IMPROVED DEPTH FILTERS FOR DISPOSABLE BIOTECHNOLOGICAL PROCESSES

CROSS-REFERENCED TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No.: 61/571,994, filed on July 8, 2011, the entire contents of which are incorporated by reference herein.

DESCRIPTION OF THE INVENTION

Field of the Invention

[0002] In general, the present invention relates to the primary clarification of feeds. In certain specific embodiments, the invention provides a primary clarification depth filtration process of feeds, feedstreams, feedstocks, cell culture broths and the like, which utilizes a primary clarification depth filtration device without the use of a primary clarification centrifugation step or primary clarification tangential flow microfiltration step. In other embodiments, the invention provides primary clarification depth filtration process of chemically treated feeds in which the cell mass has been flocculated into larger aggregates.

Background of the Invention

[0003] Manufacturing pharmaceutical-grade biomolecules, including proteins such as monoclonal antibodies (mAbs), is a complex manufacturing process comprised of multiple filtration, centrifugation, and chromatography techniques designed to produce high quality products for patients. The clarification of cell culture harvests and high-solids feedstocks can be a daunting task due to the large volumes

of harvest from modern production batch bioreactors (≤25,000 L) and high cell densities that often require primary, as well as secondary clarification prior to the subsequent chromatography operations. And as such, harvest and clarification schemes for the production processes of cell culture harvests and high-solids feedstocks, such as mammalian cells and mAbs, are the product of much evolution and evaluation carried out over the last 20 years or so.

[0004] Harvest techniques for mammalian cell culture and mAbs are now routinely expected to operate with high yields (>95%) and minimal cell disruption. As product molecule titers have increased, the higher cell mass and larger amounts of product create challenges for the downstream purification steps. Higher cell densities result in difficulties during clarification and sterile filtration. Higher product concentrations generally result in increased impurity load and the need for larger chromatography installations. As such, improvements in the form of gains in efficiency and throughput are greatly sought after.

[0005] Increasing demand and growth therapeutic mAbs have fueled efforts towards increasing product production, quality, process efficiency, and cost-effectiveness for production of industrial therapeutic monoclonal antibodies. The past decade has witnessed considerable growth in production, upstream cell culture product titers and technical advancement in the characterization of impurities and contaminants.

[0006] Primary clarification of feeds, feedstreams, feedstocks, cell culture broths and the like, including high solids feeds, such as those containing mAbs and mammalian cell culture feedstocks, remove large amounts of biomass, particularly whole cells and other larger cellular debris, followed by secondary clarification which removes smaller colloidal particulates and other particles that impair the capacity of

downstream filters. Centrifugation is typically the primary clarification step in the production processes of mAbs and mammalian cell culture broths and feedstocks.

[0007] mAb manufacturers have invested a great deal of time and effort increasing the product titer of a feedstock. However, while higher titers increase cell culture productivity, it also produces feedstocks with larger amounts of biomass and cell debris content. Feeds containing such larger amounts of biomass and cell debris can produce high turbidity centrate after centrifugation. High turbidity centrates often reduce the throughput of the secondary clarification depth filter and the subsequent sterile filter used downstream of the centrifuge. The reduced throughput causes a range of problems from increased process cost to deviations in process procedures due to plugging of filters and long processing delays. Finally, the need for primary clarification using a centrifuge requires extensive, validated cleaning procedures between runs to attempt to reduce the risk of cross contamination between batches and therapeutic molecular species.

[0008] This is particularly problematic at pilot or clinical scale biotherapeutic production where it is desirable to process multiple products in a relatively short time. The centrifuge cleaning procedures slow down the pilot plant's ability to change over to the production of a different biomolecule and greatly increase the risk of cross contamination between production runs. In addition, centrifugation cannot efficiently remove all particulates and cellular debris from these feedstocks in the primary clarification step, hence the need for the secondary clarification step utilizing depth filtration after the centrifugation step, but prior to the subsequent chromatographic steps.

[0009] Alternatively, successive filtration runs have proven useful in removing different-sized cell and cellular debris from feedstocks, but typically the volumetric

throughputs limit the application to smaller volumes (<1000L) where the filter installation has a reasonable size. The use of filtration greatly reduces the risk of cross contamination and eliminates the need for cleaning and cleaning validation between runs due the disposable nature of filtration devices. Unfortunately, the low throughput requires a large number of filter units which can reduce filtration yields because each successive step results in the loss of a portion of the feed solution through hold-up volumes of the filter device and equipment.

[0010] In order to further enhance clarification performance, throughput and downstream filtration operations, the flocculation of a cell culture harvests have been used. Flocculants are a class of materials that can aggregate and agglutinate fine particles from a solution, resulting in their settling from the liquid phase and a reduction in solution turbidity.

[0011] Flocculation can be accomplished in a variety of ways including polymer treatment, chemical treatment (changes in pH) or the addition of a surfactant. Precipitation using flocculants can be used to selectively remove impurities while leaving the protein product in the solution. However, flocculants have not been widely used in the clarification of mAbs, mammalian cells, and other bimolecular cellular materials of interest feedstocks.

[0012] Flocculation of cell culture harvests by chemicals require the use of either acids, such as acetic acid, or polymers such as chitosan, polysaccharides, polyamines or polyacrylamides. Flocculation has been used as an alternative separation technology to enhance centrifuge clarification throughput and centrate quality, thereby improving the downstream filtration operations. While chemical flocculation is quite effective in agglomerating cellular debris and cellular contaminants (host cell proteins and DNA), the resulting flocculated suspension is

generally not easily separable by ordinary filtration methods without the use of a centrifuge prior to filtration.

[0013] Flocculants precipitate cells, cell debris and proteins because of the interaction between the charges on the proteins and charges on the polymer (e.g. polyelectrolytes), creating insoluble complexes, and subsequent bridging of insoluble complexes either by residual charge interaction or through hydrophobic patches on the complexes to form larger clusters. In order to remove these large clusters, a centrifuging step or tangential flow microfiltration is the primary mode of clarification followed by the secondary clarification step whereby depth filtration is widely used in the clarification of cell culture broth prior to the capture chromatography step. Since centrifugation cannot deliver a particle-free centrate, depth filter (secondary depth filtration) and sterile filter need to be installed further downstream.

[0014] Tangential flow microfiltration (also called cross-flow microfiltration) competes with centrifugation for the harvest and clarification of mAbs and therapeutic products from mammalian cell culture. One advantage this technique offers is the creation of a particle-free harvest stream that requires minimal additional filtration. However, tangential flow microfiltration membranes used for cell culture harvests are often plagued with the problem of membrane fouling (i.e., irrecoverable declines in membrane flux) and typically require strict complex operating condition followed by a thorough cleaning regimen (as is also the case with a centrifuge) for the membranes after each use. The use of optimized membrane chemistry, with more hydrophilic tangential flow microfiltration membranes generally being somewhat less susceptible to significant fouling, to address this issue.

