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Centrifugal Analogs to Tangential Flow Microfiltration in Recombinant Cell Processing

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Figure 1- A typical purification train for microbial cell processing showing possible solid/liquid separation options

The advent of commercial biotechnology has made it possible to produce high concentrations of therapeutic proteins by the fermentation of recombinant microorganisms. In microbial fermentations, the products are typically produced either as soluble intracellular proteins or insoluble inclusion bodies. Prior to downstream purification of these products, it is common to employ a series of solid/liquid separation steps (often called harvest or cell processing operations) to separate the desired species from the fermentation media components and host cell impurities. A typical cell processing purification train is depicted in Figure 1.

The size and density of recombinant host organisms used in commercial biotechnolgy is such that both centrifugation and tangential flow microfiltration are equally viable alternatives[1]. When selecting a unit operation for recombinant cell processing it is important to have a clear understanding of the objectives of the operation as well as the process alternatives available. One potentially advantageous process alternative which is frequently overlooked is recycle in conjunction with high speed centrifugation.

This document provides an overview of sequential and recycle unit operations for microbial cell processing. It is intended to serve as a background for more in-depth comparisons between solid/liquid cell processing alternatives. A generalized materials balance model is presented herein to illustrate potential operational considerations for tangential flow filtration and its' centrifugal counterparts.

Figure 2 - A generalized Solid/Liquid Separator

Mathematical treatment of Generalized solid/liquid unit operation

In order to better illustrate the operational similarities between TFF/centrifugal cell harvesting, we will first consider a generalized solid/liquid separator. Such a separator is depicted in Figure 2.

A homogeneous feed suspension having a solids concentration x_{feed} enters the separator at a mass flow rate of W_{feed} and is separated into two streams: a solids enriched concentrate stream, and a solids depleted effluent stream.

Modes of Operation

There are four basic modes of operation for this separator:

(1) Discrete Concentration – A series of modules are connected such that the concentrate from each module in series becomes the feed for the following unit. Alternatively, a single module is used for multiple passes where the concentrate from one pass becomes the feed for the following. Clarified effluent is drawn off from each unit and not replaced.

(2) Discrete Washing – A series of modules are connected such that the concentrate from each module in series becomes the feed for the following unit. Alternatively, a single module is used for multiple passes where the concentrate from one pass becomes the feed for the following. Clarified effluent is drawn off from each unit; this mass is replaced by mixing an equal mass of wash buffer with the concentrate stream.
(3) Continuous Concentration – The concentrate from a single module is returned to the feed tank. Clarified Effluent is drawn off and not replaced.
(4) Continuous Washing – The concentrate from a single module is returned to its' feed tank. Clarified effluent is drawn off; this mass is replaced by adding an equal mass flow rate of buffer to the feed tank.

Concentration steps are utilized in recombinant cell processing for operations where the sole objective is capture of bulk solids. This includes both the recovery of whole cells from fermentation media and the harvesting of inclusion bodies from cellular lysate suspensions.

Due to the versatility of solid/liquid washing operations, washing may be employed for the harvesting of soluble products, removal of soluble impurities, and the classification of insoluble particulates. Examples for each of these being: the harvesting of soluble enzymes from cellular lysates[15], washing away soluble fermentation media components from a pre-concentrated whole cell suspensions[3], and selectively removing protein precipitates and cell wall fragments from inclusion body suspension.

Key Parameters

Regardless of the operational mode, the performance of this separator may be described in terms of two key parameters: the concentration factor and the rejection coefficient .

The Rejection Coefficient

The rejection coefficient (R) is a measure of the efficiency of the separator to partition solids into the concentrate stream and is defined as follows:

(1)

In TFF the membrane forms a physical barrier between the concentrate and clarified effluent streams. Rejection is governed by the pore size distribution of the membrane and the hydrodynamic radius of the particles in suspension. Microporous membranes provide a nearly uniform physical barrier, and as a result the rejection profile of the filter is very sharp[3]. During operation, the accumulation of solids on the membrane surface forms a "dynamic membrane" layer which can change the rejection profile of the membrane[2]. Due to the open structure of microporous membranes, the effect of the dynamic membrane on solids retention is usually minimal[3] until solids concentrations become high (in excess of 2%).

