

Fluid Purification Using Charge-Modified Depth Filtration Media

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Positioned at various stages during bioprocessing, depth filters are used as prefilters that clarify fluid streams to protect, prolong the life, and increase the efficiency of final (sterilizing) filters, chromatography columns, and other separation technologies. Depth filters are particularly well-suited for use in prefiltration during bioprocessing, removing large contaminants and microorganisms, cellular debris, nucleic acids, host-cell-derived aggregates, and other contaminants that foul downstream processing steps and lead to reduce product recoveries. Examples of applications where depth filtration is used this way include clarification of cultures following fermentation and following centrifugation or tangential flow filtration of biomass.

Charge-modified depth filters use two distinct mechanisms to remove contaminants. The first is physical



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capture within tortuous paths (Figure 1), where particles become entrapped when they enter the depth filter matrix. The second mechanism, electrokinetic adsorption, removes bacteria, fungi, viruses, and other negatively charged contaminants that are smaller than the pore size rating of the media. Filters are designed to exploit electrokinetic adsorption by using one of several amine chemistries that impart to the media a net positive charge that is maintained under typical process pH ranges and ionic strengths.

A BRIEF HISTORY

Depth filter technology has been used for many years. Early examples include deep-bed filters composed of (for example) sand or charcoal, which have been used extensively for water

purification. Early cartridge-type depth filters were made of cotton fibers wrapped around a steel or polyolefin core. Among the earliest graded-density depth filters commercially available were resin-bonded filters. Although graded-density filters do not possess discrete pores, they do exhibit a monolithic, gradient channel structure so flow path diameters decrease gradually through depth media. In such construction, channels are of larger diameter where they first contact the influent sample; apertures at the core, where fluid exits a filter, are smallest in diameter (most retentive).

Depth filtration captures particulates exhibiting a wide range of sizes and physical properties. Historically, depth filters were

PRODUCT FOCUS: RECOMBINANT PROTEINS AND OTHER FERMENTATION/CELL CULTURE PRODUCTS

PROCESS FOCUS: BIOPROCESSING, PURIFICATION, CLARIFICATION

WHO SHOULD READ: PROCESS DEVELOPMENT, PRODUCT DEVELOPMENT, MANUFACTURING, QA/QC

KEYWORDS: DEPTH FILTRATION, ELECTROKINETIC ADSORPTION, FLUX, PROCESS OPTIMIZATION, TURBIDITY

LEVEL: BASIC

Figure 1: Mechanisms of membrane and depth filtration

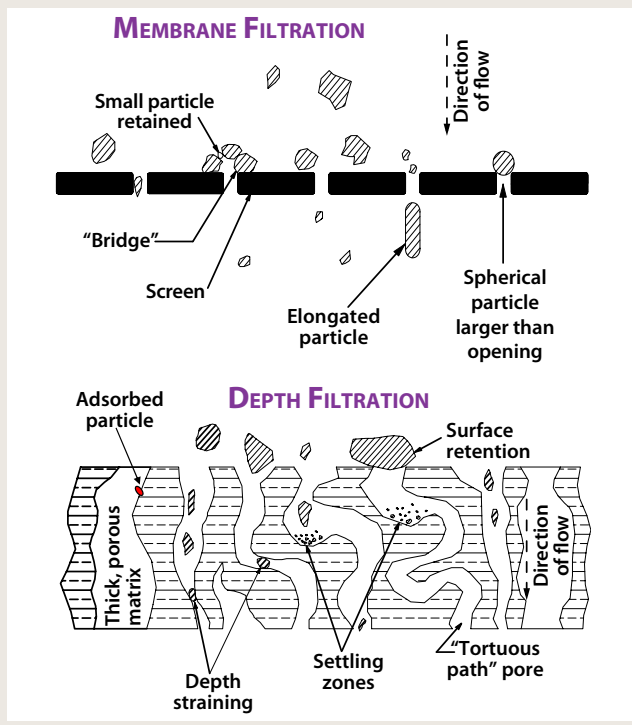
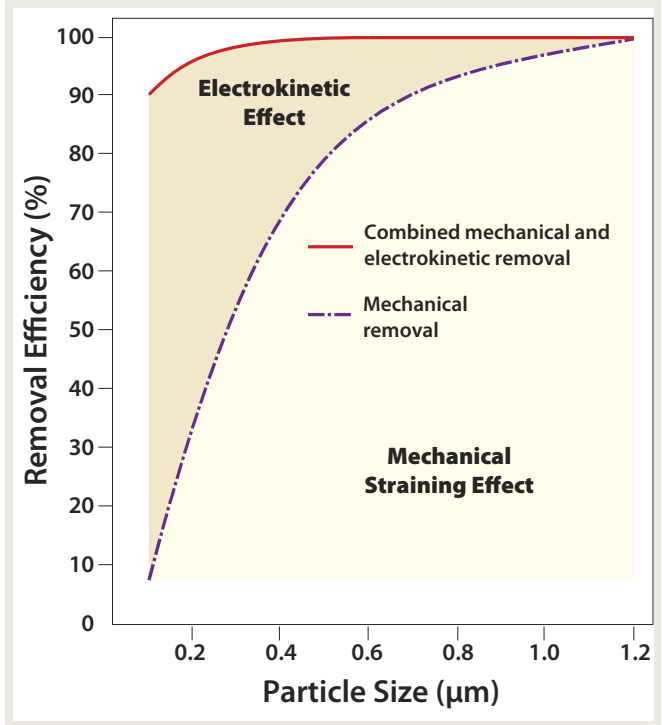