[0015] Traditionally, flocculation is generally used to agglomerate non-deformable solid particles. For example, dilute suspensions of submicron sized clay or titanium

dioxide particles, which are very difficult to filter because of their small particle size, can be chemically flocculated and easily separated easily by, because the size of these submicron particles size greatly increases by the formation of agglomerated flocs., which settle more quickly, and thereby filter faster because of large flow channels inside the cake.

[0016] However, when chemical flocculation is applied to mAb feedstocks or other biomolecule/cellular feedstocks, the resulting agglomerate is unique and quite unlike the non-deformable solid particles of earth materials and metal oxides because of the nature of the biological properties of these bimolecular materials. Most solid non-deformable particles such as earth materials or metal oxides have a density much higher than water. Therefore, once these small particles are flocculated, their particle size greatly increases, and the resulting flocs quickly settle (i.e., in minutes) by gravity. In contrast, cells, mAbs and other biomolecule species are made of amino acids and water, and have a density very close to the density of water. Therefore, flocculated cells and other biomolecules don't readily settle and often take a number of hours before settling occurs.

[0017] Another problem is the relatively low density of the flocculated cell mass which, typically form a fluffy mass that occupies significant part of the feed volume rather than forming a compacted cake. Also, because of the biological origin of the particles, the flocs are fragile and tend to break down easily under pressure.

[0018] For this reason, most conventional solid-liquid separation methods while useful for solid particle systems, fail in flocculated cell masses such as mAb feedstocks.

[0019] Particle retention is believed to involve both size exclusion and adsorption through hydrophobic, ionic and other interactions. Fouling mechanisms may include

pore blockage, cake formation and/or pore constriction. Depth filters are advantageous because they are very effective in removing contaminants and come in disposable formats thereby eliminating validation and contamination issues related to re-usable hardware installations, such as those encountered when using a centrifuge. However, depth filters are currently unable to handle the high solids feedstreams that are typical of high titer mAb processes, such that depth filters are therefore often used after centrifuging. The high particulate load and high turbidity present in unclarified cell culture supernatant adds challenges to the primary clarification by depth filtration alone.

[0020] However, depth filters are currently unable to handle the primary clarification of high-solids feedstreams, and often must be used after centrifugation or tangential flow microfiltration. The high particulate load and high turbidity present in unclarified cell culture supernatant adds challenges to primary clarification by depth filtration alone. Currently, the limited throughput results in large installations of depth filters for primary clarification which results in yield losses due to the large hold up volume and scale-up issues as discussed above.

[0021] In addition, mAb feedstocks are challenging feed streams to clarify and filter because of the presence of a higher biomass content, and result in a high turbidity centrate after centrifugation. Because of the need to remove large amounts of biomass, the high turbidity centrates shorten the life of the depth filter for clarification downstream. A need exists improve the clarification of mAbs thereby resulting in higher throughputs.

[0022] In light of the above primary clarification processes which rely on the use of a primary clarification centrifugation step or a primary clarification microfiltration step followed by a secondary clarification step which relies on depth filtration media

to remove larger particles, a need exists for a disposable, reasonably reliable, and not inordinately expensive to implement, primary clarification process that does not use a primary clarification centrifugation or microfiltration step followed by an additional secondary clarification step.

SUMMARY OF THE INVENTION

[0023] In response to the above needs and problems associated with the primary clarification processes of feeds, feedstreams, feedstocks, cell culture broths and the like, the present invention overcomes the challenges by using a primary clarification depth filtration process which utilizes a primary clarification depth filtration device without the use a primary clarification centrifugation step or primary clarification tangential flow microfiltration step.

[0024] The present invention encompasses a process for the primary clarification, by depth filtration, of feeds, feedstreams, feedstocks, cell culture broths and the like, containing a target biomolecule of interest and a plurality of cellular debris and colloidal particulates without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step, the process comprising:

[0025] a) providing a depth filtration device having a porous depth filter media;

[0026] b) providing a feed stream containing a target biomolecule of interest and a plurality of cellular debris and particulates, wherein the cellular debris and particulates have a particle size distribution of about 0.5 µm to about 200 µm;

[0027] c) contacting the porous depth filter media with the feed stream, such that the depth filter media is capable of filtering cellular debris and particulates having a particle size distribution of about 0.5 µm to about 200 µm at a flow rate of about 10 litres/m^2/hr to about 100 litres/m^2/hr; and

[0028] d) separating the target biomolecule of interest from the cellular debris and particulates without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step.

[0029] The present invention further encompasses a process for the primary clarification by depth filtration of a flocculated feed containing therein a target biomolecule of interest or biotherapeutic of interest and flocculated cellular debris, materials, and colloidal particulates using a primary clarification depth filtration device without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step, the process comprising:

[0030] a) providing a depth filtration device containing a porous depth filter media;

[0031] b) providing a chemical flocculant;

[0032] c) providing a feed containing a target biomolecule of interest and a plurality of cellular material, debris and colloidal particulates;

[0033] d) combining the chemical flocculant to the feed;

[0034] e) forming chemically flocculated cellular materials, debris and colloidal particulates in the feed, and optionally chemically flocculating the target biomolecule of interest;

[0035] f) contacting the porous depth filter media with the feed containing the chemically flocculated cellular materials, debris and colloidal particulates; and

[0036] g) separating the flocculated bimolecular species of interest and the plurality of flocculated cellular material without the use of a centrifugation clarification step or a tangential flow microfiltration clarification step.

[0037] The present invention is directed towards the primary clarification of feed using depth filtration devices without the use of a primary clarification centrifugation step or primary clarification tangential flow microfiltration step. The depth filtration

devices are able to filter high solids feeds containing particles having a particle size distribution of approximately 0.5 µm to 200 µm at a flow rate of about 10 litres/m^2/hr to about 100 litres/m^2/hr until the TMP reaches 20 psi. The primary clarification depth filter media taught herein include graded porous layers of varying pore ratings.

[0038] One preferred application of the primary clarification porous anisotropic depth filters media provided herein is the primary clarification of chemically treated flocculated high solids feeds containing a bimolecular species or biotherapeutic of interest, and a plurality of flocculated cellular debris and flocculated colloidal particulates.

[0039] In certain embodiments, the invention provides a process for using a depth filtration device having a porous filter media in the primary clarification of flocculated feeds containing mAbs, mammalian cell cultures, plant cell cultures, bacteria cell cultures, insect cell cultures, and other bimolecular cellular materials and cultures of interest, by efficiently separating flocculated aggregated cellular masses and debris from the biomolecular species of interest without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step by using a fiber based porous depth filter media capable of performing depth filtration of a high volume of feedstock containing very large particles without the unintended effect of cake filtration.

