UTILIZING MAGNETIC SEPARATION PROCESS TECHNOLOGY TO CAPTURE TARGET COMPOUNDS

CROSS REFERENCE TO OTHER APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/566,778 entitled UTILIZING A MAGNETIC SEPARATOR TO CAPTURE PROTEINS OF INTEREST filed March 18, 2024, which is incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

[0002] Separations and purification of complex molecules from crude production streams is generally a complicated, difficult, and expensive task, especially in settings of industrial-scale and cost-competitive production.

[0003] While small and simple molecules can often be separated and purified with very few and very efficient steps such as solvent extraction, crystallization or distillation, to name a few, more complex molecules and mixtures often require a cascade of steps that gradually increase the purity of the target compound.

[0004] The required purity of a target compound is dictated by its end-use application and intended price point. A broad range of industries demand high purity and functionality levels for target compounds, and thus require stringent separations and purification protocols to meet such standards.

[0005] Solid-phase adsorption (chromatography being a prime example) is among the highest fidelity purification techniques available capable of achieving high purity and functionality target compounds. However, adsorbent-based processes like chromatography tend to be expensive especially at industrial scales due to volumetric constraints of packed bed designs, expensive solid-phases, or adsorbents, and the need for batch-based processing. In particular, packed-bed designs are susceptible to clogging by accumulation of suspended solid debris. These shortcomings lead to long processing times and onerous material costs. Although chromatography is a gold-standard method for reliably isolating target compounds, it is for these reasons often

restricted in its feasibility in industrial applications. In cases where chromatography is nonetheless necessary to achieve desired target compound purity or functionality, the associated costs can drive up production expenses to the point where entry into bulk commodity markets becomes uneconomical.

[0006] A number of techniques, such as simulated moving bed, expanded bed-, annular-, or membrane chromatography, have been developed and are available to alleviate some of these constraints. However, there exists no chromatographic process technology that provides an ideal cost profile for large scale purification of process streams.

[0007] There is a lack of available adsorbent process technologies that accomplish high throughput treatment of a complex mixture and efficient and cost-effective target compound isolation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Various embodiments of the invention are disclosed in the following detailed description and the accompanying drawings.

[0009] FIG. 1 is a block diagram illustrating a system to capture target compounds utilizing magnetic separator process technology in accordance with some embodiments.

[0010] FIG. 2 is a diagram illustrating a binding stage in accordance with some embodiments.

[0011] FIG. 3 is a diagram illustrating a capture stage in accordance with some embodiments.

[0012] FIG. 4 is a diagram illustrating a portion of an elution stage in accordance with some embodiments.

DETAILED DESCRIPTION

[0013] The invention can be implemented in numerous ways, including as a process; an apparatus; a system; and/or a composition of matter. In this specification, these implementations, or any other form that the invention may take, may be referred to as techniques. In general, the order of the steps of disclosed processes may be altered within the scope of the invention.

[0014] A detailed description of one or more embodiments of the invention is provided below along with accompanying figures that illustrate the principles of the invention. The invention is described in connection with such embodiments, but the invention is not limited to any embodiment. The scope of the invention is limited only by the claims and the invention encompasses numerous alternatives, modifications and equivalents. Numerous specific details are set forth in the following description in order to provide a thorough understanding of the invention. These details are provided for the purpose of example and the invention may be practiced according to the claims without some or all of these specific details. For the purpose of clarity, technical material that is known in the technical fields related to the invention has not been described in detail so that the invention is not unnecessarily obscured.

[0015] Adsorptive magnetic separation for the isolation of target compounds from a complex mixture is an alternative adsorptive approach to currently available conventional chromatographic techniques. The technology involves a target compound binding to, and eluting from, a magnetic adsorbent. The target compound-laden magnetic adsorbents are then removed from the complex mixture using a magnetic capture step. The approach can potentially alleviate the restriction of packed beds that limit throughput and are susceptible to clogging by suspended solids contained in complex mixture input streams.

