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Concentrating Nature's Bioactivity

High Density Fermentation Harvesting Using SmartFlow® TFF

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Summary:

- Achieve up to 100% cell recovery/removal, and 99% product recovery.
- No dilution required for high density fermentation inputs enabling higher recoveries and shorter processing times
- Low shear system design eliminates product damage in shear sensitive cells or molecules.
- Integrated continuous processing design incorporates cell mass separation, product concentration, diafiltration, and wastewater treatment into a single scalable system.
- Utilizing the patented DiaSync® process can provide significant savings in process times, buffers, and waste volumes.

Overview:

SmartFlow Technologies develops integrated systems to harvest fermentation product lines while producing concentrated solids and cell free permeates. The systems can be optimized for concentration of cellular mass, extracellular products, and recovery of intracellular products after cell lysis. The continuous process system designs integrate multiple steps in traditional fermentation harvesting into a single scalable, centrally controlled operation. Contrary to traditional fermentation harvesting or residual suspended solids content after centrifugal cell separation by microfiltration, SmartFlow systems utilize appropriately sized ultrafiltration membranes in the downstream processing. Ultrafiltration membrane provide longer, sustained run times without the fouling or clogging observed in microfiltration. When used in the harvest of extracellular products, proper selection of the ultrafiltration membrane will remove cell mass and high molecular weight dissolved contaminants while allowing target product passage, resulting in improved final product purity.

High Product Recovery:

Cell Harvesting: SmartFlow's patented *ConSep* II™ modules can concentrate cellular mass to produce a typical paste containing up to 100% wet weight. Wet weight determination is obtained after centrifugation of the sample and removal of the supernatant. The net weight of the packed cell pellet is the wet weight[1]. Depending on the organism, this correlates to a 30-35% dry matter basis. Cell mass product purity can be improved by in-line diafiltration to remove the residual media components from the concentrated cell mass more efficiently than typical resuspension and re-centrifugation processes. The required diafiltration volumes are significantly reduced as the remaining intracellular volume in the highly concentrated cell mass is substantially less. The concentrated cell mass in a continuous processes is bled off and can be directed to downstream processes such as drum or spray drying. Diafiltration removal of residual media and cellular contaminants can affect the taste and product profile (ash content, mouth feel) in target applications of food components or additives. SmartFlow offers its' patented *ConSep* II™ modules that can operate up to 80 °C, which can process harvest streams directly from the fermentor and heat up the cell mass prior to drying, saving energy in the drying step.

High cell mass concentration harvesting is a concern in centrifugation. A disadvantage of centrifuges with automatic discharge of solids is that the solids must remain sufficiently wet to flow through the machine[2]. Clogging discharge ports with high cell mass solids can result in catastrophic equipment failures and disruption to other downstream processes.

Extracellular product recoveries: The extracellular product fraction of the cell separation stage can be optimized through the selection of the appropriate membrane to allow free product passage while retaining the cell mass and high molecular weight extracellular contaminants using a *ConSep* II™ filter. The product can be concentrated and further purified utilizing a second stage containing a *ConSep* II™ filter that retains the target product molecule and passes salts, sugars and low molecular weight contaminates. Product purity can be improved by incorporating in-line diafiltration to remove the low molecular weight residual media components in the product concentration stage. SmartFlow's patented DiaSync® process integrates extracellular product recovery, in-line diafiltration, and product concentration into a single integrated process.

Intracellular product recoveries after cell lysis: Recovery of intracellular products after cell lysis traditionally uses centrifugation followed by depth filtration and an ultrafiltration product capture step. The efficiency of solids removal by centrifugation has been shown to be significantly reduced, when compared to whole cell separations, due to the higher viscosity of the lysate and greater proportion of fine cell debris particles that are difficult to remove by centrifugation[3]. Separate studies have demonstrated an unacceptably high solids content (∼21% solids carry over) in the supernatants of lysed cell broth using industrial centrifugation[4]. This is the reason the depth filtration step is required.

Systems using the *patented ConSep* II™ filter can replace the two step process of centrifugation and depth filtration providing essentially complete suspended solids removal in a single step. Additional benefits of high molecular weight dissolved solids separation, with correct membrane selection, can be achieved using ultrafiltration membranes and process conditions that provide high target molecule passage. Yields can be further improved by applying in line diafiltration to the lysate filtration step to ensure complete product passage. From this point, the downstream final product concentration and diafiltration step described above could be implemented.