Figure 2: Electrokinetic adsorption efficiency



manufactured from a variety of materials including paper, asbestos, glass fibers, nylon, polyolefins, carbon, and ceramics (1). Asbestos offered a net positive surface charge, which made it particularly attractive as a filtration media because most contaminants are negatively charged. But use of that material in filtration and other applications was suspended in the 1970s due to its toxicity.

Naturally occurring materials such as perlite (derived from volcanic rock) and diatomaceous earth (phytoplanktonic skeletal remains) can be deposited on a septum and function as depth filters alone. Alternatively, such materials (filter aids) can be incorporated into filter sheets with cellulose (as described below) and wet-strength resins. Those filter aid materials not only offer a large amount of internal surface area that augments flow rates and increases particulate loading capacities, but they also capture contaminants by adsorptive mechanisms.

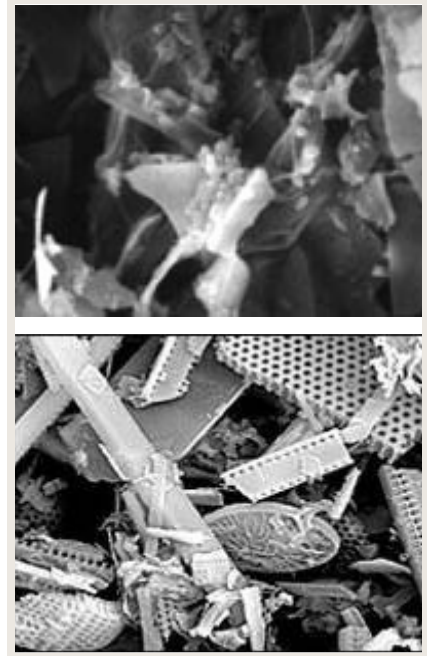
To gain the most benefit from filter aid materials, a balance between permeability, shape, and packing density must be achieved. As mentioned above, adsorptive retention of contaminants coupled with mechanical sieving further increases depth filter performance. To exploit

this phenomenon, methods were developed involving chemical treatment of aids and filtration media to impart a positive charge (2). Media of high-density positive charge are very effective in removing contaminants and can retain particles much smaller than their physical pore size. For example, CUNO Zeta Plus filter media (incorporating a charge modifier and filtration aids) was introduced in 1973 to exploit the advantage of adsorptive and mechanical contaminant removal by depth filtration.

