*, forming a uniform concentrated feed stream composition) by mixing at the union of stream line **151** and **152**.

[0055] In an example, as shown in Figs. 5 and 6, the device homogenizes the non-uniform feed stream (e.g., the ProA elution stream) by a co-current circulation. A co-current circulation is achieved by a bypass line (*i.e.*, stream line **152**) diverting a predefined percentage of the circulating feed stream (defined as a percent feed rate) from a first location in the flow path to a second location downstream of the first location within the closed-circuit flow path such that the result is a decreased flow rate in a portion of the stream lines.

[0056] Referring to Fig. 5(b), in an example, the PFR **100** includes two chambers in fluid communication with one another. As discussed earlier, the size of a PFR depends on the volume of the Protein A elution stream. In this example, once the first set of valves **142 and 143** are closed and the second set of valves **141, 144, 145, 146, 147 and 148** are opened, the first pump **134** pushes the ProA elution stream through the line **155** towards Chamber 2 of the PFR **100**. A bypass line **152** diverts —from about 30% to about 90%, such as from about 50% to about 80%, for example, about 75% of— the ProA elution stream from a first location in the PFR to a second location within the closed-circuit flow path which includes the PFR and lines **151, 155 and 156**. The remaining ProA elution stream continues to flow into Chamber 1 of PFR **100**, which can then exit the PFR **100** via stream lines **156 and 151** towards the first pump **134**. Before reaching the first pump **134**, the bypass line **151** merges with the stream line **152** and the ProA elution in each of the two stream lines **151 and 152** mixes. The first pump **134** then guides the combined ProA elution stream through line **155** into the PFR **100** again for yet another circulation round.

[0057] In an embodiment, the first pump **134** and the second pump **136** have different flow rates. For example, the flow rate of the second pump **136** is less than the flow rate of the first pump **134**. In another embodiment, the flow rate of the second pump **136** which is defined by being the pump which operates at a flow rate less than the flow rate of the first pump **134** is such that it can divert from about 1% to about 99% of the total flow rates in the reactors (i.e., in this particular example, the total going through the first pump **134**), such as from about 10% to about 90% of the total flow rates in the reactors, or from about 50% to about 80%, such as a flow rate of about 75% of the total flow rate in the reactors.

[0058] In an embodiment, a PFR **100** may be arranged such that, when a non-uniform

concentrated feed stream such as that shown in Fig. 1 is introduced into the PFR **100** and circulated a predetermined number of times in the closed-circuit flow path represented by fluidic lines **155**, **156**, **151**, and **152** and pump **134** such that the non-uniform concentrated feed stream becomes substantially homogenized. See Fig. 12. In an embodiment, the nonuniform concentrated feed stream is fed through the PFR of the present invention and becomes homogenized by at least 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90% or 100%.

[0059] In an example, in Fig. 6, a co-current circulating system for homogenizing a non-uniform feed stream (e.g., ProA elution feed stream) is shown. The co-current circulation method or device may be characterized by a PFR in which a bypass line **152** diverts a percentage of the fluid flow from a first location in the PFR to a second location which is downstream of the first location such that the result is a decreased flow rate in a portion of the stream lines. As shown, the first pump **134** drives the feed stream through line **151** into the PFR **100**. With the assistance of second pump **136**, the bypass line **152** diverts approximately 50% of the flow rate from a first location (e.g., first chamber) to a second location (e.g., fifth chamber) downstream of the first location in the PFR **100**. Given that 50% of the feed stream was diverted from the first chamber in the PFR **100**, the second, the third, and the fourth chambers contain only 50% of the flow rate as represented by “1x”. The diverted feed stream and the non-diverted feed stream merge in the fifth chamber in PFR **100**. The combined feed stream then flows to the sixth chamber, where the bypass line **152** again diverts the combined feed stream from chamber 1 to chamber 5 and thus repeating the above flow pattern once again. In this example, the flow rate of the first pump **134** is approximately 2 times the flow rate of the second pump **136**.

[0060] The co-current circulation process may be repeated for a sufficient number of times, such that the maximum peak concentration of the non-uniform ProA elution is dampened from

about 70% to about 90%. For example, the circulation process can repeat from about 3 times to about 20 times, such as from about 5 times to about 10 times, for example, 6 times. Alternatively, the circulation process can repeat for a predetermined amount of time, such as from about 10 minutes to about 3 hours, for example, for about 30 minutes.