In centrifugation there is no physical barrier between the concentrate and effluent streams; separation is performed on the basis of the sedimentation rate of the particles. When the sedimentation rates are small (as they are with microbial cell suspensions), the terminal velocities may be approximated by the Stokes' equation[4]:

(2)

Where D is the Stokes' diameter of the solids, $\Delta\rho$ is the density difference between the solids and the fluid, a is local centrifugal acceleration, and μ is the fluid viscosity. Relating the rejection profile (or grade efficiency) of disk stack centrifuges to the hydrodynamics within the centrifuge bowl has been the subject of much research[4],[5],[6],[7]. For solid/liquid separators typically used in biotechnology, an approximate correlation which has good agreement with experimental data is[8]:

(3)

Where ω is the rotational rate, Q is the volumetric feed rate to the centrifuge and k is a proportionality constant.

The Concentration Factor

The concentration factor (α) is a measure of the separators' efficiency to partition liquid into the effluent stream and is defined as follows:

(4)

Where $W_{effluent}$ is the mass flow rate of the clarified effluent stream. In recent years a number of papers have been published on predicting the mass efflux from tangential flow microfiltration systems[9],[10],[11],[12],[13]. These models assume that efflux rate may be related to the D'Arcy Equation[13]:

(5)

here A is the filter area, ΔP is the pressure drop across the membrane, ρ_l is the fluid density and R_m is the membrane resistance. The cake resistance (R_c) is assumed to vary with the solids concentration, the dynamic membrane thickness and porosity, the particle size and orientation, and the fluid shear[9].

Solids-ejecting disk stack centrifuges intermittently discharge the concentrated solids from the accumulation space within the bowl. Modern disk stack centrifuges are capable of producing shots of highly reproducible volume[14] (V_{shot}). Typically the centrifuge operator is able to specify the shot frequency (t_{shot}), and thereby select the concentration factor:

(6)

where ρ_s is the density of the solid particles.

CONTINUOUS vs. recycle CONCENTRATION

By definition, a single pass through the separator increases the concentration by a factor of α. If further concentration is desired one might envision linking multiple separators in series to achieve a overall concentration many times higher than α. Such a system is depicted in Figure 3.

Figure 3 - Sequential configuration for solids concentration

Table 1 – Maximum achievable solids concentrations for various solid/liquid separators

Solid/Liquid Separator	Maximum Feed Solids Fraction (wt/wt)	Maximum Concentrate Solids Fraction (wt/wt)	Typical Concentration Factors
Intermittent Discharge (solid ejecting) Disk Stack Centrifuge	5%	15%	1-15
Continuous Discharge (nozzle) Disk Stack Centrifuge	10%	10%	1-10
0.22 μ Microfilters	3%	3%	1 - 1.05

In an ideal separator, the concentration factor of each unit would remain constant and the concentration after N sequential operations would be $x_{feed}\alpha^N$. In practice, solids/liquid separators have a maximum achievable solids concentration, x_{max}. As the concentration in the feed approaches this limit, the rate of efflux approaches zero and no further concentration can be achieved. As a result, each additional separator in series contributes less than the one before it. The maximum solids

concentration for several solid/liquid separation devices is summarized in Table 1.

Solids may alternatively be concentrated with a single separator by recycling the concentrate stream to the feed tank. If the feed tank is well mixed, the rate of efflux is equal to:

(7)

Because the efflux rate drops off to zero as the concentration approaches the maximum solids concentration, the removal of each successive volume of effluent takes slightly longer than the one preceding it.

Concentration in recycle mode is generally more desirable than sequential concentration when the concentration factor is nearly unity. As shown in table 1, this happens to be the case in crossflow microfiltration. Even though the concentration factor for solids-ejecting disk stack centrifuges is much higher, they may also be configured for recycle concentration of solids.

Unlike TFF microfilters, the solids concentration in the concentrate stream of centrifuges is frequently very close to the maximum achievable solids concentration. When only a small increase in concentration is desired, recycle concentration could allow the operator to achieve the desired α in less time and with less manual manipulation than a series concentration with resuspension.

Two operational challenges exist in continuous concentration with disk stack centrifuges:

(1) Breakthrough – Equation (3) indicates that the rejection profile in the disk stack is dependent on the fluid dynamic properties of the feed suspension. Since the fluid density and viscosity are dependent on the concentration in the feed tank, the rejection profile will be changing as a function of time. If the feed rate into the centrifuge is not adjusted accordingly, breakthrough of the product into the effluent may occur.
(2) Shot Frequency – in the intermittent discharge disk stack, the solids are ejected from the accumulation space at discrete intervals. As the concentration in the feed gets higher, the shot frequency needs to be adjusted in accordance with the rate of solids accumulation.