[0016] Although conventional, non-magnetic industrial chromatography is a gold-standard method for reliably isolating target compounds, its high material costs and limited volumetric throughput often restrict its feasibility in industrial applications. In cases where chromatography is required, the associated costs can increase production

expenses to the point where entry into bulk commodity markets becomes impractical. One example for this situation is industrial biotechnology, which includes the bulk production of enzymes, recombinant proteins, biopolymers, and other biochemicals. Herein, microbial hosts act as "cell factories" to produce organic molecules in fermentation tanks. In such processes, microorganisms (often bred or genetically modified for optimal suitability) are grown and cultivated (fermented) to efficiently produce complex target compounds from simple inputs. Once produced, target compounds must be isolated from the fermentation broth, a complex mixture which, in addition to the target compounds, comprises the aforementioned inputs, the microorganisms and their disintegration parts, their metabolic waste, byproducts and water. Separations and purification unit operations are a key driver of production costs, especially in cases where a high-degree purity and functionality of the product is desired by the consumer or required by regulators. While chromatography is often viewed as the most reliable means of achieving such purity standards, it is nonetheless viewed as a purification step of last resort due to the onerous cost burden. However, even in cases where separations and purifications cascades do not require chromatography to reach purity and functionality standards, the cumulative cost burden of extant unit operations– which can include homogenizers, centrifuges, microfiltration membranes, ultrafiltration membranes, nanofiltration membranes, precipitation, flocculation, aqueous two-phase systems, and chromatography–is nonetheless substantial. Thus, a lack of techno-economically optimal separations and purification technologies represents a significant bottleneck in industrial biotechnology, as well as across a range of other industrial applications.

[0017] Magnetic separation is a versatile laboratory method widely used at small-scales in the life sciences industry for the isolation of target compounds like DNA/RNA, proteins, and cells. Attempts to translate the substantial benefits of magnetic separations from lab-scale to and implement it at industrial scale have been sparse so far and of limited success. In order to allow enough time for the target compound to interact with and bind to the adsorbent surface, industrially-geared magnetic separations approaches have historically employed very small particles (nanoparticles or small microparticles) since they offer inherently quicker binding, or short residence

times, in a given vessel. However, the use of such small particles, which due to their size exhibit very low magnetic moments, necessitates high magnetic field gradients to effectively separate the adsorbents from crude production stream. This means that academia as well as industry has historically looked to high gradient magnetic separators (HGMS) to reliably achieve target compound isolation in industrial settings. Such necessity for high gradients can only be accomplished with very strong magnets. And given the expense and physical limitations of such electromagnets (as well as strong permanent magnetic arrangements), they are in practice either confined to relatively small sizes or require excessive machinery to be implemented (as applied in the mining industry). When one contrasts the volumetric possibilities of HGMS and the required machinery with the potential range of applications, wherein volumetric requirements for industries like chemicals, industrial biotechnology, and industrial refining regularly exceed 10s or even 100s of m^3 per hour, it becomes clear that the applicability of high-gradient approaches is fundamentally limited.

[0018] To achieve high volumetric throughput capacity with reasonably sized equipment, an optimal magnetic separation process should operate substantially continually. HGMS systems, coupled with nanoparticles, are not suited to accomplish substantially continual magnetic separation. Such systems require adsorbent to be separated from the fluid they are contained in after each sub-process step (e.g. after binding, washing, elution, regeneration, conditioning). Small magnetic particles such as nanoparticles or even small microparticles have to be actively captured to achieve such a separation within reasonable timeframes. This means active (magnetic) separation devices would be needed for most intended changes in (chemical) environment drastically exacerbating the (for HGMS systems already unfavorable) machinery requirements. The result is that currently no magnetic separations technology exists which can substantially continually process crude production streams using simple and economical equipment. Furthermore, HGMS systems contain magnetizable matrix materials (e.g. steel wool) which make proper cleaning, sterilization, and maintenance complicated. This is especially so in settings where complex (often biological) input streams interface with strict requirements of cleanliness. As a consequence, a magnetized volume –embodying an HGMS chamber, or vessel– of 0.1 m^3 is considered

by practitioners to be a large instantiation, which is again incompatible with throughput requirements encountered in industries like chemicals, industrial biotechnology, and industrial refining.

[0019] Systems and methods to capture target compounds utilizing magnetic separation process technology are disclosed herein. The systems and methods disclosed herein may be able to accommodate high volumetric flow rates while employing granular magnetic adsorbents. In some embodiments, the target compound is isolated from a complex mixture of where one or a set of compounds is a target compound. In some embodiments, the target compound is a protein, an enzyme, a nucleic acid or another complex organic molecule (e.g., alkaloids, antibiotics, lipids, vitamins, etc.), a synthetic oligo- or polymer or a metal ion (e.g., Lithium). Many such target compounds are produced and purified in large quantities and their production could be substantially simplified and economically improved by an adsorbent based purification process technology with commensurate throughput capacity. Furthermore, the typically fragile nature of target compounds such as enzymes and proteins command swift processing of a complex mixture, such as fermentation broth, usually within a matter of several hours whereas the volume of complex mixtures to be treated frequently ranges from roughly 5 to 2000 m^3 and more. The systems and methods disclosed herein are especially well suited to accommodate a volumetric throughput in the range of 0.25 m^3 up to 250 m^3 per hour. The systems and methods disclosed herein may be designed for other volumetric throughputs.