[0040] In certain embodiments, the invention provides a depth filtration device including a porous depth filter media having multiple graded layers for use in primary clarification and the removal of aggregated cellular biomass, including flocculated cellular debris and colloidal particulates with a size larger than about 10 microns (µm) or smaller particles with the use of a flocculating agent.

[0041] In still other embodiments, the invention provides a depth filtration device including a porous depth filter media having open graded layers for use in primary clarification depth filtration that enables the depth filtration of cellular debris and colloidal particulates having particle sizes varying from 0.5 µm to 200 µm, thereby improving the throughput for the unclarified feedstreams without the unintended effect of cake filtration.

[0042] In certain embodiments, the present invention provides a depth filtration media:

[0043] a) having large pores for the large flocs of cellular debris to penetrate without the unintended effect of cake filtration or the formation of floc bridging inside the pores of the filter;

[0044] b) with very high depth to spread out the cell masses to prevent internal floc bridging, which would lead to internal cake filtration inside the media, external to or internal to the media in order to avoid concentration of the pressure drop which could cause floc breakdown;

[0045] c) having an anisotropic depth filter layer, i.e. with a gradual reduction in pore size that matches the population of the floc size in the feedstock. For certain feedstocks with significant amount of fine flocs such as those produced from acid flocculation instead of polymers, the depth filter layer includes a composite media having a combination of felt material, DE and chopped fibers for fine removal is needed;

[0046] d) for the primary clarification of bimolecular species of interest with mean particle sizes greater than 10um, typical of flocculant and chemically treated feed streams where the depth filter includes graded layers of non-woven fibers with open

nominal pore size ratings capable of filtering flocculated feed streams with high amount of solids;

[0047] e) of composite graded layers of non-woven fibers and cellulose/diamatoceous earth with open nominal pore size ratings capable of filtering polymer flocculant treated feed streams with high amount of solids;

[0048] f) having good retentive properties for the polymer flocculant (eg. smart polymer (SmP), chitosan etc) treated feeds despite the greater permeability;

[0049] g) having good retentive properties for the polymer flocculant (eg. acid precipitation, caprylic acid etc) treated feeds despite the greater permeability;

[0050] h) used in the process for primary clarification of cell and cell debris containing cultures using a depth filter comprising graded layers of non-woven fibers; and

[0051] i) used in the process for primary clarification of flocculated cell and cell debris containing cultures using a depth filter comprising graded layers of non-woven fibers.

[0052] In certain embodiments, the invention provides a depth filtration media prefilter for depth filters having a nominal pore size < about 25 µm comprising graded layers of non-woven fibers.

[0053] In still other embodiments, the invention provides a depth filtration media prefilter for depth filters with a nominal pore size < 25 about µm comprising at least three graded layers of non-woven fibers.

[0054] In still other embodiments, the invention provides a depth filtration media including a depth filter including at least two layers of graded non-woven fibers with a nominal pore size rating > about 25 µm capable of filtering flocculated feed streams with > about 3% solids.

[0055] In still other embodiments, the invention provides a depth filtration media including a depth filter including at least two layers graded non-woven fibers with a nominal pore size rating > about 25 µm capable of filtering polymer flocculated feed streams with > about 3% solids.

[0056] In still other embodiments, the invention provides a depth filtration media including a depth filters comprising of at least two layers of graded non-woven fibers with a nominal pore size rating > about 25 µm capable of filtering polymer flocculated feed streams with > about 3% solids resulting in a turbidity output < 20 NTU.

[0057] To overcome the present challenges associated with the depth filters, the present invention is directed towards the development of a primary clarification depth filter media and process of using the same which can filter high solid containing fluid streams having a particle size distribution of approximately 0.5 microns to 200 microns at a flow rate of about 10 litres/m^2/hr (10 LMH) to 100 litres/m^2/hr (100 LMH) until the TMP reaches 20 psi. The primary clarification depth filter includes graded layers having various pore ratings with the application in primary clarification for polymer and chemically treated flocculated feeds.

[0058] The present invention is directed towards depth filters for disposable primary clarification processes. The use of open graded layers for depth filtration enables the filtration of feeds containing large clusters with the potential to eliminate centrifugation, and enables the filtration of higher solids having particle sizes varying from about 0.5 microns to about 200 microns, thereby improving the throughput for these unclarified feedstreams.

[0059] Additional features and advantages of the invention will be set forth in the detailed description and claims, which follows. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be

apparent to those skilled in the art. It is to be understood that the foregoing general description and the following detailed description, the claims, as well as the appended drawings are exemplary and explanatory only, and are intended to provide an explanation of various embodiments of the present teachings. The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way.

BRIEF DESCRIPTION OF THE DRAWINGS

[0060] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate the presently contemplated embodiments of the invention and, together with the description, serve to explain the principles of the invention.

[0061] **Figures 1A, 1B, 1C, 1D, 1E, and 1F** depict different schematic embodiments of examples of primary clarification depth filters according to the invention, wherein Figure 1A (eight layers), Figure 1C (seven layers) and Figure 1E (eight layers) depict primary clarification depth filters for use with polymer flocculant (smart polymer) treated feeds, and Figures 1B, 1D and 1F depict primary clarification depth filters having at least eight layers for use with chemically treated feeds (acid treatment); and

[0062] Figure 2 is a schematic representation of an exemplary primary clarification depth filter purification process, as described herein. The purification process shown uses a bioreactor for cell culture followed by the following process steps: primary clarification depth filtration; Protein A bind and elute chromatography (capture); virus inactivation; flow-through purification; and formulation. As shown, each of the process steps employs one or more devices used to achieve the

intended result of the process step. As shown, clarification employs a graded clarification depth filtration as taught herein and depicted in Figures 1A to 1F; Protein A bind and elute chromatography is performed using continuous multicolumn chromatography (CMC); virus inactivation employs two in-line static mixers; flow-through purification employs activated carbon (AC) followed by anion exchange (AEX) chromatography followed by a pH change using an in-line static mixer and a surge tank followed by flow-through cation exchange (CEX) chromatography and virus filtration; and formulation employs a diafiltration/concentration tangential flow filtration device followed by sterile filtration. One or more sterile filters are also employed throughout the process.

DESCRIPTION OF THE EMBODIMENTS

[0063] All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

[0064] For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities of ingredients, percentages or proportions of materials, reaction conditions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about" whether or not explicitly indicated. The term "about" generally refers to a range of numbers that one would consider equivalent to the recited value (i.e., having the same function or result). In many instances, the term "about" may include numbers that are rounded to the nearest significant figure.

[0065] Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0066] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass all subranges subsumed therein. For example, a range of "1 to 10" includes any and all subranges between (and including) the minimum value of 1 and the maximum value of 10, that is, any and all subranges having a minimum value of equal to or greater than 1 and a maximum value of equal to or less than 10, e.g., 5.5 to 10.