COMPARING TYPES OF FILTERS

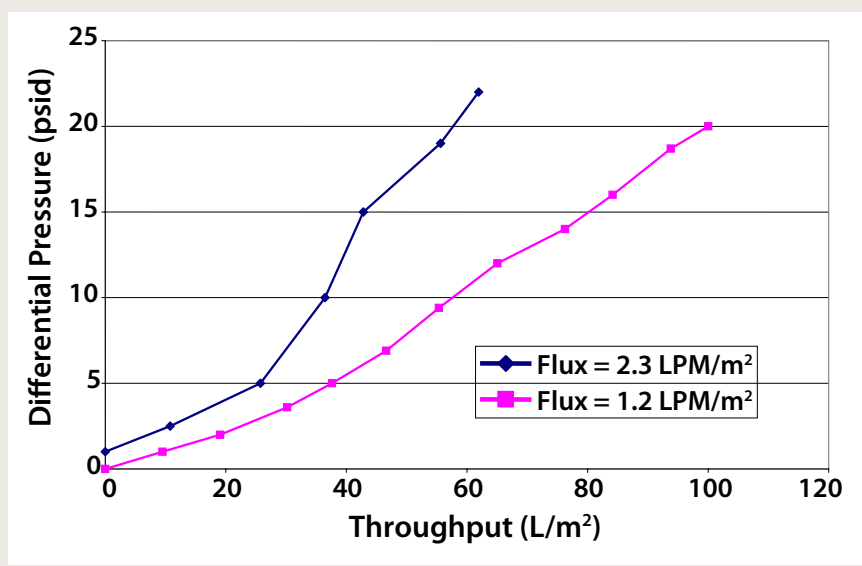
Standard microporous membranes consist of a solid matrix containing pores of sizes ranging from low nanometers to midmicrons in diameter. Such membranes retain particulates primarily by physical interception at their surface or within their internal pore structures if particles are smaller than apertures at the membrane surface. Charge-modified microporous media capture by charge-based retention of contaminants (e.g., charge-modified nylon membrane for removal of bacterial endotoxin) within their internal porous structures. Membrane filters are useful for purification and/or bioburden reduction of relatively clean

Figure 3: Scanning electron micrographs of (top) bacteria retained by electrokinetic capture on depth filter and (bottom) diatomaceous earth filter aid



fluid streams and as final filters for sterilization by removing bacteria, viruses, nucleic acids, and other contaminants. Membrane filters can be inefficient when the fluid to be filtered has a high contaminant load and/or a broad particle size distribution. They themselves have tight pore size distributions, and their pore sizes can be readily measured (3).

Figure 4: Differential pressure and throughput volume for filtration of CHO cell centrate at two different flux rates



WHO SELLS DEPTH FILTERS?

Here are web sites for some depth filter manufacturers and product lines.

Cuno, Inc. (a 3M company, www.cuno.com): ZetaPlus, PolyNET, Betafine

ErtelAlsop (www.ertelalsop.com): Disk-Pak, AccuScale, PharmaScale

Filtrox AG (www.filtrox.ch): PuraFix

GE Infrastructure Water and Process Technologies (www.gewater.com): Osmonics Z-Plex, Hytrex, Purtrax

Meissner Filtration Products, Inc. (www.meissner.com): AlPHA, Vanguard, DeltaMax, DeltaDepth, Duraclear

Millipore Corporation (www.millipore.com): Clarigard, Polygard, Millistak

Pall Corporation (www.pall.com): SUPRADisc, SUPRAclean

Sartorius AG (www.sartorius.com): Sartoclear, Sartofine

US Filter (a Siemens business, www.usfilter.com): Prepure, Postpure

By contrast, depth filters are more effectively characterized by their particle removal efficiency. Their “nominal” pore size is based on empirically measured retention characteristics. Functionally, depth filters achieve particle separation through capture of contaminants within the tortuous paths and channels of their media. An element of surface capture occurs; however, depth media should be selected to limit the amount of surface filtration and optimize the use of their *internal void volume* (the interstitial space within the filter matrix) (see Figures 1 and 3). This provides greater capacity for removing contaminants.

As described above, construction of depth filters provides certain advantages over filters that primarily use surface capture. First, the more “open” depth media provide reduced flow restriction. Second, larger pores enable greater contaminant loading before flow through the media becomes so restricted as to require replacement (4). The cross-section illustration in Figure 1 compares surface (membrane) and depth filters. Without a positive charge on the media, particles are captured only by a sieving (depth straining) mechanism as they migrate through. Significant enhancement of contaminant capture capabilities is imparted by incorporating filter aids and/or charge modification chemistries that give a positive charge to the media because most particles targeted for removal are

negatively charged (2). Those characteristics make depth filters an excellent choice for trapping contaminants having a broad particle size distribution.