[0020] US 2006/0016732 describes a process that relies on nanoparticles that have to be separated with a high gradient magnetic field. However, such a field can only be generated with electromagnets within comparatively small volumes (e.g. up to ~0.1 m^3). It also necessitates the use of a dedicated chamber and discontinuous (cyclical) operation. The combination of these two constraints limits the volumetric throughput capabilities of the process to a maximum of several hundred liters per hour. This is incompatible with the volumetric throughputs required by many industrial processes for instance in industrial biotechnology, industrial chemistry, molecular farming or direct metal ion extraction from complex brines.

[0021] US 7,291,272 describes a process that is designed to purify an incoming feed liquid (unpurified water) from contaminants to yield a more pure liquid (purified water) deprived of contaminants. However, the process lacks two very important features: a step that is designed to isolate the particles from the treated liquid in a sufficiently concentrated manner so as to yield a sufficiently concentrated stream of target compound as well as a desorption stage where the compounds bound to the particles are selectively desorbed to yield a purified target compound stream separate from an individual impurity stream.

[0022] The systems and methods disclosed herein are designed to accommodate a substantially continual inflow of target compound-laden complex mixture while, in turn, keeping magnetic adsorbents contained in the process by continually cycling (repeatedly with or without short interruptions) through the various process stages. Conceptually, the two streams are interacting (are brought into contact) with each other in the binding stage(s) by adding target compounds to the inflow and are actively separated from each other by a magnetic separator. In some embodiments, the complex mixture is a fermentation broth or cell culture broth, chemical reaction mixture, liquid plant cell homogenate, food (such as egg and dairy) or juice streams, saltwater brine, biotechnological- or industrial waste streams, etc. The systems and methods disclosed herein are designed to be highly tolerant to suspended solid debris and other particulate contaminant material. The active magnetic separator ensures that high volumetric flow rates of adsorbents can be maintained while limiting the amount of contamination of the adsorbent flow with the solution they are extracted from as compared to systems utilizing passive separation via sedimentation (as described in US 7,291,272) or active adsorbent capture via other methods such as filtration.

[0023] The systems and methods disclosed herein utilize magnetic adsorbents that are tailored such that target compounds adsorb to these adsorbents under binding conditions after which the target compounds can be desorbed to isolate a purified and preferably concentrated solution. The systems and methods disclosed herein consist of three process stages: binding, capture, and elution. The stages are designed in a way that allows magnetic adsorbents to be cycled between these process stages and

optional additional process stages without leaving the system. The systems and methods disclosed herein are designed such that no more than one active magnetic capture step is necessarily required to afford the intended advantages described herein while keeping the magnetic adsorbents cycling continually through and within the bounds of the system. This requires the adsorbents to possess distinct physical and chemical properties such as the ability to passively settle in liquid environments with high terminal velocity, the ability to be efficiently separated from the liquid they are contained in by the application of comparatively low magnetic fields (in contrast to high gradient magnetic separation) as well as a large internal surface area to which target compounds can adsorb with desired specificity and affinity and from which target compounds can be selectively desorbed. In some embodiments, the adsorbents are subject to a magnetic field have a strength between 0.01 T and 1.5 T.

[0024] In the binding stage, a substantially continual complex mixture inflow containing a target compound is brought into contact with such magnetic adsorbents (e.g., magnetic core shell microparticles with a magnetic core and a porous functional shell with high binding capacity preferably with selectivity towards the target compounds under the applied process conditions) in a vessel, preferably in a continuously stirred tank reactor or a plug flow reactor, and the target compounds adsorb to the magnetic adsorbents.

[0025] In the capture stage, target compound-laden adsorbents are actively and substantially continually captured by a magnetic separator separating the inflowing mixture into an adsorbent sludge with low liquid content and a target compound-depleted complex mixture stream without adsorbents. These two output streams are diverted to different stages of the process: the adsorbent sludge enters the elution stage and the depleted complex mixture is either fully or partially ejected as waste or fully or partially re-enters the process at the binding stage or at a second binding stage or fully or partially re-enters the process that produces the untreated complex mixture inflow.

[0026] In an optional binding stage, the complex mixture depleted of the target compound is brought into contact with fresh magnetic adsorbents coming from the final

(elution and regeneration) stage of the process. Remaining target compound in the depleted complex mixture is allowed to bind to the fresh magnetic adsorbents. The vessel allows the passive separation of adsorbents which, via their settling properties, accumulate at the bottom and are diverted back into the first binding stage whereas the fully depleted complex mixture is diverted as overflowing spent complex mixture.