[0067] Before describing the present invention in further detail, a number of terms will be defined. Use of these terms does not limit the scope of the invention but only serve to facilitate the description of the invention.

[0068] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related. The following terms are defined for purposes of the invention as described herein.

[0069] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0070] The term "bioreactor," as used herein, refers to any manufactured or engineered device or system that supports a biologically active environment. In some instances, a bioreactor is a vessel in which a cell culture process is carried out which involves organisms or biochemically active substances derived from such organisms. Such a process may be either aerobic or anaerobic. Commonly used bioreactors are typically cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel. In some embodiments described herein, a bioreactor is made of a material other than steel and is disposable or single-use. It is contemplated that the total volume of a bioreactor may be any volume ranging from 100 mL to up to 10,000 Liters or more, depending on a particular process. In some embodiments according to the processes and systems described herein, the bioreactor is connected to a unit operation such as a depth filter. In some embodiments described herein, a bioreactor is used for both cell culturing as well as for precipitation, where a precipitant may be added directly to a bioreactor, thereby to precipitate one or more impurities.

[0071] The term "cell culture," refers to cells grown in suspension, roller bottles, flasks and the like, as well as the components of the suspension itself, including but not limited to cells, cell debris, cellular contaminants, colloidal particles, biomolecules , HCP, host cell proteins (HCP) and DNA, mAbs, flocculants. Large scale approaches, such as bioreactors, including adherent cells growing attached to microcarriers in stirred fermentors, are also encompassed by the term "cell culture." Moreover, it is possible to not only to culture contact-dependent cells, but also to use the suspension culture techniques in the methods of the claimed invention.

Exemplary microcarriers include, for example, dextran, collagen, plastic, gelatin and cellulose and others as described in Butler, Spier & Griffiths, Animal cell Biotechnology 3:283-303 (1988). Porous carriers, such as, for example, Cytoline® or Cytopore®, as well as dextran-based carriers, such as DEAE-dextran (Cytodex 1®), quaternary amine-coated dextran (Cytodex 2®) or gelatin-based carriers, such as gelatin-coated dextran (Cytodex 3®) may also
be used. Cell culture procedures for both large and small-scale production of proteins are encompassed by the present invention. Procedures including, but not limited to, a fluidized bed bioreactor, hollow fiber bioreactor, roller bottle culture, or stirred tank bioreactor system may be used, with or without microcarriers, and operated alternatively in a batch, fed-batch, or perfusion mode.

[0072] The terms "cell culture medium," and "culture medium" refer to a nutrient solution used for growing animal cells, e.g., mammalian cells. Such a nutrient solution generally includes various factors necessary for cell attachment, growth, and maintenance of the cellular environment. For example, a typical nutrient solution may include a basal media formulation, various supplements depending on the cell type and, occasionally, antibiotics. In some embodiments, a nutrient solution may include at least one component from one or more of the following categories: 1) an energy source, usually in the form of a carbohydrate such as glucose; 2) all essential amino acids, and usually the basic set of twenty amino acids plus cystine; 3) vitamins and/or other organic compounds required at low concentrations; 4) free fatty acids; and 5) trace elements, where trace elements are defined as inorganic compounds or naturally occurring elements that are typically required at very low concentrations, usually in the micromolar range. The nutrient solution may optionally be supplemented with one or more components from any of the following categories:

1) hormones and other growth factors as, for example, insulin, transferrin, and epidermal growth factor; 2) salts and buffers as, for example, calcium, magnesium, and phosphate; 3) nucleosides and bases such as, for example, adenosine and thymidine, hypoxanthine; and 4) protein and tissue hydrolysates. In general, any suitable cell culture medium may be used. The medium may be comprised of serum, e.g. fetal bovine serum, calf serum or the like. Alternatively, the medium may be serum free, animal free, or protein free.

[0073] The term "cell culture additive," as used herein, refers to a molecule (e.g., a non-protein additive), which is added to a cell culture process in order to facilitate or improve the cell culture or fermentation process. In some embodiments according to the present invention, a stimulus responsive polymer, as described herein, binds and precipitates one or more cell culture additives. Exemplary cell culture additives include anti-foam agents, antibiotics, dyes and nutrients.

[0074] The terms "Chinese hamster ovary cell protein" and "CHOP," as used interchangeably herein, refer to a mixture of host cell proteins ("HCP") derived from a Chinese hamster ovary ("CHO") cell culture. The HCP or CHOP is generally present as an impurity in a cell culture medium or lysate (e.g., a harvested cell culture fluid containing a protein or polypeptide of interest (e.g., an antibody or immunoadhesin expressed in a CHO cell). Generally, the amount of CHOP present in a mixture comprising a protein of interest provides a measure of the degree of purity for the protein of interest. Typically, the amount of CHOP in a protein mixture is expressed in parts per million relative to the amount of the protein of interest in the mixture. It is understood that where the host cell is another mammalian cell type, an E. coli, a yeast cell, an insect cell, or a plant cell, HCP refers to the proteins, other than target protein, found in a lysate of the host cell.

[0075] The terms "contaminant," "impurity," and "debris," as used interchangeably herein, refer to any foreign or objectionable material, including a biological macromolecule such as a DNA, an RNA, one or more host cell proteins (HCPs or CHOPs), endotoxins, viruses, lipids and one or more additives which may be present in a sample containing a protein or polypeptide of interest (e.g., an antibody) being separated from one or more of the foreign or objectionable molecules using a stimulus responsive polymer according to the present invention. In some embodiments, a stimulus responsive polymer described herein binds and precipitates a protein or polypeptide of interest from a sample containing the protein or polypeptide of interest and one or more impurities. In other embodiments, a stimulus responsive polymer described herein binds and precipitates one or more impurities, thereby to separate the polypeptide or protein of interest from one or more impurities.

[0076] The term "surge tank" as used herein refers to any container or vessel or bag, which is used between process steps or within a process step (e.g., when a single process step comprises more than one step); where the output from one step flows through the surge tank onto the next step. Accordingly, a surge tank is different from a pool tank, in that it is not intended to hold or collect the entire volume of output from a step; but instead enables continuous flow of output from one step to the next. In some embodiments, the volume of a surge tank used between two process steps or within a process step in a process or system described herein, is no more than 25% of the entire volume of the output from the process step. In another embodiment, the volume of a surge tank is no more than 10% of the entire volume of the output from a process step. In some other embodiments, the volume of a surge tank is less than 35%, or less than 30%, or less than 25%, or less than 20%, or less

than 15%, or less than 10% of the entire volume of a cell culture in a bioreactor, which constitutes the starting material from which a target molecule is to be purified.