IMPLEMENTING DEPTH FILTRATION

Table 1 lists some applications in which depth filtration can be used successfully. Figure 2 shows the advantages of positively charged media for the efficiency of contaminant capture. Two identical filters, differing only by the presence or absence of electropositive charge-modification, were compared in their removal efficiencies for negatively charged particles across a broad size distribution. Both filters show equivalent performance in removing large particulates; however, the charge-modified filter far outperforms the other in capturing submicron particulates smaller than the channels of the depth media. When applied to bioprocess streams, that ability significantly improves protection of downstream sterilizing-grade filters and increases the performance and longevity of chromatography media by removing host-cell-derived and other contaminants (5). Data presented at a recent bioprocess symposium indicated that the adsorptive properties of depth filters effectively remove host-cell contaminants from downstream process streams (6).

Removal of contaminants significantly smaller than a filter’s pore-size rating makes charge-modified depth filters effective for removing bacteria, fungi, endotoxin, viruses, and even prions. Figure 3 shows electrokinetic capture of microbes on a charge-modified depth filter. As discussed in the following section, fluid characteristics (pH, ionic strength) affect the efficiency of charge-modified media. So depth media possessing the appropriate amine functionality (secondary, tertiary, or quaternary) must be tested empirically to achieve optimal removal of biological contaminants.

Factors Affecting Performance:

Operating conditions and the nature of a fluid to be filtered influence the selection of media pore size, total surface area, and flux rate. Mathematical expression of flux is

complicated by the need to consider flow orientation relative to the filter surface. The strict formula is

$$Q = A \times v \times \cos\Theta$$

where Q is flux, A is a given surface area, v is fluid velocity, and $\cos\Theta$ is the angle of flow relative to the surface. Assuming flow is perpendicular, then $\Theta = 0$ and $\cos\Theta = 1$, so $Q = Av$. The simplest equation would be

$$\text{flux} = \frac{\text{flow rate}}{\text{unit area}}$$

To ensure optimal performance of an appropriately selected depth filter, flux should be as low as is reasonable while ensuring that a process does not become prohibitively slow. When flux is decreased, media residence time increases, thereby increasing interactions between particles and media. Depth filtration depends on diffusion of a sample material throughout the matrix of a filter to either sieve or bind particulates sought for removal from a liquid stream. Even as low flux aids in binding of general contaminants found in fluid streams, sample materials with high solids content and proportional viscosity also require very low flux rates to compensate for their inherently slow diffusion rate. Throughput (determined by the amount of fluid passed through a depth filter before it reaches a preestablished pressure drop) increases significantly by reducing flux (Figure 4).

It is important to understand the effects of solution pH on depth media performance. One primary mechanism of capture depends on the electrochemical charge of contaminants in a fluid stream. Changes in pH can modify the surface charge of contaminants and media. So pH can be adjusted to enhance and optimize the performance of charge-modified depth media. Using different types of charge modifier can augment performance when pH adjustment is not an option. Such alternatives allow researchers to determine empirically which combination of filter aid, charge modifier, and pore size provides optimal performance for their applications.

During filtration, several parameters are typically monitored: pressure drop across a prefilter, nephelometric turbidity (NTU) of depth filter effluent (compared with NTU readings of its influent), and

Table 1: Applications for depth filtration

Bioprocess	Biologicals	Pharmaceutical
Cell culture clarification	Clarifying plasma-derived biologicals (blood fractionation)	Small-volume parenteral (SVP) prefiltration
Tangential flow filtration prefiltration or replacement	Purifying serum and cell culture media feeds	Clarifying oral liquid pharmaceuticals
Clarifying cell lysates	Virus and prion reduction	
Prefiltration and protection of sterilizing filters and chromatography columns	Clarifying toxoid broths for vaccines	
Prefiltration of fermentation feed streams	Clarifying/purifying allergenic extracts	
Bioburden reduction; virus and prion reduction; clarification of sera, reagents, and buffers	Lipid removal	

pressure drop across the downstream (sterilizing grade) filter. It is very important to measure both pressure drop and NTU across the depth filter. You may not observe an increase in pressure across a depth filter, but NTU can rise as adsorptive sites in the media become saturated. Bench-scale and pilot-scale testing will help reduce chances that filters will become overwhelmed before a batch run is complete.