[0027] In the elution stage, impurities that are loosely (e.g. with lower affinity than the target compounds) bound to the magnetic adsorbents, can be desorbed and removed in an optional washing counter current contactor. In this process step, the adsorbents ejected from the active magnetic separator are added to a washing solution that allows the selective desorption of non-target compounds that co-adsorbed to the adsorbents in the binding stage and would lower the purity of the target compound when co-eluted in the subsequent target compound desorption vessel. A concentrated impurities solution is withdrawn at the top of the vessel whereas a concentrated washed adsorbent is continually withdrawn from the bottom of the counter current contactor.

[0028] In the elution stage, the target compound loaded adsorbents from the capture stage are fed into a counter current contactor with an elution solution that allows the target compound to selectively desorb from the adsorbent shell while impurities are still bound to the shell. The counter current setup allows for a high concentration of the target compound solution to be withdrawn from the top of the counter current contactor in a purified form for storage, whereas eluted adsorbents (i.e., a sludge made up of magnetic adsorbents with bound impurities) are drawn from the bottom.

[0029] The adsorbent sludge withdrawn from the bottom of the target compound desorption vessel in the elution stage then enters the regeneration sector of the elution stage where, utilizing a regenerant solution, remaining impurities are desorbed and the adsorbents settle, through a quiescent zone, into a storage solution. This storage solution- adsorbent sludge is then substantially continually pumped either to an adsorbent reservoir, from which the adsorbents are brought into the next cycle or directly back into the binding stage of the process.

[0030] Thus, a dedicated separation solution using magnetism is disclosed herein to enable the isolation of target compounds from a complex mixture at high volumetric throughputs. This may require at least two components: a magnetic separation unit which can extract and separate magnetic adsorbents from a complex mixture, and magnetic adsorbents. This enables 1.) the selective binding of given target compounds contained in the complex mixture to the adsorbents and 2.) the target compound to be separated in the magnetic separator before being released, or desorbed, from the adsorbents in a purified form. This separation solution employs adsorbents that can be chosen based on the individual properties of the target compounds. In addition to providing a potentially competitive cost profile and large volumetric throughput capabilities, the ability of the system disclosed herein to utilize tailored adsorbents minimizes the need to design entirely new separation and purification configurations for different target compounds produced in a given facility.

[0031] FIG. 1 is a block diagram illustrating a system to capture target compounds utilizing a magnetic separator process technology in accordance with some embodiments. In the example shown, system 100 includes vessel 102 (e.g., stainless steel tank) that includes an agitator. In some embodiments, a microbial fermentation process is undertaken in vessel 102. In some embodiments, vessel 102 is a storage vessel for temporarily storing a complex mixture, also referred to throughout as a fluid, a liquid or a broth. In some embodiments, the complex mixture can be received from a fermentation process carried out in a different vessel or from a different unit operation that has previously processed the complex mixture (e.g. from a filtration process, a centrifugation process). The microorganisms produce large quantities of a target compound (single compounds or a combination of target compounds such as proteins, enzymes, nucleic acids or another complex organic molecules such as alkaloids, antibiotics, lipids, vitamins, etc.), which accumulate in a fermentation broth. Preferably these target compounds are accumulating in the extracellular medium. Intracellularly produced target compounds can be liberated by lysing the cells to form a lysate.

[0032] The complex mixture input containing the target compound as well as a host of unwanted impurities is provided to vessel 108 and selected magnetic adsorbents

are added to the complex mixture input. The selected magnetic adsorbents are provided to vessel 108 preferably from an adsorbent reservoir 104 via vessel 106. The magnetic adsorbents stored in adsorbent reservoir 104 have an inherent magnetic moment (e.g., an average volumetric saturation magnetization between 480 A/m and 480,000 A/m). They also have an enhanced sedimentation velocity, due to aggregation induced by intrinsic magnetic properties. The adsorbents are large enough to have strong magnetic properties and to exhibit sufficient settling properties. The strong magnetic properties are utilized during adsorbent capture and preferably, on drum washing, while the settling behavior is crucial for high throughput counter current contactors that are used in the binding stage as well as the elution stages of the process, minimizing losses to the waste and keeping virtually 100% of the adsorbents in the circular process.

[0033] The target compounds adsorb (bind) to particular magnetic adsorbents with selected specificity. In some embodiments, vessel 108 is a continuously stirred tank reactor (a vessel which enables a substantially continual flow of reactants to be inputted, mixed, and outputted) and vessel 106 is a counter current contactor (a vessel where adsorbents are substantially continually added in the top section and settle to the bottom of the vessel while complex mixture is continuously added in the bottom section of the vessel and the majority travels to and is removed from the vessel in the top section).