[0061] In another example, as shown in Fig. 7, a counter-current circulating system for homogenizing a non-uniform feed stream (e.g., ProA elution feed stream) is shown. The counter-current circulation method or device may be characterized by a PFR in which a bypass line diverts a percentage of the fluid flow from a first location in the PFR to a second location which is upstream of the first location such that the result is an increased flow rate in a portion of the stream lines. As shown, in a counter-current example, the flow rate of the second pump **136** is defined by being the pump that operates at a flow rate less than or equal to the flow rate of the first pump **134**. With the assistance of pump **136**, the bypass line **152** diverts anywhere from about 1% to about 100% of the total flow rate from a first location (e.g., chamber 4) to a second location (e.g., chamber 1) in the PFR **100**. In an embodiment, the bypass line diverts from about 10% to about 90% of the total flow rate, or from about 50% to about 80%, or about 40%, or about 50%, or about 60%, or about 75% of the flow rate from a first location to a second location upstream of the first location in the PFR. The combined feed stream then flows through the PFR in chambers two through 4, where the bypass line **152** again diverts the combined feed stream from chamber 4, repeating the above flow pattern once again.

[0062] The counter-current circulation process may be repeated for a sufficient number of times, such that the maximum peak concentration of the non-uniform ProA elution is dampened by from about 70% to about 90%. For example, the circulation process can repeat from about 3 times to about 20 times, such as from about 5 times to about 10 times, for example, 6 times.

Alternatively, the circulation process can repeat for a predetermined amount of time, such as from about 10 minutes to about 3 hours, for example, for about 30 minutes.

[0063] Alternatively or in addition, Fig. 7 illustrates a counter-current circulation device for homogenizing a ProA elution stream exiting a column chromatography. In this example, the PFR **100** has six chambers. The first pump **134** drives the ProA elution stream into the PFR **100** at chamber one via stream line **151**. The ProA elution stream then travels from chamber 1 to chamber 4 where the bypass line **152** (with the help of the second pump **136**) diverts 1/3 (or 1X) of the maximum flow rate of the ProA elution stream from there to the second chamber to create a combined (or 3X) ProA elution stream. The combined (3X) ProA elution stream then flows into the second, third, and fourth chambers of the PFR **100**. At the end of the fourth chamber of the PFR **100**, the bypass line **152** again diverts (1X) of the elution stream back to chamber 2, and the same flow pattern repeats itself. This process repeats for a sufficient number of times, such that the maximum peak concentration of the non-uniform ProA elution is dampened by from about 70% to about 90%. For example, the circulation process can repeat from about 3 times to about 20 times, such as from about 5 times to about 10 times, for example, 6 times. Alternatively, the circulation process can repeat for a predetermined amount of time, such as from about 10 minutes to about 3 hours, for example, for about 30 minutes. In this example, the flow rate of the first pump **134** is approximately 2 times the flow rate of the second pump **136**.

[0064] In each of the co-current and counter-current circulation devices, either pump can be any combination of centrifugal and positive displacement pumps such that they both are positive displacement pump, both are centrifugal pumps, or one is centrifugal and one positive displacement. More preferably, the first pump **134** (*i.e.*, faster-operating pump) may be a

centrifugal pump and the second pump **136** (*i.e.*, slower operating pump) may be a positive displacement pump. These pumps govern the fluid dynamics of the design (*e.g.*, prevention of pressure and pump deadheading issues). Furthermore, each of the co-current and counter-current circulation devices may include a loading flow rate of at least 200 mL/min and an output flow rate into an incubation chamber of about 43 mL/min or more. Moreover, each of the co-current and counter-current circulation devices may include a loop volume of at least 3 times the volume of the chromatography column and include a maximum flow rate of about 3.5 times or more of the elution flow (*e.g.*, a flow rate of about 700 mL/min or more) or 16.3 times the flow rate relative to loop chase.

Examples

Example 1

Homogenizing a non-uniform feed stream using a tubing system

[0065] To homogenize a non-uniform feed stream, one solution would be to circulate material around a length of tubing to homogenize the composition. Mechanistically, this occurs due to the axial dispersion experienced by the flowing liquid in a tube. Fig. 8 illustrates an experiment in which protein was eluted into a length of tubing and circulated. Once the protein was circulated about 6 times, the UV A280 trace, which translates to protein concentration, becomes relatively flat. This change is indicative of complete mixing.

[0066] Once the mixing is completed, the mixed protein required a push out of the tubing and to the next unit operation in-line without significant dispersion of the contents. Due to the same mechanism that caused the mixing to occur (*i.e.*, axial dispersion), significant product tailing occurred—this ultimately led to a loss in time and product. Fig. 9 shows the washout of the mixing loop, where CV is column volume relative to that of the eluting Protein A (ProA)

column. Also, this tailing added complexity for sanitizing and rinsing the device since the quantity required would exceed 7.5 CV—further increasing the time and volume needed for the step.