Continuous processing:

Large scale industrial fermentation requires harvesting systems to operate in continuous processing modes to maintain production efficiencies. PuroSep® filtration systems are often designed for integrated continuous operations covering downstream processing steps from harvest through product concentration and wastewater treatment and any scale. Target cell mass concentrations are able to be standardized, independent of day to day variations in the fermentor output, for optimized downstream processes such as drying or other downstream processes. For extracellular products, the cell mass concentrates can be returned to the fermentor if desired. Alternatively, cells can be removed from the fermentor to maintain the cell population at optimal growth densities and fermentor sterility. In this case, the cell mass can be dried for sale into appropriate markets.

Direct loading into the system:

High density fermentation output can be fed directly into SmartFlow systems independent of cell densities. Continuous process PuroSep® systems are designed to match the process output from fermentation at any volume.
When using centrifugation in the cell separation stage, high density fermentation cell mass streams must be diluted before loading into a stacked disc centrifuge for optimal operations[5]. This increases the separation time and reduces product yield. Salte demonstrated the highest solids concentration that can be loaded into a centrifuge is 9% to achieve a target product concentration of 94%[6]. This required dilution significantly increases the process time required to perform the cell separation. This dilution adds to the total process wastewater production and adds cost to the product.

High viscosity processing:

ConSep II™ filters open channel design enable the SmartFlow TFF systems to process high viscosity fluid streams. For viscous product processing, increasing the channel height in the fluid flow path of the *ConSep* II™ module reduces the pressure drop along the length of the channel. In traditional TFF formats the limited number of channels heights and spacer geometries available do not offer the product specific module configurations to minimize the pressure drop within the filter. Product viscosity can also be reduced by processing at elevated temperatures. The SmartFlow "High Temperature Tangential Flow Filtration"[7] white paper demonstrates that filter flux can double by elevating the processing temperature from 40-80 °C. The increase in filter flux rate is due to the lower viscosity of water at 80 °C. If the target product is tolerant to high temperatures, filtering viscous solutions at 80 °C can reduce the filter area required significantly. Associated system and OPEX cost savings also accrue with the improved flux rates observed during high temperature processing.
In stacked disc centrifuges, cell disruption has been shown to increase with increased viscosity in centrifugation and is correlated to the turbulence (Reynolds number) created [8]. The increase in viscosity also significantly reduces solid–liquid separation efficiency in centrifugation[5] Stokes' law, demonstrates the centrifuge efficiency is dependent on the viscosity of the fluid[9]. The sedimentation rate of the particle decreases as the viscosity of the continuous phase increases.[10]
SmartFlow's open channel design enables shear and turbulence concerns to be addressed in process development and implemented through the selection of the proper channel height of the filtration module. The resulting module configuration is linearly scalable over any process volume.

Low shear operation:

The patented *ConSep* II™ filter systems open channel design utilize shear control as a critical process parameter in optimizing the membrane separation. For shear sensitive products, the SmartFlow process design stage incorporates the shear constraints into the determination of channel height and recirculation rates to assure the process runs within the shear constraints of the product. *ConSep* II™ filter channel heights are available from 0.5 to 3.0mm, offering a wide range of shear rates

generated. Typically, SmartFlow optimizes shear in process development and typically is in the 5-10X 10^3 s^{-1} shear range. For products that are extremely shear sensitive, SmartFlow has demonstrated separations of insect cell cultures with shear rates of 1400 $^{-1}$ sec.[11]
The use of Stacked disc centrifuge has been reported to produce high shear rates in the inlet and outlets of the centrifuge[12]. Salte used a shear rate of up to 10^6 s^{-1} when examining the difference between cells exposed to shear to cells not stressed by high shear rates[6]. Chan utilized a shear rates of 6.3 x 10^5 $s^{-1}$when examining the cell disruption as a function of residence time in a stacked disc centrifuge[8].
The use of *ConSep* II™ filters provide the opportunity to process shear sensitive products reducing shear induced product damage during processing. .

Integrated system design:

In a totally integrated system, multiple stages are employed. We have previously described the different individual stages to address product recoveries for processes where the product is the whole cells themselves, extracellular products, or intracellular product recovery after cell lysis. In industrial processes there are often ancillary products resulting from the outputs of the different stages in the process. SmartFlow has designed and commissioned systems that integrate control of the fermentation process from media prep to harvesting and CIP/SIP procedures into the filtration control panel. Downstream drying or product formulation can also be integrated into the single process control panel.
When the target product is extracellular, the cell mass may be concentrated and dried to produce animal feed or non-specific protein supplements. SmartFlow can integrate the total process, optimizing not only the target product process, but the ancillary product processes as well. One often overlooked integration potential is treating the permeate from the product concentration stage. The permeate can be processed to recover the sugars and other low molecular weight media components passed through the product concentration membrane. This stage produces a high sugar and amino acid concentrate and a low BOD water permeate. The water permeate from this step can be used for diafiltration in upstream processes, in plant washdown, cleaning, or discharged to drain without excess waste water fines or fees. This should be a consideration as wastewater has been estimated to contribute 10-15% of plant CapEx[13].
Warner has stressed the importance of an integrated process, starting with the integration of the pilot scale process before full production deployment[14]. SmartFlow can provide a fully integrated pilot scale downstream processing system utilizing multiple skids from our fleet of pilot skids, integrate them into a single computer controlled process.

