

Bioseparation: The Integration Imperative

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Bioletics already represent 15 percent of a \$550bn pharma market and the development pipelines are full. Therapeutic anti-

Mammalian Cell Culture Manufacturing Not Yet Fully Exploited

Cultivated mammalian cells have become the dominant system for the production of recombinant

proteins for clinical applications. Today about 60–70% of all recombinant protein pharmaceuticals are produced in mammalian cells and many candidate proteins are currently in company pipelines, most of them banking on the promise of mon-

oclonal antibodies. Regarding fermentation technology, the preferred system is suspension culture, operated either as batch/fed-batch or as continuous perfusion culture. The individual use of the cultivation mode depends on the properties of the cell line and the product of choice. One of the features in mammalian cell culture technology is developed serum-free and protein-free culture media for hybridoma, BHK and CHO cells. Batch and fed-batch technology is used for high expres-

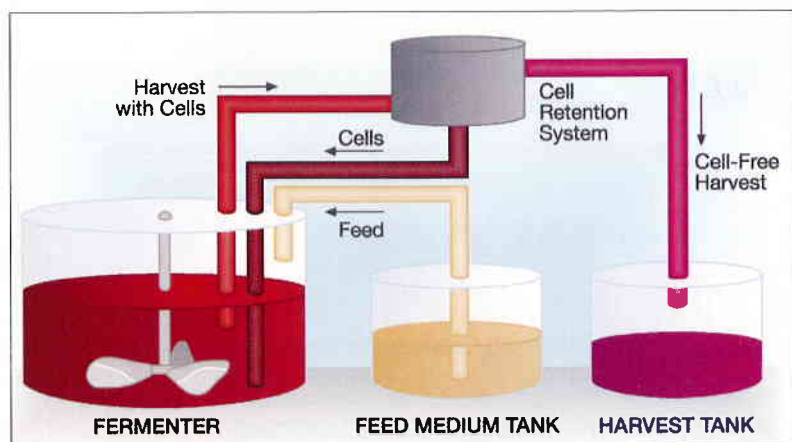


Fig. 1: Continuous perfusion culture: Capture at the frontline

bodies will reach 6 to 8 launches per year and approach US\$20bn near term. More than US\$10bn worth of biologic drugs are going off patent over the next 5 years. It has to be seen, if this products will be manufactured inside or outside ICH-level (International Conference on Harmonization) countries. Biomanufacturing is an expensive business. Approximately 75% is related to fixed costs and the remainder from variable costs related to process consumables. Manufacturing costs make 5 – 25% of total cost-of-goods (CoG). Requirements for efficacy, safety, and quality are steadily increasing and dictate competitiveness and payability. Thus, there are good reasons to tackle the backlog in bioseparation, where process determines product, and comparability determines quality.

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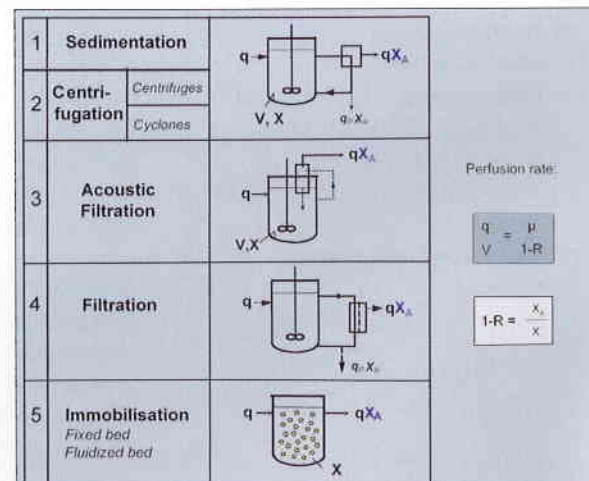


Fig. 2: Different methods of product harvesting



Fig. 3: Principle of mammalian perfusion culture

months. During this time period, culture fluid containing the released protein is continuously removed from the fermenter and replaced by fresh medium. Thus, cultures are operated under physiological steady-state conditions allowing a high degree of process control. High cell density is maintained by separating cells from the harvest fluid in an external cell retention device and recycling them back to the fermenter, yielding cell densities up to 30-fold higher than in batch cultures. A perfusion rate of 2–10 fermenter volumes per day is adjusted according to the actual cell density in the fermenter so that the specific perfusion rate as indicator of physiological steady state conditions is kept constant. In such a case product quality

and biochemical composition is constant over fermentation time. If the targeted maximal cell concentration in the culture is reached, it is kept constant by removing excess cells separately out of the fermenter or from the cell return line coming from the cell retention device. The major technological limitation in the scaling-up of perfusion systems is the need for robust retention devices to enable perfusion of medium as needed (Fig. 2). For this, currently available cell retention techniques are presented, namely, cross-flow filters, hollow fibers, controlled-shear filters, vortex-flow filters, spin-filters, gravity settlers, centrifuges, acoustic settlers, and hydrocyclones. Depending on the characteristics of the utilized production cells (Fig. 3), these retention techniques are compared and evaluated for their respective advantages and their potential

like spin-filters, continuous centrifuges, acoustic devices, etc, key features of the inclined plate settler, are scalability to perfusion rates of at least 3.000 l per day, reliable long term operation (with no moving parts or membrane transfers) and - most important - the selective removal of dead cells and cell debris out of the fermenter, because of their lower density compared to viable cells. The key know-how is to adjust the perfusion rate in a way that viable cells are retained and dead cells are removed by the settler. Therefore, fermentation results are very impressive resulting in long term performance of a culture without deterioration for up to 6 months including high viability of >95 % throughout the entire cultivation time and no

System	Capacity		Operation time	Maintenance	Investment	Availability
	q [L/d]	q/V[1/d]				
Filter	-	< 4	short	high	mean	yes
Centrifuges	>3000	-	short	high	high	yes
Cyclones	medium	< 1-2	long	low	low	yes
Acoustic Sys.	< 250	-	long	low	high	yes
Inclined Settler	medium	<15	100 d	low	mean	no

Critical points for realisation of perfusion culture

Fig. 4: Evaluation of retention techniques.