Example 2

Homogenizing a non-uniform feed stream using a PFR system

As described in PCT/US2019/054959

[0067] Materials: The PFR is characterized by its repeating serpentine flow paths. The PFR was 3D printed using stereolithography (SLA) technology at 3D Systems (Rock Hill, SC). The riboflavin used in creating the mobile phases and pulse tracer was purchased through Thermo Fisher Scientific (Suwanee, GA).

[0068] To homogenize a non-uniform feed stream, another solution may be to use A PFR system such that shown in Fig. 10. The PFR was designed to disrupt axial dispersion by generating radial mixing through Dean vortices. This radial mixing is perfect for allowing the vessel to be filled and washed out to minimize time and volume requirements, but far from optimal for circulation. To confirm that a simple solution would not work, an experiment was conducted where 1 L of riboflavin was inserted into a 3 L PFR and circulated. Fig. 10 illustrates the experimental setup.

[0069] As illustrated in Fig. 11, after approximately 17 passes, peak max was only dampened by about 10%.

Example 3

Homogenizing a non-uniform feed stream using a PFR system of the present invention

[0070] In an attempt to invent a more effective means of mixing the peak in the PFR while

maintaining the ability to empty the PFR, the two designs illustrated in Figs. 6 and 7 were created.

[0071] The co-current design (Fig. 6) is characterized by the creation of a bypass line to divert a fraction of the circulating liquid for displacement farther downstream, such that the result is a decreased flow rate in a portion of the stream lines. The counter-current design (Fig. 7) is characterized by the creation of a return line to divert a fraction of the circulating liquid for displacement farther upstream, such that the result is an increased flow rate in a portion of the stream lines. Fig. 12 illustrates the result of the experiment conducted using the co-current method. The variables tested in these designs included the following: location within the closed-loop of the bypass/return line, changes/alternations between co-current and counter-current methods during the experiment, and flow rate ratio of the pumps.

[0072] The co-current design, on the other hand, generated less pressure. As for the spacing of the bypass line, equal volume distance of the bypass line was chosen since this would require two identical PFRs, as seen in Figs. 5(a) and 5(b). The faster-operating pump was a centrifugal pump and is called the primary circulating pump (PCP). The slower operating pump was a positive displacement pump called the secondary mixing pump (SMP). The selection and placement of these pumps are critical to the fluid dynamics of the design (*e.g.*, prevention of pressure and pump deadheading issues).

[0073] Testing the design shown in Figs. 5(a) and 5(b) with Protein, Figs. 13, 14, and 15 show traces from the elution peak sourced from a ProA column, the mixing within the reactors, and the subsequent washout, respectively. The ProA elution curve shown in Fig. 14 is sourced from a 1 L ProA column. The peak max of the elution peak had a high enough protein concentration such that the UV detector of the chromatography skid became saturated, and the A280 curve appears flat maxing out at ~4000 mAu. However, during the mixing portion of the operation, shown in

Fig. 14, a variable path length UV detector (FlowVPE) was used and therefore was not susceptible to saturation, so it was able to capture the 60 g/L peak max before mixing.

[0074] The key takeaway points from this set of experimental data are that a protein peak maximum of ~60 g/L was dampened to 8-14 g/L and that the subsequent protein washout took 3.8 CVs.

[0075] From the preceding description, those skilled in the art can appreciate that the present teachings can be implemented in a variety of forms. Therefore, while these teachings have been described in connection with particular embodiments and examples thereof, the true scope of the present teachings should not be so limited. Various changes and modifications may be made without departing from the scope of the teachings herein.

[0076] The scope of this disclosure is to be broadly construed. It is intended that this disclosure disclose equivalents, means, devices, and methods to achieve the devices, activities, and mechanical actions disclosed herein. For each device, article, method, mean, mechanical element, or mechanism disclosed, it is intended that this disclosure also encompasses in its disclosure and teaches equivalents, means, devices, and methods for practicing the many aspects, mechanisms, and devices disclosed herein. Additionally, this disclosure regards a coating and its many aspects, features, and elements. As such a device can be dynamic in its use and operation, this disclosure is intended to encompass the equivalents, means, devices, and methods of the use of the device and/or article of manufacture and its many aspects consistent with the description and spirit of the operations and functions disclosed herein. The claims of this application are likewise to be broadly construed.

[0077] The description of the inventions herein in their many embodiments is merely exemplary and, thus, variations that do not depart from the gist of the invention are intended to be within the

scope of the invention. Such variations are not to be regarded as a departure from the spirit and scope of the invention.