Dynamic Depth-Filtration: Proof of Principle

Microfiltration with Biopharmaceutical Liquids

Technical Note AMC06
Version 3.2

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Summary

This technical note introduces the concept of dynamic depth-filtration, a constant flow, pressure filtration technique. This document is a proof of principle protocol and an optimization guide. Terms in **bold** appear in the Glossary.

Background

Dynamic depth-filtration is a new **microfiltration** technique that combines **filter aid filtration** with **depth filtration**. Both techniques have been widely used in biopharmaceutical separations since the early 1900s.

> Dynamic depth-filtration typically increases the speed and solids capacity of conventional depth filtration by 5-10X, while halving both the disposal weight/volume and the process footprint.

Dynamic depth-filtration is among the most robust of separation technologies for compressible solids (*e.g.*, bacterial debris), especially when feedstock solids loading is greater than 1%. The advantages of dynamic depth-filtration include high solids capacity, low cost, and ease of scale-up (De Jonge et. al., 1993; Hunt, 1999; Gadam, et. al., 2001).

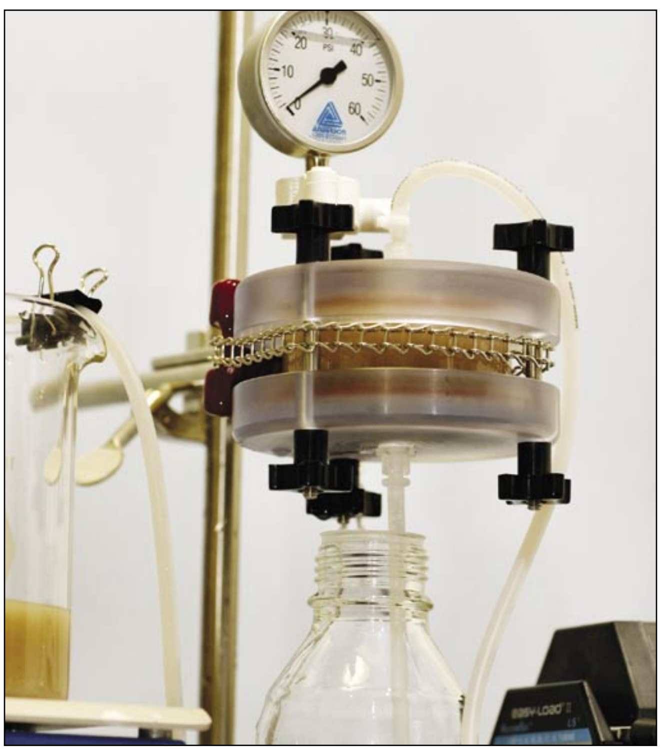


Figure 1. Bench Scale Dynamic-Depth Filter

Method

The principal filter media in this technique is Celpure®, a USP-NF grade **diatomite** filter aid. Diatomite is a silica powder composed of diatoms which are rigid, porous, and irregular in shape.

Dynamic depth-filtration's uniqueness lies in the suspending of filter media (*e.g.*, diatomite) in the feedstock during filtration. It can be contrasted with dead end and crossflow filtration in which the filter media is entirely immobilized in a filter element. When suspended in the feedstock filter media is called **body feed**.