[0077] The term "static mixer" refers to a device for mixing two fluid materials, typically liquids. The device generally consists of mixer elements contained in a cylindrical (tube) housing. The overall system design incorporates a method for delivering two streams of fluids into the static mixer. As the streams move through the mixer, the non-moving elements continuously blend the materials. Complete mixing depends on many variables including the properties of the fluids, inner diameter of the tube, number of mixer elements and their design etc.

[0078] As used herein the term "depth filter" (e.g., gradient-density depth filter) achieves filtration within the depth of the filter material. A common class of such filters are those that comprise a random matrix of fibers bonded (or otherwise fixed), to form a complex, tortuous maze of flow channels. Particle separation in these filters generally results from entrapment by or adsorption to, the fiber matrix. The most frequently used depth filter media for bioprocessing of cell culture broths and other feedstocks consists of cellulose fibers, a filter aid such as DE, and a positively charged resin binder. Depth filter media, unlike absolute filters, retain particles throughout the porous media allowing for retention of particles both larger and smaller than the pore size. Particle retention is thought to involve both size exclusion and adsorption through hydrophobic, ionic and other interactions. The fouling mechanism may include pore blockage, cake formation and/or pore constriction. Depth filters are advantageous because they remove contaminants and also come in disposable formats thereby eliminating the validation issues.

[0079] The term "affinity chromatography matrix," as used herein, refers to a chromatography matrix which carries ligands suitable for affinity chromatography.

Typically the ligand (e.g., Protein A or a functional variant or fragment thereof) is covalently attached to a chromatography matrix material and is accessible to the target molecule in solution as the solution contacts the chromatography matrix. One example of an affinity chromatography matrix is a Protein A matrix. An affinity chromatography matrix typically binds the target molecules with high specificity based on a lock/key mechanism such as antigen/antibody or enzyme/receptor binding. Examples of affinity matrices are matrices carrying protein A ligands like Protein A SEPHAROSE™ or PROSEP®-A. In the processes and systems described herein, an affinity chromatography step may be used as the bind and elute chromatography step in the entire purification process.

[0080] The terms "ion-exchange" and "ion-exchange chromatography," as used herein, refer to the chromatographic process in which a solute or analyte of interest (e.g., a target molecule being purified) in a mixt mixture, interacts with a charged compound linked (such as by covalent attachment) to a solid phase ion exchange material, such that the solute or analyte of interest interacts non-specifically with the charged compound more or less than solute impurities or contaminants in the mixture. The contaminating solutes in the mixture elute from a column of the ion exchange material faster or slower than the solute of interest or are bound to or excluded from the resin relative to the solute of interest.

[0081] "Ion-exchange chromatography" specifically includes cation exchange, anion exchange, and mixed mode ion exchange chromatography. For example, cation exchange chromatography can bind the target molecule (e.g., an Fc region containing target protein) followed by elution (e.g., using cation exchange bind and elute chromatography or "CIEX") or can predominately bind the impurities while the target molecule "flows through" the column (cation exchange flow through

chromatography FT-CIEX). Anion exchange chromatography can bind the target molecule (e.g., an Fc region containing target protein) followed by elution or can predominately bind the impurities while the target molecule "flows through" the column, also referred to as negative chromatography. In some embodiments and as demonstrated in the Examples set forth herein, the anion exchange chromatography step is performed in a flow through mode.

[0082] The term "ion exchange matrix" refers to a matrix that is negatively charged (i.e., a cation exchange media) or positively charged (i.e., an anion exchange media). The charge may be provided by attaching one or more charged ligands to the matrix, e.g., by covalent linkage. Alternatively, or in addition, the charge may be an inherent property of the matrix (e.g., as is the case of silica, which has an overall negative charge).

[0083] The term "anion exchange matrix" is used herein to refer to a matrix which is positively charged, e.g. having one or more positively charged ligands, such as quaternary amino groups, attached thereto. Commercially available anion exchange resins include DEAE cellulose, QAE SEPHADEX™ and FAST Q SEPHAROSE™ (GE Healthcare). Other exemplary materials that may be used in the processes and systems described herein are Fractogel® EMD TMAE, Fractogel® EMD TMAE highcap, Eshmuno® Q and Fractogel® EMD DEAE (EMD Millipore).

[0084] The term "cation exchange matrix" refers to a matrix which is negatively charged, and which has free cations for exchange with cations in an aqueous solution contacted with the solid phase of the matrix. A negatively charged ligand attached to the solid phase to form the cation exchange martrix or resin may, for example, be a carboxylate or sulfonate. Commercially available cation exchange matrices include carboxy-methyl-cellulose, sulphopropyl (SP) immobilized on

agarose (e.g., SP-SEPHAROSE FAST FLOW™ or SP-SEPHAROSE HIGH PERFORMANCE™, from GE Healthcare) and sulphonyl immobilized on agarose (e.g. S-SEPHAROSE FAST FLOW™ from GE Healthcare). Preferred is Fractogel® EMD SO3, Fractogel® EMD SE Highcap, Eshmuno® S and Fractogel® EMD COO (EMD Millipore).

[0085] The term "impurity" or "contaminant" as used herein, refers to any foreign or objectionable molecule, including a biological macromolecule such as DNA, RNA, one or more host cell proteins, endotoxins, lipids and one or more additives which may be present in a sample containing the target molecule that is being separated from one or more of the foreign or objectionable molecules using a process of the present invention. Additionally, such impurity may include any reagent which is used in a step which may occur prior to the method of the invention. An impurity may be soluble or insoluble in nature.

[0086] The term "insoluble impurity," as used herein, refers to any undesirable or objectionable entity present in a sample containing a target molecule, where the entity is a suspended particle or a solid. Exemplary insoluble impurities include whole cells, cell fragments and cell debris.

[0087] The term "soluble impurity," as used herein, refers to any undesirable or objectionable entity present in a sample containing a target molecule, where the entity is not an insoluble impurity. Exemplary soluble impurities include host cell proteins (HCPs), DNA, RNA, viruses, endotoxins, cell culture media components, lipids etc.

[0088] The term "continuous process," as used herein, refers to a process for purifying a target molecule, which includes two or more process steps (or unit operations), such that the output from one process step flows directly into the next

process step in the process, without interruption, and where two or more process steps can be performed concurrently for at least a portion of their duration. In other words, in case of a continuous process, as described herein, it is not necessary to complete a process step before the next process step is started, but a portion of the sample is always moving through the process steps. The term "continuous process" also applies to steps within a process step, in which case, during the performance of a process step including multiple steps, the sample flows continuously through the multiple steps that are necessary to perform the process step. One example of such a process step described herein is the flow through purification step which includes multiple steps that are performed in a continuous manner, e.g., flow-through activated carbon followed by flow-through AEX media followed by flow-through CEX media followed by flow-through virus filtration.

[0089] In some embodiments, a depth filter, as described herein, is used for clarification, following which the clarified cell culture can continuously flow onto the next step in the purification process, e.g., a bind and elute chromatography step (e.g., Protein A affinity chromatography).