CONTINUOUS VS. RECYCLE WASHING

Solid/liquid wash steps may be used for the washing away of soluble impurities, the harvesting of soluble product, and the classification of polydisperse suspensions. A discussion of solid/liquid washing for soluble impurities removal is presented below.

From the material balances derived above, the fraction of soluble impurities remaining in the concentrate after a single pass through the generic solid/liquid separator would be:

(8)

Again, we can envision a sequential arrangement of solid/liquid separators which would have a removal efficiency many times greater than this value. Once such system is depicted in Figure 4.

Figure 4 - Flow diagram for sequential solid/liquid washing

If the rejection coefficient is one and the mass fraction of insolubles is constant, the concentration factor should be nearly constant throughout the series. Assuming this to be the case, the fraction of soluble impurities remaining after N sequential washes is given by:

Figure 5 - Fraction of insoluble impurities remaining after N sequential wash operations for various feed solids concentratoins. M equal to M_{feed}; R equal to one.

(9)

Let us consider for a moment a solid/liquid washing operation where only a fixed mass of buffer (M) was available. Using the sequential washing scenario, we could:

(1) add all of M to the feed stream and use a single solid/liquid separator to remove M in one step
(2) add fraction (M/N) to the feed stream of each of N solid/liquid separators in series.

Figure 5 depicts the fraction of the soluble impurities remaining for various values of N and x_{feed}. In order to obtain the most efficient washing for a given mass of wash buffer, N must be very large. In fact, as N goes to infinity, the fraction of solubles remaining at the end of the sequential train approaches:

(10)

Where M_{feed} is the mass of the feed stream (not including the buffer).

Alternatively, solid/liquid separators may be used for continuous washing by returning the solids to the feed vessel and adding fresh buffer at a rate equal to the efflux rate. A materials balance on the soluble impurities results in the following expression for the rate of change of the concentration:

(11)

When the rejection coefficient is one, integration of Equation (11) yields the same result as for an infinite number of sequential wash steps. Since it is physically impossible to perform an infinite number of steps, continuous washing should always provide more effective removal of soluble impurities for a given mass of wash buffer.

Although continuous washing is frequently associated with crossflow filtration operations, several industries use the centrifugal analog for purification of a diverse array of products[16]. It is the author's intent to present a detailed discussion of continuous washing applications in biotechnology and a comparison of TFF microfiltration and it's centrifugal counterparts.

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LITERATURE CITED

1. Bjurstrom, E., *Chem. Eng. Prog*., **81**, 2, 126 (1985).
2. Brown, D. E. and Kavanagh, P.R., *Process Biochem.*, **22**, 4 ,96 (1987).
3. Lasky, M. and Grant, D., *Amer. Biotecnol. Lab.*, **3**. 6. 16 (1985).
4. Ambler, C., J. *Chem. Eng. Prog..*, **48,** 150, (1952).
5. Frampton, G.A., *Chem. Process Eng.*, Aug, 402, (1963).
6. Jury, S.H., Locke, W.L., *AIChE Journal,* **3**, 4, 480, (1957).
7. Bohman, H., "Hydrodynamics of the liquid flow in the disk of a centrifugal Separator," presented at the 1st European Conference on Mixing and Centrifugal Separation, Cambridge (1974)
8. Brunner K-H, Molerus, O., *Germ. Chem. Eng.*, **2**, 228, (1979).
9. Defrise, D., Gekas, V., *Process Biochem.*, Aug., **23**, 4, 105, (1988).
10. Tanaka, T., Abe, K-I., Nakanishi, K., *Biotechnol. Techniques.,* **8**, 10, 743, (1994).
11. Foley, G., *Biotechnol. Techniques*, **8**, 10, 743, (1994).
12. Nagata, N., Herouvis, K., Dziewulski, D, Belfort, G., *Biotechnol. Bioeng*., **34**, 447, (1989).
13. Kroner, K.H., Schutte, H., Husted, H., Kula M.R, *Process Biochem.*, **19**, 2, 67 (1984).
14. De Loggio, T. , Ketki, A., *Chem. Eng.*, **101**, 1, 70, (1994).
15. Kroner, K.H., Husted, H., *Process Biochem.* **19**, 6, 170, (1984).
16. Murkes, J., *Filtration & Separation*, Sept/Oct, 485, (1967).