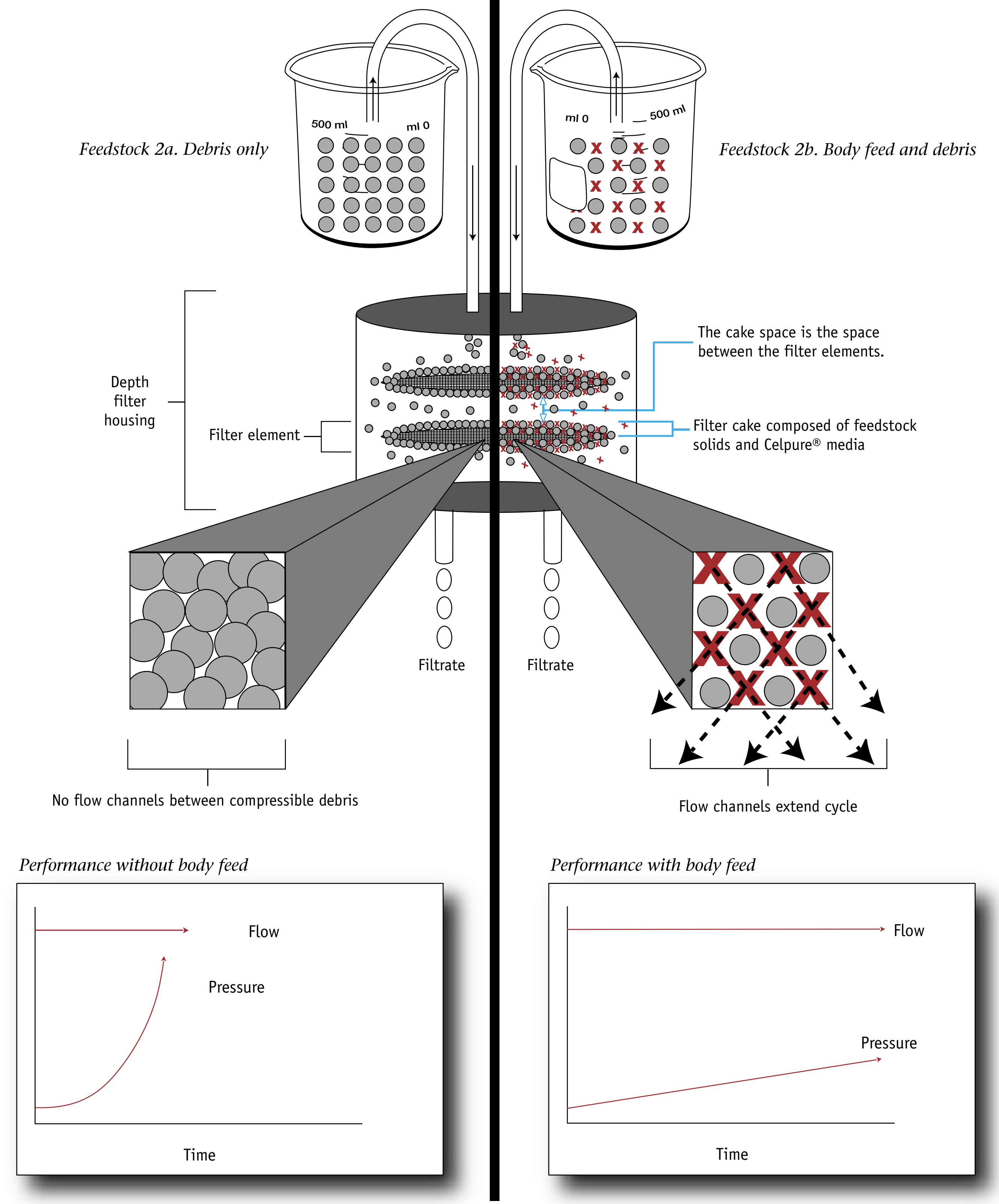
The filter media suspension deposits diatomite alongside the compressible solids throughout filtration. The rigid and porous diatomite particles prevent the compressible debris from forming an impermeable mass (Figure 2b), which is what gives dynamic depth-filtration its extraordinary solids holding capacity and speed. This also continuously regenerates the filter surface.

Dynamic depth-filtration can be contrasted with conventional depth filtration, in which the plugging of the filter surface begins almost immediately, rendering much of the "depth" in depth filters as theoretical solids capacity, not functional capacity. Without body feed, impermeable debris accumulates within finite flow channels. The result is a rapid pressure rise and the requirement for greater filtration surface area (Figure 2a).