[0034] A complex mixture containing the target compounds as well as the magnetic adsorbents bound to the target compounds, is transferred directly from vessel 108 into a magnetic separator 110. In some embodiments, magnetic separator 110 may be a magnetic drum separator, a magnetic decanter, a carousel-type high-gradient magnetic separator, or a magnetic extractor. The magnetic separator 110 separates the majority of the target compound-laden absorbents from the majority of the broth into two streams: 1) an adsorbent cake with a high concentration of the target compounds bound to the magnetic adsorbents and 2) a depleted/intermediate liquid which contains a residual concentration of the target compounds. A single binding stage (vessel 108) is inefficient in binding substantially all of the target compounds contained in the inflowing

complex mixture to the magnetic adsorbents. Therefore, depleted/intermediate broth is diverted from the magnetic separator 110 into the second binding vessel 106. The depleted/intermediate broth is then brought into contact with fresh functionalized adsorbents provided from vessel 118 or from adsorbent reservoir 104, depleting the broth to a greater extent of target compounds by adsorption to the supplied regenerated adsorbents. The adsorbents in vessel 106, now laden with remaining target compounds from the depleted/intermediate liquid, are fed back into vessel 108 while the complex mixture maximally depleted of target compounds is transported to vessel 112 for waste.

[0035] The magnetic adsorbents with bound target compounds are fed from the magnetic separator 110 into vessel 114 (e.g., a counter current contactor) which substantially continually provides an elution solution (such as an elution buffer with higher ionic strength than the complex broth under binding conditions) that allows the target compounds to selectively desorb from the adsorbent while impurities are still bound to the adsorbents. The counter current setup allows for a concentrated target compound solution to be withdrawn from the top of the vessel 114 in a purified form for storage in purified target compound storage 116, whereas eluted adsorbents (i.e., a sludge made up of magnetic adsorbents with bound impurities) are drawn from the bottom of vessel 114. In some embodiments vessel 114 includes an overflow section comprised of inclined plate settlers to enable the concentrated compound solution to be withdrawn from the top of vessel 114. In some embodiments, overflow sections employing inclined plate settlers may be replaced with a hydrocyclone, a separate riser/decanter section where adsorbents settle. In some embodiments, overflow sections employing inclined plate settlers may be replaced with an active adsorbent capture mechanism, such as continuous filtration, continuous centrifugation, continuous decanter, magnetic traps that are periodically cleared, or substantially continual magnetic capture (e.g., with a small dedicated drum separator.).

[0036] This adsorbents sludge drawn from the bottom of vessel 14 then enters the regeneration vessel 118 of the elution stage where, utilizing a regenerant solution, remaining impurities are desorbed from the adsorbents. The adsorbents then settle into a storage solution 120. This sludge of storage solution and adsorbents is then

substantially continually pumped to the adsorbent reservoir 104, from which the adsorbents are brought into the next cycle. In some embodiments, vessel 118 includes an overflow section comprised of inclined plate settlers to enable the regenerant with impurities to be withdrawn from the top of vessel 118. In some embodiments, overflow sections employing inclined plate settlers may be replaced with a hydrocyclone, a separate riser/decanter section where adsorbents settle. In some embodiments, overflow sections employing inclined plate settlers may be replaced with an active adsorbent capture mechanism, such as continuous filtration, continuous centrifugation, continuous decanter, magnetic traps that are periodically cleared, or continuous magnetic capture (e.g., with a small dedicated drum separator.).

[0037] FIG. 2 is a diagram illustrating a binding stage in accordance with some embodiments. Due to the nature of the protein binding to porous magnetic adsorbents, a single contactor is likely insufficient to achieve high adsorbent binding capacity utilization and high levels of target compound depletion in the treated complex mixture. Therefore, the binding stage comprises two steps that ensure both (however, in some embodiments, the binding stage is comprised of a single continuously stirred tank reactor or a plug flow reactor or a counter current contactor). In the example shown, vessel 108 is used to achieve a primary depletion of target compounds from the broth by adsorption to magnetic adsorbents. The adsorbents are captured from the broth by a magnetic separator 110 and further transferred to the elution stage. The broth, after the primary depletion stage and devoid of adsorbents again, is pumped to the second binding step. In vessel 106, fresh magnetic adsorbents 208 (e.g., 50-90% magnetic adsorbent volume fraction) are brought into contact with the depleted/intermediary broth discharged from magnetic separator 110 to allow the majority of remaining free target compounds to bind to unloaded adsorbents. The treated complex mixture is discharged at the top 210, keeping the loss of adsorbents at a minimum (the top outlet section is designed to ensure a high degree of retention of adsorbents in the system; e.g. sections employing inclined plate settlers, hydrocyclones, a separate riser/decanter section, magnetic separators or magnetic traps). Simultaneously, the adsorbents settle through the broth and exit that stage via 212 as a concentrated sludge at the bottom outlet with an initial amount of target compounds bound to the adsorbents (e.g., 20% of adsorbent