Typically, bench-scale testing runs under conditions that mimic a full-scale process. For example, flux (flow rate/unit area) and throughput (volume/unit area of media) are evaluated and used to make recommendations for total filter area and flux in a full scale system. Those metrics are critical in choosing suitable depth filter media and required filtration area. When determining appropriate filter area, an additional "safety factor" should be included to compensate for variabilities in process fluids. Values of 50% over minimal required filtration areas are not uncommon when ensuring protection of key downstream processing steps.

CELLULOSE-BASED DEPTH MEDIA

A variety of (wood pulp) cellulose based depth filtration media have been designed specifically for prefiltration and clarification of bioprocess, biological, and pharmaceutical fluid streams. Multiple combinations of pore size, filter aids (with different porosities and surface areas), and wet-strength resins (which impart their intrinsic positive charge to filter media) exist

Figure 5: Depth media cartridge construction

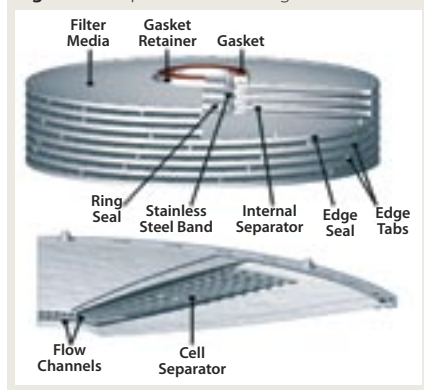
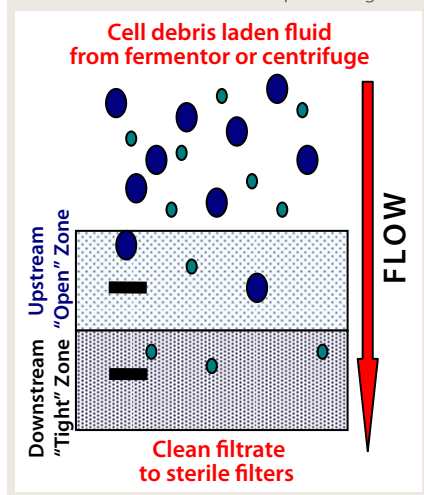


Figure 6: Schematic of performance attributes of Cuno Zeta Plus Maximizer and Maximizer EXT depth media; dual zone construction creates a true graded density (upstream open porosity, downstream tight porosity) for post-fermentation clarification in bioprocessing



to provide the most efficient filter attributes for performance in any given application. Individual pore size grades of cellulose-based depth media are generated by varying the handling of pulp during processing and preparation

of a slurry, in which the pulp is combined with a wet-strength resin (charge-modifier) and filter aid. Figure 3 shows a scanning electron micrograph of filtration aids contained in CUNO Zeta Plus depth media. Various wet-strength resins are used to optimize capture of fluid-stream contaminants and enable process development, scale-up, and manufacturing personnel to exploit the best amine functionalities for their process fluids. A felting process forms sheets of cellulose, which are then cut into squares or discs depending on the final product format desired. Figure 5 shows a cut-away view of a Zeta Plus media cartridge. This readily modifiable technology incorporates anion-exchange media, carbon, and other affinity resins into a depth filter format.

Graded-density media increase contaminant loading capacities, improve throughput, and provide a smaller overall footprint. For example, new Zeta Plus filter media take advantage of the graded density technology and increased media mass to augment performance (Figure 6). The medium has two distinct layers, with the upstream layer more open than the downstream one. Those layers can be selected independently and combined to optimize performance.

A COMPLEMENTARY TECHNOLOGY

Depth filtration effectively removes bioburden, cell debris, protein aggregates, viruses, and prions from fluid streams. Its complementary mechanisms of contaminant removal — adsorption and sieving — allow removal of large and submicron particulates, making depth filters a good choice for prefiltration applications. Depth media used at various stages in bioprocessing, biological, and pharmaceutical applications can reduce costs of final filtration, protect chromatographic media, increase productivity, and improve final product yields.

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