Constant Flow Filtration:
Depth vs. Dynamic Depth

Figure 2a. Depth Filtration

Figure 2b. Dynamic Depth-Filtration



Pre-Evaluation Determinations

1. Selecting Bench-Scale Hardware Selection

Filter Housing

Dynamic depth-filtration requires sufficient **cake space** (Figure 2b) for the accumulating Celpure® media and feedstock solids. A cake height of 1 – 3 cm over the filter surface is usually sufficient. The bench scale filter depicted below (Figure 3) satisfies this requirement.

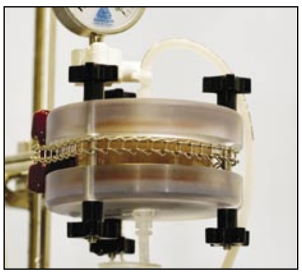


Figure 3a. This 50-cm², 3-cm tall filter housing may be ordered from www.ertelsop.com (part # LAB 50P). Accessories are listed at the back of this document (Table 5).

Figure 3b. Disassembled filter (part # LAB 50P).

Pump

Choose a reversible, peristaltic pump capable of pumping 5–100 mL/min. with size 16 tubing (Masterflex®).

> The remaining determinations are empirical. Their purpose is to allow you to quickly generate of the first set of data, which you can then use to direct optimization.

2. Selecting Body-Feed Grade

Celpure grade selection (Table 1) is the primary factor affecting filtrate clarity. Most biopharmaceutical applications will require Celpure 65.

Celpure® Grade	Permeability (mDarcy)	Surface Area (m^2/g)	99% Retention* (µm)
65	40-80	6.0-7.0	< 0.20
100	70-140	5.0-6.0	> 0.30
300	150-300	3.0-4.0	> 0.45
1000	750-1,250	1.0-2.0	> 1.0

*Table 1. Listed in order of increasing permeability (Celpure® 65 is the least permeable). *Nominal*

3. Selecting Body-Feed Concentration

The performance benefits of body feed are illustrated in Figure 4. To maximize this benefit, the cake volume created by the body feed should be at least equal to the volume of the feedstock solids. This ensures the following:

- The amount of Celpure is sufficient to maintain flow channels in the cake.
- The differential pressure is primarily influenced by the Celpure and not the feedstock debris.

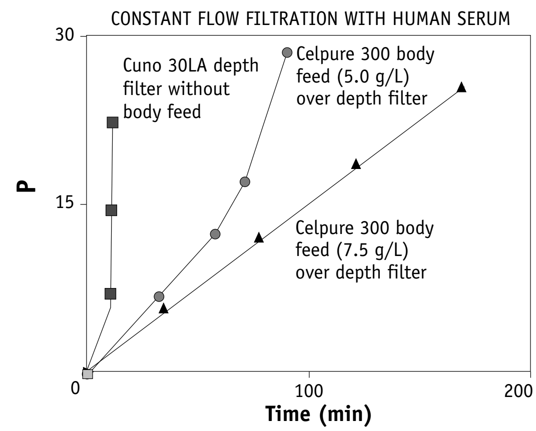


Figure 4. Body feeding over a depth filter with Celpure® media significantly improve overall performance.

Insufficient body feed results in the feedstock solids increasing the resistance to flow excessively, making for a less predictable process. Conversely, an optimized body feed (or a slightly high body-feed concentration) results in greater scalability. A body-feed concentration that occupies 2 times the feedstock solids' *volume* (not weight), is a good starting point.

Estimate the feedstock solids *volume* by centrifuging (~1,000 g force) a 10-mL sample (fully suspended feedstock) and then determining the percent of solids by volume. Use Table 2 to establish a starting body-feed concentration.

Body Feed Calculator	
Feedstock Solids (% by volume)	Body Feed (g/L)
≤ 2	12
4	24
6	36
8	48
10	60
12	72
14	84
16	96
18	108
20	120

Table 2. These body feed rates are for proof of principle. Optimized rates will typically be ½ these initial rates. In general, solids concentrations of up to 50% are easily clarified with Celpure media; however, processes approaching this upper limit are best evaluated with the assistance of Advanced Minerals.

4. Determining Capacity and Batch Size

Each gram of body feed produces 3–4 cm^3 of cake volume. Table 3 lists filter configurations and cake capacities.