binding capacity saturated with bound target compounds). This sludge is transferred to the primary broth depletion in vessel 108 to utilize the remaining binding capacity of the adsorbents . In some embodiments, overflow sections employing inclined plate settlers may be replaced with a hydrocyclone, a separate riser/decanter section where adsorbents settle. In some embodiments, overflow sections employing inclined plate settlers may be replaced with an active adsorbent capture mechanism, such as continuous filtration, continuous centrifugation, continuous decanter, magnetic traps that are periodically cleared, or continuous magnetic capture (e.g., with a small dedicated drum separator.).

[0038] A high concentration of adsorbents in the adsorbents-sludge stream that is added to the binding stage at the top of vessel 108 is necessary to omit dilution (of the inflowing complex mixture) that would be detrimental for adsorption efficiency and, thus, the degree of depletion. Concentrating the adsorbents is accomplished by passive settling of the adsorbents in a section at the bottom of vessel 106 (the bottom section may feature closely spaced surfaces that have an inclination angle that is sufficiently steep to allow accumulated adsorbents to slide downwards (an inclined plate settler section).

[0039] FIG. 3 is a diagram illustrating a capture stage in accordance with some embodiments. In the example shown, the capture stage comprises a co-current rotation magnetic drum separator (other magnetic separators may be used) that includes two rubber-drums 302, 304 that squeeze out interstitial liquid from the adsorbent cake and replace the interstitial liquid with a washing solution. A suspension with a particular percentage of adsorbents volume fraction (e.g., 20-25%) is inserted. The adsorbents are separated from the suspension due to their high magnetic moment, forming a cake on the drum surface of the magnetic drum separator. The rubber drum 302 minimizes interstitial broth lifted out along with the cake. The rubber drum 304 squeezes the washing solution (provided by wash buffer spray 308) out of the adsorbent cake. The rubber drum 304 will not carry liquid in the direction of the center-drum rotation due to its rotation direction (different rotation directions may be used). The result is an adsorbent cake on the drum surface having a high percentage of loaded adsorbents

(e.g., 50-95%). In some embodiments, the washing solution is an aqueous buffer with similar ionic strength to the broth. The squeezed-out washing solution will flow downwards to the first rubber-drum 302 which, due to its rotation direction, will divert the washing liquid away from the drum surface and can either be captured by a dedicated trough (not shown) or drip back into the feeding pan 306. In some embodiments, the liquid content of the captured magnetic adsorbent sludge is reduced to less than 50% of the total sludge volume. In some embodiments, scrapers/blades are used instead of rubber-drums. In some embodiments, gas jets are used instead of rubber drums.

[0040] FIG. 4 is a diagram illustrating a portion of an elution stage in accordance with some embodiments. In order to mitigate the need for additional steps, in the regeneration section 118 of the elution stage the adsorbents settle, in a counter-current fashion, through a regenerant solution environment and pass the injection location of regenerant concentrate where they settle into a quiescent zone of storage liquid 402. In some embodiments, the adsorbents are concentrated into a sludge (reconditioning solution) with less than 60% of interstitial liquid volume before they are recycled for use in the binding stage. The regenerant concentrate flows upward from regenerate concentrate input and used regenerant is removed from the counter current contactor at 404. The removed regenerant solution may be diverted as waste or may be partially reused by mixing with fresh regenerant concentrate and subsequent injection into the counter current contactor 404 through the regenerant inlet.

[0041] In some embodiments, substantially continual operation (that is: substantially continual fluid flow at some or all in- and outlets) is achieved for any given process stage or any given vessel-equivalent by the alternating use of at least two or more discontinuously operated vessels. Discontinuities in one or more in- or outflows of one of the two or more given vessels can be substituted by temporally staggered discontinuous operation of one or more substantially identically operated vessels.

Adsorbents

[0042] Ideal functionalized magnetic adsorbents for the present invention need to be synthesized. Such adsorbents need to enable target compound adsorption and

desorption at a high efficiency and specificity. Ideal magnetic adsorbents for the present invention have a magnetic core and a porous polymer or swollen gel shell.