[0090] The term "semi-continuous process," as used herein, refers to a generally continuous process for purifying a target molecule, where input of the fluid material in any single process step or the output is discontinuous or intermittent. For example, in some embodiments according to the present invention, the input in a process step (e.g., a bind and elute chromatography step) may be loaded continuously; however, the output may be collected intermittently, where the other process steps in the purification process are continuous. Accordingly, in some embodiments, the processes and systems described herein are "semi-continuous" in nature, in that they include at least one unit operation which is operated in an intermittent matter,

whereas the other unit operations in the process or system may be operated in a continuous manner.

[0091] The term "connected process" refers to a process for purifying a target molecule, where the process comprises two or more process steps (or unit operations), which are in direct fluid communication with each other, such that fluid material continuously flows through the process step in the process and is in simultaneous contact with two or more process steps during the normal operation of the process. It is understood that at times, at least one process step in the process may be temporarily isolated from the other process steps by a barrier such as a valve in the closed position. This temporary isolation of individual process steps may be necessary, for example, during start up or shut down of the process or during removal/replacement of individual unit operations. The term "connected process" also applies to steps within a process step, e.g., when a process step requires several steps to be performed in order to achieve the intended result of the process step. One such example is the flow-through purification process step, as described herein, which may include several steps to be performed in a flow-through mode, e.g., activated carbon; anion exchange chromatography, cation exchange chromatography and virus filtration.

[0092] The term "fluid communication," as used herein, refers to the flow of fluid material between two process steps or flow of fluid material between steps of a process step, where the process steps are connected by any suitable means (e.g., a connecting line or surge tank), thereby to enable the flow of fluid from one process step to another process step. In some embodiments, a connecting line between two unit operations may be interrupted by one or more valves to control the flow of fluid through the connecting line.

[0093] The terms "purifying," "purification," "separate," "separating," "separation," "isolate," "isolating," or "isolation," as used herein, refer to increasing the degree of purity of a target molecule from a sample comprising the target molecule and one or more impurities. Typically, the degree of purity of the target molecule is increased by removing (completely or partially) at least one impurity from the sample.

[0094] The term "precipitate," precipitating" or "precipitation" as used herein, refers to process used in clarification, in which the properties of the undesirable impurities are modified such that they can be more easily separated from the soluble target molecule. This is typically accomplished by forming large aggregate particles and/or insoluble complexes containing the undesirable impurities. These particles have properties (e.g. density or size) such that they can be more easily separated from the liquid phase that contains the soluble target molecule, such as by filtration or centrifugation. In some cases, a phase change is effected, such that the undesirable impurities can be more easily separated from the soluble target molecule. Precipitation by phase change can be effected by the addition of a precipitating agent, such as a polymer or a small molecule. In a particular embodiment, the precipitant is a stimulus responsive polymer, also referred to as a smart polymer. In some embodiments described herein, the precipitant or precipitating agent is a flocculant. Flocculation, as used herein, is one way of performing precipitation where the performance typically depends on the flocculant concentration used ("dose dependent"). Typical flocculating agents are polyelectrolytes, such as polycations, that complex with oppositely charged impurities.

[0095] In some embodiments described herein, clarification employs the addition of a precipitant to a sample containing a target molecule and one or more impurities followed by depth filtration. In some cases, a change in solution conditions (such as

temperature, pH, salinity) may be used to initiate the precipitation, such as in the case of stimulus responsive polymers. The precipitated material which contains the one or more impurities as well as the precipitating agent is removed thereby recovering the target molecule in the liquid phase, where the liquid is then typically subjected to further process steps in order to further purify the target molecule.

[0096] Precipitation may be performed directly in a bioreactor containing a cell culture expressing a target molecule to be purified, where a precipitant is added directly to the bioreactor. Alternatively, the precipitant may be added to the cell culture, which typically contains the target molecule, in a separate vessel.

[0097] There are many ways known to those skilled in the art of removing the precipitated material, such as filtration or settling or any combinations thereof.

[0098] The term "settling," as used herein, refers to a sedimentation process in which the precipitated material migrates to the bottom of a vessel under the influence of gravitational forces. Settling can be followed by decanting or filtering of the liquid phase or supernatant.

[0099] As used herein the term "smart polymer" (SmP), (also known as stimuli-responsive polymers or intelligent polymers or Affinity Macro Ligands (AML)), as used herein means a group of polymers that are biologically, chemically, or physically responsive to an external stimulus such as to changes in environmental conditions such pH, temperature, light, ionic strength, radiation, voltage, external pressure, solvent composition, or other stimulus. Smart polymers respond with large property changes to small physical or chemical stimuli, and can reversibly change their physical or chemical properties in response to these environmental stimuli (Roy and Gupta, 2003; Kopecek, 2007). Smart polymers can take many forms; they may be dissolved in an aqueous solution, adsorbed or grafted on aqueous-solid

described herein. In some embodiments, the degree of purity of the target molecule in a sample is increased by removing (completely or partially) one or more impurities from the sample by using a stimulus responsive polymer, as described herein. In another embodiment, the degree of purity of the target molecule in a sample is increased by precipitating the target molecule away from one or more impurities in the sample.

[0129] In some embodiments, a purification process additionally employs one or more "chromatography steps." Typically, these steps may be carried out, if necessary, after the separation of a target molecule from one or more undesired entities using a stimulus responsive polymer according to the present invention.

[0130] In some embodiments, a "purification step" to isolate, separate or purify a polypeptide or protein of interest using a stimulus responsive polymer described herein, may be part of an overall purification process resulting in a "homogeneous" or "pure" composition or sample, which term is used herein to refer to a composition or sample comprising less than 100 ppm HCP in a composition comprising the protein of interest, alternatively less than 90 ppm, less than 80 ppm, less than 70 ppm, less than 60 ppm, less than 50 ppm, less than 40 ppm, less than 30 ppm, less than 20 ppm, less than 0 ppm, less than 5 ppm, or less than 3 ppm of HCP. As used herein "primary clarification" includes the removal of aggregated cellular biomass, including flocculated cellular debris and colloidal particulates with a size larger than about 10 microns (µm) or smaller particles with the use of a flocculating agent.

[0131] The terms "clarify", "clarification", "clarification step," as used herein, generally refers to one or more steps used initially in the purification of biomolecules. The clarification step generally comprises removal of cells and/or cellular debris

$$K = \left[\frac{V_f}{V_m}\right] \times \left[\frac{V_{sc}}{V_s}\right] \times \left[\frac{V_s}{V_f}\right] \times 100$$

[0166] (2)

[0167] The following examples will demonstrate the usefulness of Equations (1) and (2) and the K parameter in determining and comparing the efficiency of particle depth filters when used with particular feedstocks.