Filter Area (cm^2)	Filter Height (cm)	Cake Space (cm^3)
50	3	150
200	10	2,000

Table 3. Example hardware configurations.

Choose batch volumes that will fill one-half the cake capacity. After mastering this technique, maximize the capacity by completely filling the space in subsequent trials.

5. Selecting the Septum

The septum is a depth filter used to support and retain the body feed. Depth filters with a particle retention rating tighter than 0.2 microns can unnecessarily inhibit filtration flux. The following Ertel (www.ertelalsop.com) depth filters will work well:

- M-503 Die 196 (5 micron)
- M-453 Die 196 (2 micron)
- M-703 Die 196 (0.45 micron)

6. Selecting the Flux Rate

The technique described is a constant flow filtration. Constant flow maximizes control over the permeability of the filter cake and minimizes body-feed usage.

Table 4 suggests flux rates based on volumetric solids-content. Greater rates are possible and should be attempted after mastering this technique. Feedstocks with high viscosity may require slower rates.

Solids (% by volume)	Flux Rate (L/m^2 -min.)
< 2	10.0-15.0
5-3	5.0-10.0
15-5	3.0-5.0
50-15	1.0-3.0

Table 4. Suggested flux and recirculation rates based on solids content.

An optimized flux allows the differential pressure to increase linearly, as in Figure 5a, which will maximize scalability. A flux rate that is too high constricts the flow channels and extrude the trapped solids into the filtrate.

Using Pressure *vs.* Time To Direct Optimization With Constant Rate Filtration

STRAIGHT LINE

Figure 5a.

As long as clarity is satisfactory, this can be ideal. This is especially the case where process validation and scalability are paramount.

GENTLE UPWARD CURVE

Figure 5b.

In some cases, this indicates an optimized filtration process. This curve also indicates slight under-dosing of body feed (a practical way to minimize filter aid usage).

STEEP UPWARD CURVE

Figure 5c.

A steep curve is usually a sign of too great of a flux and/or too little body feed.

SUDDEN CHANGE IN SLOPE AT END OF CYCLE

Figure 5d.

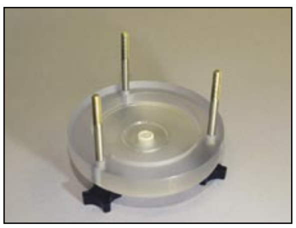
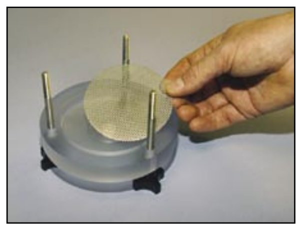
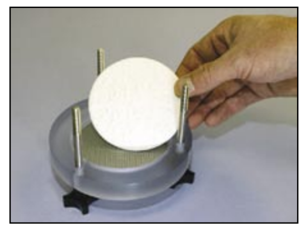
The sudden change at the end of the pressure curve indicates that there is no more cake space in the filter.

Preparing the Filter

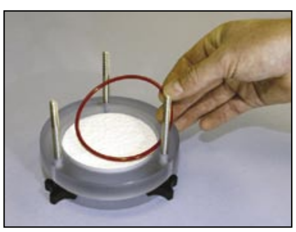
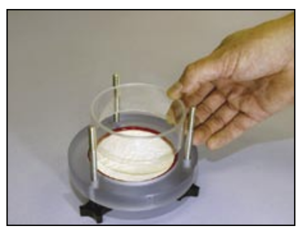
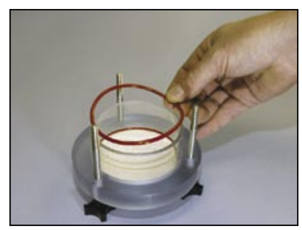
1. Wrap Teflon tape around three threaded NPT luer lock fittings (Cole-Parmer part number NCI-00125-NG). Insert the fittings into the bottom and top of the filter housing and then tighten. Note that top of the housing has two fitting holes and the bottom only has one.