High density fermentation system example:

High density fermentation provides significant economic advantages for commercial production. Increasing the cell density from 3-5% cell mass to 20-35% cell mass for the same fermentation volume can increase the product yield up to 10 fold. The *ConSep* II™ open channel design directly accepts the output of a high density fermentation process for immediate processing. Using traditional harvest techniques, a portion of the increased production density is lost in the product harvest step as described above. This is more significant in operations where the product is extracellular. Any lost or unrecovered liquid volume in the cell concentration step contributes to production yield losses. SmartFlow® TFF systems can significantly improve production yields in high density fermentation processes where the product is in the liquid fraction by implementing the DiaSync® process.

Typical stacked disc centrifuges will produce a paste of 70% wet weight in a fermentation harvesting application. This correlates to a 2X concentration of an original 35% wet weight high density fermentation run, excluding the inherent dilution required. The volume of recovery of the product containing centrate in this case is a 67.5% yield (Table 1).

Production yield can be improved to 95% by using the SmartFlow TFF modules. The SmartFlow technology has been demonstrated to achieve 90-95% wet weight pastes in fermentation harvesting applications. This results in an initial yield improvement to 90%. Further product recovery through the DiaSync® process can improve the product recovery to 95%.

Table 1.

High Density Fermentation			
Wet Weight	35%		35%
Dry Weight	20%		20%
	Disc Stack Centrifuge		SmartFlow
Wet Weight	70%		95%
Concentration factor	2		2.71
Product (Volume) recovered	67.50%		90%
In Line Diafiltration- DiaSync®	N/A		95-100%

Figure 1

Continuous Process Fermentation cell harvesting system with integrated process and CIP controls.

Commercial Process:

SmartFlow has developed and implemented integrated fermentation harvesting systems for over 30 years in pharmaceutical, food, and nutraceutical applications. Large scale installations utilize continuous process designs enabling 27/7 operation and fermentation output balancing, compensating for day to day variations in fermentation density. The introduction of the new *ConSep* II™ product line in 2019 has expanded the implementation of downstream processing of fermentation based products to enable high temperature operations. In one example of the high temperature advantage of the new patented *ConSep* II™ product line, one of the first production scale systems to implement the new module has been in continuous operation 24/7 for over 20 months with only planned shutdowns. The operation is running at 80°C and utilizes hot caustic CIP protocols to maintain filter performance. The integrated system control program rotates stages through operation and off-line caustic CIP processes to assure uninterrupted operation. During the period of operation there has be no degradation of filter performance due to plugging. This demonstrates high temperature industrial scale TFF systems can operate at scale cost effectively. Though a consumable, the *ConSep* II™ product line enhancements were engineered to provide a targeted 5 year life. Long filter module life minimizes down time and lowers total product cost.

Discussion:

Utilizing SmartFlow TFF for fermentation harvesting provides advantages over traditional separation methods in terms of yield, purity, and economics of operation whether the product is expressed or contained intracellularly. Benefits of continuous process design, direct loading, and integrated system design add to the process efficiencies realized in use.

When applied to high density fermentation, the advantages demonstrated increase dramatically. The integrated product recovery, in-line diafiltration, and product concentration embodied in the patented DiaSync® process enable continuous fermentation harvest in a completely automated system

With SmartFlow® TFF systems, target product concentrations are able to be standardized, independent of day to day variations in the fermentor output, for optimized downstream processes such as drying. Continuous processing featuring direct product loading from the fermentor has been described for both traditional and high density fermentation operations. Processing high viscosity or shear sensitive products can be efficiently processed with PuroSep® systems, where traditional fermentation harvest methods exhibit difficulties. By integrating other production processes into the PuroSep automation environment, full process integration from fermentation to drying or other final product preparation is available.

With the patented *ConSep* II™ filter's high temperature capabilities, SmartFlow TFF systems can optimize the downstream processing of fermentation products without being constrained by lower temperature limits required in typical spiral/hollow fiber TFF systems. This enables higher membrane flux rates, processing above the bacterial danger zone, altering enzyme activity, and effective microbial kill processes during processing. These high temperature benefits can add efficiency and reduce the cost of the total downstream processing operation.

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