[0168] The following examples are provided so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make the compositions of the invention and how to practice the methods of the invention and are not intended to limit the scope of what the inventor regards as his invention. Efforts have been made to insure accuracy with respect to numbers used (e.g. amounts, temperature, etc.), but some experimental errors and deviation should be accounted for. Unless indicated otherwise, temperature is in degrees C, chemical reactions were performed at atmospheric pressure or transmembrane pressure, as indicated, the term "ambient temperature" refers to approximately 25°C. and "ambient pressure" refers to atmospheric pressure.

[0169] **Comparative Examples**

[0170] Settling often fails because of long settling time (hours). Disposing large volume of unconsolidated cell floc mass causes low mAb yield as a significant fraction of the mAb is trapped in the cell mass. (Refer to Example 5)

[0171] Cake filtration fails because the cell mass breaks down under pressure. In other words, cell debris break apart from the polymer under stress and flow through as high turbidity in the filtrate. (refer to Example 6)

[0172] Depth filtration fails because the flocs, particularly those from polymer systems, are quite large, in the order of 100 microns. Instead of penetrating into the

[0204] **Example 9A.**

[0205] Depth filters for the removal of aggregated and large biomolecule particulates.

[0206] A depth filtration device **10** was assembled using seven (7) layers of non-woven fibers (polypropylene) having a total thickness of all the layers of 1.6 cm. The layers are arranged in the depth filtration device from most open nominal pore size 200 µm (2 layers) followed by nominal 50 µm (2 layers), nominal 40 µm (2 layers) to a single nominal 8 µm layer (see **Figure 1**).

[0207] The individual properties of the seven (7) layers of (needle felt) non-woven fibers (Rosedale Products, Inc., Ann Arbor, MI) are shown in **Table 3** (2 layers × 200 µm, 2 layers × 50 µm, 2 layers × 40 µm, and 1 layer × 8 µm). After assembling the stack of layers, polypropylene hose barb end caps were added to the top and bottom and the entire assembly overmolded into a single, integral Mini Cap filtration device (available from Millipore Corporation, Billerica, MA USA). The depth filtration device was tested for water permeability resulting in a value of 0.45 L/min at 4 psi.

[0208] **Table 3:** Characterization of physical properties of non-woven felt material from Rosedale Products, Inc. and Midwest Filtration Company

Supplier	Layer	Nom. Pore (µm)	Av. Pore (µm)	Basis Weight (g/m2)	Water Flow Rate (gallons/min)
Rosedale	Needle Punch	200	100	425	555
Rosedale	Needle Punch	100	85	380	529
Rosedale	Needle Punch	50	70	309	524
Rosedale	Needle Punch	40	70	347	514
Rosedale	Needle Punch	30	60	320	523

Rosedale	Needle Punch	25	50	266	492
Rosedale	Needle Punch	20	40	413	330
Rosedale	Needle Punch	10	35	396	360
Rosedale	Melt Blown	8	8	288	264
Midwest	UniPro 760 PP (Needle Punch)	≥ 200	110	260	552
Midwest	Needle Punch	200	80	336	550
Midwest	Needle Punch	100	70	369	497
Midwest	Needle Punch	50	65	335	476
Midwest	Needle Punch	25	50	390	436
Midwest	Needle Punch	10	35	390	410
Midwest	Needle Punch	5	31	368	400
Midwest	Needle Punch	1	30	486	350
Midwest	UniPro 350 MM (Melt Blown)	≤ 1	15	187	338

[0209] **Example 9B.**

[0210] Depth filters for the removal of aggregated and large biomolecule particulates.

[0211] The graded depth filter of Example 9B consists of graded non-woven fibers, having a depth of 1.6 cm, and are capable of filtering an acid flocculated feed stream comprising particles in the range of about 0.5 µm to about 200 µm. The graded depth filter consists of seven (7) layers of non-woven fibers from Rosedale Products, Inc., Ann Arbor, Ml (2 layers × 200 µm, 2 layers × 100 µm, 2 layers × 50 µm, and 1 layer × 8 µm). After assembling the stack of layers, polypropylene hose barb

streams comprising particles in the range of about 0.5 µm to about 100 µm. The graded depth filter comprises six (6) layers of non-woven fibers from Midwest Filtration Company, Cincinnati, Ohio (2 × 10 µm, 2 × 5 µm, 2 × 1 µm), followed by a layer of cellulose (CE25), and a layer of diatomaceous earth (DE40). After assembling the stack of layers, polypropylene hose barb end caps were added to the top and bottom and the entire assembly overmolded into a single, integral Mini Cap filtration device (available from Millipore Corporation, Billerica, MA USA). The device was tested for water permeability resulting in a value of 0.4 L/min at 4 psi.

[0261] **Example 12.**

[0262] Filtration performance of depth filters removal of aggregated and small biomolecule particulates.

[0263] Filter devices from Examples 11A-11I were tested for filtration performance using the following method. The unclarified cell culture harvest was treated with 1M glacial acetic acid to adjust the pH to 4.8 and stirred for 30 minutes. Depth filters were run with untreated and acid treated unclarified feed after flushing out with the Milli-Q water with the TMP across each filter monitored by pressure transducers. The depth filters were first flushed with > about 50 L of Milli-Q water for each square meter of filter area at 600 $L/m^2/h$ to wet the filter media and flush out extractables. Untreated and acid precipitated unclarified harvest were loaded at 100 $L/m^2/h$ until the TMP across any one filter reached 20 psig.

[0264] Table 6 compares the filter throughput of two Millistak® filters (X0HC and D0HC) with primary clarification depth filter for the acid treated feed. X0HC and D0HC gave a throughput of 5 L/m^2 and 20 L/m^2 whereas throughput of primary clarification depth filter was 210 L/m^2.

WHAT IS CLAIMED IS:

1. A process for the clarification of a chemically flocculated feed containing a target biomolecule of interest and a plurality of cellular debris and/or colloidal particulates by depth filtration, the process comprising:

a) providing a depth filtration device having a porous depth filter media having a first non-woven fiber layer, a second non-woven fiber layer, a third non-woven fiber layer, a fourth non-woven fiber layer, a fifth non-woven fiber layer, and a sixth non-woven fiber layer, said first and second non-woven fiber layers having a pore size of 100 microns, said third and fourth non-woven fiber layers having a pore size of 50 microns, and said fifth and sixth non-woven fiber layers having a pore size of 25 microns;

b) providing a chemically flocculated feed containing a target biomolecule of interest and a plurality of cellular debris and/or colloidal particulates;

c) contacting the porous depth filter media with the chemically flocculated feed; and

d) separating the target biomolecule of interest from the cellular debris and the colloidal particulates in the chemically flocculated feed.

2. The process of claim 1, wherein the clarification process is a primary clarification process, and the porous depth filter media further comprises a seventh layer comprising cellulose and an eighth layer comprising diatomaceous earth.