2. Insert bolts into the bottom of the housing. Place the metal septum support into the bottom of the housing. Place the septum (*i.e.,* depth filter) on the septum support.

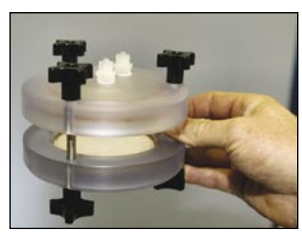




3. Place one gasket below and one on top of the cylindrical filter body.

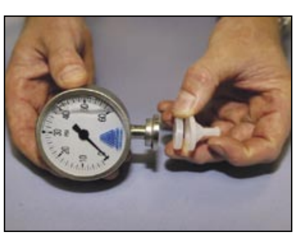




4. Place the top of the housing on the body and tighten the wing nuts. (Finger tightening is sufficient.)

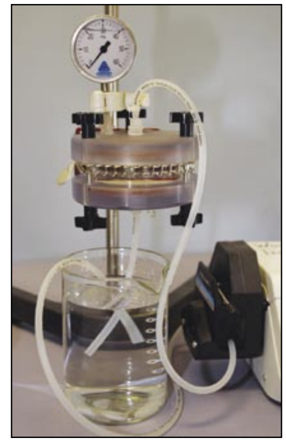




5. Attach the pressure gauge to the sanitary luer lock fitting using the gasket and clamp. Mount the filter to the stand and insert the sanitary pressure fitting into one of the luer lock fittings on top of the filter.



6. Insert the tube-end luer lock fittings on the inlet and outlet tubing and clamp. Secure inlet and outlet tubing to the filter via luer lock fittings.



7. Fill the filter (using the pump) with a process-compatible solution. Fill from the bottom, allowing the air to rise to the top of the housing where it is vacated. All air pockets should be removed. Check for leaks and re-tighten the housing bolts as necessary. (Finger tightening is sufficient.)

   Next, switch the direction of the pump and form a recirculation circuit by placing the intake and outlet tubing in the same flask.

   Reduce the flux to a rate recommended in Table 4. At this point, the differential pressure should be ≤ 2 psi. If the pressure is greater, the septum may be too "tight" or the flux may be too great.

Performing the Filtration Test

1. Prepare the Feedstock

Using the guidelines in *Determining Capacity and Batch Size* (page 4), prepare enough feedstock to perform the first trial. Then, using the guidelines in *Selecting Body-Feed Grade* (page 3) and *Selecting Body-Feed Concentration* (page 3), weigh out the Celpure® body feed and suspend it in the feedstock. Maintain all solids (Celpure media and debris) in suspension with a stir plate.

2. Initiate Filtration

At this point, the filter should be in recirculation at a flux rate recommended in Table 4 (page 4).

Transfer the inlet to the body-fed feedstock. When you see feedstock entering the filter, transfer the outlet to the filtrate container and start the timer. Record the differential pressure and cumulative volume every 2-5 minutes depending on the rate of pressure increase. These readings will be analyzed to determine the conditions for subsequent tests.

3. Terminate the test

End the test when the differential pressure reaches its limit. Dynamic depth-filtration is best performed at < 40 psi. Proceed to *Assessment and Optimization* (next page).

Assessment & Optimization

1. Clarity

If the pooled filtrate clarity is unacceptable, repeat the experiment with a less permeable grade of Celpure® media until the clarity is acceptable (Table 1).

2. Differential Pressure & Body Feed

After determining the appropriate Celpure grade, maximize the capacity by ensuring that the cake space is filled at the same time the terminal differential-pressure limit is reached.

> ***Capacity vs. Cake Height***
> The greater the cake height the greater the filter capacity.
>
> The references to cake height limitations below are for GMP process validation. If the process is for the production of research material (*i.e.,* scalability is not an issue) do not limit cake height options. Cake heights of up to 20 cm are possible.

Pressure Increase Too Rapid

If the pressure reaches the limit (*e.g.,* 40 psi) before 50% of the cake space is filled, the body feed rate is too low or the flux rate is too high.