[0043] Wet low-intensity magnetic separators that can be operated in a substantially continual fashion with large volumetric throughput capacity require particulate magnetic material to be larger than 10 µm in order to afford efficient capture. This is due to the necessary force of attraction to cause sufficient velocity of the adsorbent towards the capturing surface (magnetic material) given a short residence time of the adsorbent-carrying fluid in such separators. Thus, the diameter of the adsorbents' magnetic core needs to be large enough to enable the adsorbents to be efficiently captured by a magnetic separator with a low magnetic field strength. This design element is highly important to the functionality of the invention.

[0044] The adsorbents should also possess the ability to passively settle in liquid environments with sufficient terminal velocity. This is accomplished by virtue of the size of the adsorbent core and adsorbent shell, and the resulting mass and magnetic moment of the adsorbents. The magnetic moment of each adsorbent results in the formation of adsorbent aggregates which further enhances the adsorbent settling. Together, these phenomena will enable adsorbents to be passively separated from the liquid they are contained in within vessels 106, 108, 114, and 118 without the need for active adsorbent capture and reintroduction for each vessel, and thus enabling substantially continual operation of the system. This design element is highly important to the functionality of the invention.

[0045] The porous shell surrounding the core provides pore sizes that allow efficient mass transfer into and out of the shell while also providing large enough internal surface area for a high binding capacity to the target compound.

[0046] In order to afford the required target compound binding and elution specificity, different properties of the (internal) surface of the shells have to be established to comport with the properties of different target compounds. To this end, the porous shell inner surface needs to have specific physicochemical properties that allow for reversible adsorption and desorption (e.g., low surface energy hydrophilic

surfaces). Furthermore, the surface needs to have chemical cues that confer a certain specificity towards the physicochemical interactions (adsorption) between the surface of the adsorbent and the exposed surface of the target compounds. This can be implemented by functionalizing the porous shell inner surface with complex molecules (ligands) that are designed to confer the required specificity.

Adsorbent Structure

[0047] The magnetic adsorbents comprise magnetic cores with an average diameter of e.g. 50 µm. These cores will be encapsulated with a porous shell with an average thickness of 10 to 100 µm that, when affixed with ligands, can accommodate the adsorption of target compounds. In some embodiments, the magnetic adsorbents have a size between 20 – 1000 µm.

[0048] To enable the necessary interaction with the magnetic separator, as well as the necessary settling properties of the adsorbents, the average core diameter is 10 – 300 µm, and preferably 50 – 100 µm.

[0049] Target compounds will be able to adsorb to the inner (and, negligibly, to the outer adsorbent) surface of the shell with high selectivity under specifically chosen conditions. The average shell thickness that will be targeted ranges from 10 to 100 µm. Shells thinner than that will not afford enough total internal surface area for protein to adsorb to and result in insufficient binding capacity per adsorbent. On the other hand, thicknesses above 100 µm will result in long diffusion times from the outer surface of the adsorbent to the inside. Given the process parameters of the systems described herein, the binding capacity of thicker shells will not be usable as the characteristic time for target compounds to reach the inside will be longer than the time that adsorbents are in contact with the solution containing the target compound. The effect will be reduced adsorbent material utilization rate.

[0050] Thus, the adsorbents will preferably have a core-shell structure with a core diameter of 50- 150 µm and a shell thickness of preferably 10 – 100 µm resulting in adsorbents with a diameter of 125 – 350 µm. This very specific combination of

parameters/properties is unique, not commercially available, and not described in available literature.

[0051] Although the foregoing embodiments have been described in some detail for purposes of clarity of understanding, the invention is not limited to the details provided. There are many alternative ways of implementing the invention. The disclosed embodiments are illustrative and not restrictive.

[0052] WHAT IS CLAIMED IS:

CLAIMS

1. A system, comprising:

a first vessel configured to:

receive a substantially continual inflow of a liquid containing target compounds during operation of the system;

adsorb the target compounds to magnetic adsorbents;

a magnetic separator configured to, during operation of the system, substantially continually separate a mixture that includes the target compounds bound to the magnetic adsorbents into an adsorbent sludge that includes the target compounds and a complex mixture depleted of the target compounds; and

a second vessel configured to:

substantially continually desorb, during operation of the system, the target compounds from the adsorbent sludge received from the magnetic separator during operation of the system to substantially continually isolate the target compounds;

obtain magnetic adsorbents with a regenerated ability to bind the target compounds; and

provide a substantially continual outflow of the magnetic adsorbents with the regenerated ability to bind the target compounds back to the first vessel.

2. The system of claim 1, wherein the magnetic separator is a magnetic drum separator, a magnetic decanter, a carousel type high-gradient magnetic separator, or a magnetic extractor.