3. The process of claim 2, wherein the non-woven layers have a total thickness of 0.3 cm to 3 cm.

4. The process of claim 1, wherein the cellular debris and the colloidal particulates have a particle size distribution from 0.5 μm to 200 μm, and a mean particle size greater than 10 μm.

5. The process of claim 1, wherein the target biomolecule of interest is selected from a monoclonal antibody, a polyclonal antibody, and a biotherapeutic.

6. The process of claim 1, wherein the chemically flocculated feed is generated by addition of a polymer or an acid.

7. The process of claim 6, wherein the polymer is a smart polymer.

8. The process of claim 7, wherein the smart polymer is a modified polyamine.

9. The process of claim 6, wherein the acid is acetic acid.

10. The process of claim 2, wherein the non-woven fibers comprise polypropylene, polyethylene, polyester, or nylon.

11. The process of claim 1, wherein the porous depth filter media provides throughputs > 100 $L/M^2/hr$ and removes flocculated cellular debris and colloidal particulates having a particle size distribution of 0.5 µm to 200 µm.

12. The process according to claim 1, wherein the feed is a cell culture feed located in a bioreactor and is directly loaded from the bioreactor into the depth filtration device for primary clarification processing, following addition of a polymer or acid.

13. A process according to claim 12, wherein the primary clarification processing is followed by a protein A bind and elute chromatography step for chromatographic processing.

14. A process for the primary clarification of a chemically flocculated feed including a target biomole of interest and a plurality of cellular materials and/or colloidal particulates by depth filtration without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step, the process comprising:

a) providing a depth filtration device comprising porous non-woven fiber depth filter media having a first non-woven fiber layer, a second non-woven fiber layer, a third non-woven fiber layer, a fourth non-woven fiber layer, a fifth non-woven fiber layer, and a sixth non-woven fiber layer, said first and second non-woven fiber layers having a pore size of 100 microns, said third and fourth non-woven fiber layers having a pore size of 50 microns, and said fifth and sixth non-woven fiber layers having a pore size of 25

microns;

b) providing a chemical flocculant;

c) providing a cell culture feed containing a target biomolecule of interest and a plurality of cellular materials and/or colloidal particulates;

d) adding the chemical flocculant to the cell culture feed thereby resulting in a chemically flocculated feed including flocculated cellular materials and/or flocculated colloidal particulates;

e) contacting the porous depth filter media with a chemically flocculated cell culture feed, thereby to separate the target biomolecule of interest from the flocculated cellular materials and/or flocculated colloidal particulates.

15. The process of claim 14, wherein the porous depth filter media further comprises a seventh layer comprising cellulose and an eighth layer comprising diatomaceous earth.

16. The process of claim 15, wherein the non-woven layers have a total thickness of 0.3 cm to 3 cm.

17. The process of claim 14, wherein the cellular materials and colloidal particulates have a particle size distribution from 0.5 µm to 200 µm, and a mean particle size greater than 10 µm.

18. The process of claim 14, wherein the target biomolecule of interest is selected from a monoclonal antibody, a polyclonal antibody, and a biotherapeutic.

19. The process of claim 14, wherein the chemical flocculant is a polymer or an acid.

20. The process of claim 14, wherein the chemical flocculant is a smart polymer.

21. The process of claim 20, wherein the smart polymer is modified polyamine.

22. The process of claim 20, wherein the acid is acetic acid.

23. The process of claim 14, wherein the porous depth filter media provides throughputs > 100 $L/M^2/hr$ and removes the flocculated cellular debris and flocculated colloidal particulates.

24. The process of claim 1, wherein the feed is selected from a transgenic mammalian cell culture, a bacterial cell culture, a nontransgenic mammalian cell culture, a tissue culture, a microbial fermentation batch, a plant extract, biofuel, a seawater culture, a freshwater culture, wastewater cultures, treated sewage, untreated sewage, a milk culture, a blood culture, and combinations thereof.

26. A process according to claim 14, wherein the cell culture feed is located in a bioreactor and is directly loaded from the bioreactor into the depth filtration device for primary clarification processing.

27. A process according to claim 26, wherein the primary clarification processing is followed by a protein A bind and elute chromatography step for chromatographic processing.

28. A depth filtration device for the primary clarification of a chemically flocculated feed containing a target biomolecule of interest and a plurality of cellular debris and/or colloidal particulates without the use of a primary clarification centrifugation step or a primary clarification tangential flow microfiltration step, wherein the device comprising porous depth filter media is selected from the following:

(a) from top to bottom, having two layers of non-woven fibers with a nominal pore size of 100 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 50 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 25 µm, followed by one layer of cellulose and one layer of diatomaceous earth;

(b) from top to bottom, having 2 layers of non-woven fibers with a nominal pore size of 25 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 10

µm, followed by 2 layers of non-woven fibers with a nominal pore size of 5 µm, followed by one layer of cellulose and one layer of diatomaceous earth;

(c) from top to bottom, having 2 layers of non-woven fibers with nominal pore size of 200 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 100 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 50 µm, followed by one layer of non-woven fibers with a nominal pore size of 8 µm;

(d) from top to bottom, having 2 layers of non-woven fibers with nominal pore size of 50 µm, followed by 2 layers of non-woven fibers with nominal pore size of 25 µm, followed by 2 layers of non-woven fibers with a nominal pore size of 10 µm, followed by one layer of cellulose and one layer of diatomaceous earth;

(e) from top to bottom, having 2 layers of non-woven fibers with a nominal pore size of 100 µm, followed by 2 layers of non-woven fibers of 50 µm, followed by 2 layers of non-woven fibers with a nominal pore size 25 µm, followed by one layer of diatomaceous earth and one layer of cellulose; and

(f) from top to bottom, having 2 layers of non-woven fibers with a nominal pore size of 35 µm, followed by 2 layers of non-woven fibers of 15 µm, followed by 2 layers of non-woven fibers with a nominal pore size 10 µm, followed by one layer of diatomaceous earth and one layer of cellulose.

29. The device of claim 28, wherein the non-woven fibers comprise polypropylene, polyethylene, polyester, or nylon.

30. The device of claim 28, wherein the cellular debris and the colloidal particulates have a particle size distribution from 0.5 µm to 200 µm, and a mean particle size greater than 10 µm.

31. The device of claim 28, wherein the media provides throughputs > 100 $L/M^2/hr$ and removes flocculated cellular debris and colloidal particulates having a particle size distribution of 0.5 µm to 200 µm, following addition of a chemical flocculant to the feed.

32. The device of claim 31, wherein the feed has > 3% solids resulting in a turbidity output < 20 NTU when a chemical flocculant is added to the feed.

33. The device of claim 28, wherein the target biomolecule of interest is selected from a monoclonal antibody, a polyclonal antibody, and a biotherapeutic.