Begin by ruling out that the body feed concentration is too low. Confirm by doubling the body feed and repeating the experiment using the guidelines below.

- If the resulting pressure increase is less rapid, and within desired limits, advance to the *Pressure Increase Below Optimal* section.
- If there are no differences in the resulting rate of pressure increase, investigate whether the flux is too great (next paragraph).

After ruling out the above, and if the pressure increase is still too rapid, it is likely that the flux rate is too high. Repeat the experiment, but reduce the flux by 25%. If the pressure increase is less rapid, and within desired process performance, advance to *Pressure Increase Optimal* section. If not, repeat again, but further reduce the flux by 25%.

Pressure Increase Below Optimal

If the differential pressure is less than 50% its limit and the filter is more than 50% full of cake, there is an opportunity to improve the filtration capacity (at the same flux rate). Repeat the experiment using the same Celpure media grade at 75% the amount of body feed.

Pressure Increase Optimal

If the differential pressure is approximately 50% the limit, with 50% the cake capacity filled, then the body feed is near optimum (at that flux rate). Confirm with an extended run on a larger volume of feedstock (at the same flux rate).

> After optimizing for body feed usage, the ratio of solids to body feed should be maintained. A slightly over body-fed process is typically more robust than an under body-fed process. Therefore, tests with the highest anticipated solids content can establish conditions to manage variations.

3. Recovery

At the end of filtration, the filter cake will retain residual filtrate and therefore some product. This can be displaced with a post wash of compatible solution. In most cases, one or two portions equal to the volume of cake will be sufficient. Post wash should be applied before the feedstock uncovers the filter cake.

4. Scale up

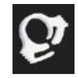
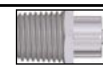
Given the filtration capacity determined by an extended run, the required filter area for the next scale will be directly proportional to the feedstock volume (for a given cake height). For example, if a 50-cm^2 filter processed 600 mL of feedstock, 1.0 m^2 will be required for 120 L.

Technical Support

Contact Advanced Minerals Technical Support at:

info@advancedminerals.com

Table 5. Bench Scale Filter Accessories

ITEM #	VENDOR	DESCRIPTION	PICTURE
TUBING & CONNECTORS			
EW-96440-16	Cole-Parmer	Tubing: Masterflex® platinum silicone tubing, L/S® 16, 25 ft.	
A-06359-37	Cole-Parmer	Luer lock fitting: Female luer x 1/8" hose barb; 25/pack	
EW-06832-01	Cole-Parmer	Luer lock fitting: Plastic hose clamps, white, 0.228" x 0.256", Acetal Copolymer; 100/pack	
NCI-00125-NG	Cole-Parmer	Luer lock fitting: 1/4" NPT (Male) to luer lock, polypropylene; each	
EW-08782-27	Cole-Parmer	Teflon PTFE Thread sealer tape (package of 10)	
PRESSURE GAUGE & ACCESSORIES			
EK06901100212	Anderson Instrument	Sanitary fitting: "Mini" pressure gauge, 3/4" Tri-Clamp	
EW-30541-00	Cole-Parmer	Sanitary fitting: Gasket, silicone, 3/4" (package)	
A-31200-50	Cole-Parmer	Luer lock/Sanitary fitting: 1/2" mini to female luer lock, Polypropylene; each	
A-30508-20	Cole-Parmer	Sanitary fitting: Nylon clamp, 1/2" to 3/4"	
STANDS & CLAMPS			
A-50001-92	Cole-Parmer	Mixer support stand	
A-08041-20	Cole-Parmer	Stand clamp	
A-08047-32	Cole-Parmer	Chain clamp	
PUMPS & ACCESSORIES			
07523-60	Cole-Parmer	Economy Variable-Speed Digital Drives: Size 16 tubing will give 8-140 mL/min; Size 17 will give 28-1700 Size 18 will give 38-2300	
EW-77200-60	Cole-Parmer	Easy-Load® II Pump Heads for Precision Tubing: Accepted tubing L/S 13, L/S 14, L/S 16, L/S 25, L/S 17, L/S 18	

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Glossary

Body Feed
Filter media suspended in the feedstock during filtration.