3. The system of claim 1, wherein the magnetic adsorbents have a size between 20 – 1000 μm.

4. The system of claim 1, wherein the magnetic adsorbents have an average volumetric saturation magnetization between 480 A/m and 480,000 A/m.

5. The system of claim 1, wherein a magnetic field strength experienced by the magnetic adsorbents in the magnetic separator is between 0.01 T and 1.5 T.

6. The system of claim 1, wherein the liquid is a fermentation broth, a cell culture broth, a chemical reaction mixture, liquid plant cell homogenate, liquid food stream, a juice stream, or saltwater brine.

7. The system of claim 1, wherein the first vessel is configured to substantially continually stir the target compounds and the magnetic adsorbents during operation of the system.

8. The system of claim 1, wherein the magnetic separator includes one or more devices configured to mold the adsorbent sludge into an adsorbent cake and remove interstitial liquid included in the adsorbent sludge.

9. The system of claim 8, wherein the one or more devices are a combination of blades and drums installed with a clearance from a surface of the magnetic separator.

10. The system of claim 9, wherein a wash solution spray provides a washing solution to the adsorbent cake and one of the drums is configured to squeeze the washing solution out of the adsorbent cake.

11. The system of claim 1, wherein the magnetic adsorbents with the regenerated ability are provided back to the first vessel via an adsorbent reservoir.

12. The system of claim 1, wherein a portion of the complex mixture depleted of the target compounds that is substantially no longer laden with the target compound is removed as waste.

13. The system of claim 1, wherein the second vessel includes an elution solution that allows the target compounds to selectively desorb from adsorbents shells included in the absorbent sludge.

14. The system of claim 1, wherein the isolated target compounds are withdrawn from a top portion of the second vessel and eluted adsorbents are drawn from a bottom portion of the second vessel.

15. The system of claim 14, wherein the eluted adsorbents is a sludge comprised of magnetic adsorbents with bound impurities.

16. The system of claim 15, further comprising a third vessel configured to substantially continually utilize a regenerant solution to desorb the bound impurities from the magnetic adsorbents.

17. The system of claim 16, wherein the magnetic adsorbents settle into a storage solution to form the magnetic adsorbents with the regenerated ability.

18. The system of claim 1, further comprising a third vessel configured to mix the complex mixture depleted of the target compounds with the magnetic adsorbents with the regenerated ability to bind target compounds, wherein remaining target compounds included in the complex mixture binds to the magnetic adsorbents with the regenerated ability to bind target compounds.

19. The system of claim 18, wherein the third vessel is configured to provide the first vessel the magnetic adsorbents with regenerated ability bound to the target compounds.

20. The system of claim 19 wherein the third vessel includes an overflow section to accumulate waste.

21. The system of claim 20, wherein the overflow section includes inclined plate settlers, a hydrocyclone, a separate riser/decanter section, continuous filtration, continuous centrifugation, continuous decanter, magnetic traps that are periodically cleared, or continuous magnetic capture.

22. The system of claim 1, wherein the second vessel includes an overflow section to accumulate the isolated target compounds.

23. The system of claim 1, wherein any magnetic adsorbents included in the adsorbent sludge is included in the substantially continual outflow of the magnetic adsorbents with the regenerated ability to bind the target compounds.

24. A method, comprising:

receiving, at a first vessel, a substantially continual inflow of a liquid containing target compounds during operation;

adsorbing, at the first vessel, the target compounds to magnetic adsorbents;

substantially continually separating, by a magnetic separator during the operation, a mixture that includes the target compounds bound to the magnetic adsorbents into an adsorbent sludge that includes the target compounds and a complex mixture depleted of the target compounds; and

substantially continually desorbing, by a second vessel during the operation, the target compounds from the adsorbent sludge received from the magnetic separator during the operation to substantially continually isolate the target compounds;

obtaining magnetic adsorbents with a regenerated ability to bind the target compounds; and;

providing a substantially continual outflow of the magnetic adsorbents with the regenerated ability to bind the target compounds back to the first vessel.

25. The method of claim 24, wherein the magnetic separator is a magnetic drum separator, a magnetic decanter, a carousel type high-gradient magnetic separator, or a magnetic extractor.

26. The method of claim 24, wherein the magnetic adsorbents have a size between 20 – 1000 µm.

27. The method of claim 24, wherein the magnetic adsorbents have an average volumetric saturation magnetization between 480 A/m and 480,000 A/m.

28. The method of claim 24, wherein a magnetic field strength experienced by the magnetic adsorbents in the magnetic separator is between 0.01 T and 1.5 T.