Cake Height
Thickness of the filter cake.

Cake Space
The volume of filter space that can accommodate the filter cake (*e.g.,* Celpure® media and cell debris).

Celpure® Media
A highly purified silica filter aid obtained from diatomite.

Darcy's Law
An empirical law that governs flow through porous media and describes the relationship among flow rate, pressure drop, and resistance. Filter aid products are usually processed to provide a range of filtration rates that are closely related to permeability as reported in Darcy units.

Depth filtration
Depth filters trap particles within a three-dimensional matrix. Capacity is determined by the depth of the matrix.

Diatom
See Diatomite.

Diatomite
Obtained from diatomaceous earth, a sediment of biogenic silica in the form of siliceous shells of diatoms, a diverse array of microscopic, single-cell algae. Diatomite products are characterized by an inherently intricate and highly porous structure composed primarily of silica.

Dynamic Depth-Filtration
Dynamic depth-filtration is a new microfiltration technique. It combines filter aid filtration with depth filtration, both of which have been widely used in biopharmaceutical separations since the early 1900s. Dynamic depth filtration typically increases the speed and solids capacity of conventional depth filtration by 5-10 times, while having the overall disposal (weight and volume).

Filter Aid
Inorganic mineral powders or organic fibrous materials used in combination to enhance filtration performance. Filter aids have been in use for over 75 years in the production of pharmaceuticals, biopharmaceuticals, and chemicals.

A process trade-off exists in filter aid technology between the permeability of the porous media and its turbidity removal capabilities. Filter aid products are produced in grades over a wide range of permeability ratings. As in depth filtration, the objective is to select a filter aid that removes only the size of turbidity necessary to achieve the desired clarity without unnecessarily reducing throughput performance.

Filter Aid Filtration
Filter aid filtration is a century-old solid-liquid separation technique typically used with compressible, low permeability solids (*e.g.,* bacterial debris). Its uniqueness lies in the suspending of filter media (*e.g.,* diatomite) in the feedstock prior to and/or during filtration. It can be contrasted with dead end and crossflow filtration in which the filter media are entirely immobilized in a filter element.

Filter Cake
Retained solids and filter media on the filter element.

Microfiltration
Microfiltration is a pressure-driven solid-liquid separation process. In general, microfiltration is capable of removing suspended solids in the 0.10-1.0 micron range. In comparison, ultrafiltration is generally used with solids in the 0.01-0.10 micron range.

Permeability
For use in filtration, diatomite products are usually processed to provide a range of filtration rates that are closely related to their permeability reported in units of Darcies. The selection of a filter aid with a particular permeability depends on the flow rate and degree of fluid clarification desired for the particular application.

Precoat
A precoat is a thin layer, 1.5 to 3.0 mm, of filter aid that is applied to the septum before the actual filtration process. A precoat is usually unnecessary when using a depth filter as the septum.

If a precoat is needed, weigh out the appropriate Celpure grade to produce a precoat of approximately 3.0-mm thick; this corresponds to 0.1 g/cm^2 of surface area. Prepare a Celpure suspension of approximately 2.5-5.0% by weight. (Concentrations below 0.3% take too long to precoat and above 15% are difficult to pump.) Maintain the suspension with enough stirring to suspend the particles, but not so much that a vortex reaches the bottom of the container.

Apply the precoat by recirculating the suspension through the filter at a rate of 20-40 L/m^2-min on 50 cm^2 (100-200 mL/min). Within approximately 10 minutes the solution will become clear, and an even precoat should be visible on the septum. If the precoat is not relatively flat, re-precoat with a different flux according to the following criteria:

- If the precoat is thicker in the center of the filter surface a greater flux rate is needed.
- If the precoat is thinner in the center a slower flux rate is needed.

Pressure Precoat Filtration

See Filter Aid Filtration.