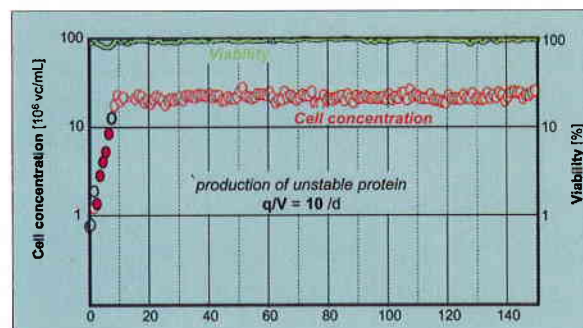


Fig. 5: Long term continuous fermentation with optimized settling systems

variation in product glycosylation or other biochemical product features. A typical perfusion culture at the 200 l scale consists of a target cell density of 20 – 40 million cells/ml, a duration of 2 – 4 months and a perfusion rate of 2 – 5 fermenter volumes per day. An example of such a culture is shown in Fig. 5. Suffice to say, that

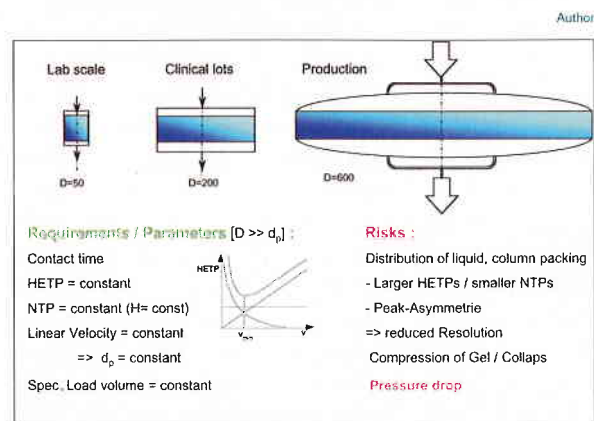


Fig. 6: Conventional scale up in chromatography

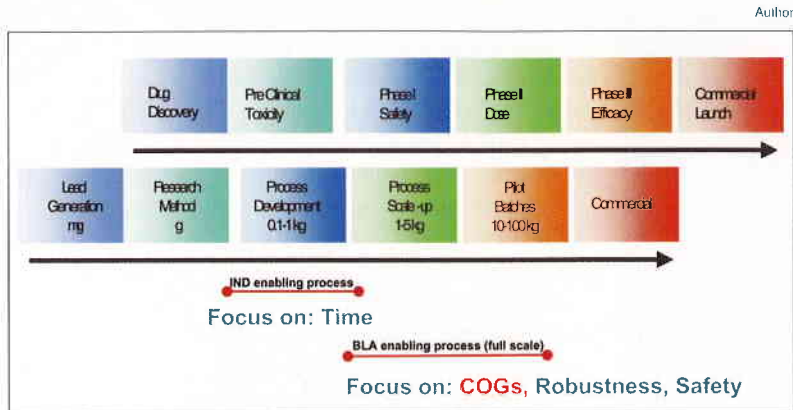


Fig. 7: The Focus is shifting during development ...

productivity from such a system is very high, since 400 – 1.000 l of product containing harvest are collected every day. The antihemophilic Factor VIII is reliably being manufactured using perfusion technology with suspension-cultivated BHK cells. Factor VIII is probably the largest (2.332 amino acids) and most complex protein ever produced in bioreactors. Bayer HealthCare, Berkeley CA, manufactures about 200 g per year with FDA-approved GMP-standards.

«Think big» is not enough

Advancements in genetic engineering and cell culture technology have driven protein expression levels higher than ever. So, the productivity of mammalian cells cultivated in bioreactors has reached the gram per liter range in a number of cases. Rapid progression of cell line and

low the upstream revolution. Reviewing simple, robust, and controllable technology is one option to overcome current challenges. 'Think big' is not enough, when conventional column scale-up is reaching its physical limits and the cost of column fillings is soaring beyond the limit (Fig. 6). Innovations in downstream processing are needed to accommodate improvements in expression titers and throughput. It is to "pass the lim-

fermentation development allows routine production of complex glycosylated proteins in high concentrations and leads to very large scale applications. Very often available purification technologies are not able to fol-

low purification processes capable of yielding up to kilogram quantities of e.g. purified MAb at the lowest possible cost. Productivity has to be increased through integration, reduced cycle times and built in automation with reduction of interfaces. An example for a highly integrated capture step is shown in Fig. 8. High-throughput capture and multi-tasking polishing platforms with robust, generic and modular unit operations are key to modern downstream processing.

Disposable technologies offer a novel manufacturing option. Bags instead of tanks make sense when the potential for cross-contamination is of greater concern and flexibility is



Fig. 9: Disposable manufacturing approach already has become real

solutions have to be well-balanced with reconsideration of "old" technologies like crystallization, extraction and precipitation. Time-to-market is still the most important driver, however, during development the focus is shifting to related costs (Fig. 7). Thus, the challenge is to develop and scale

needed. A common practice is the use of pre-sterilized flexible bags for medium and harvest (Fig. 9). The advantages of such a set up are: (a) flexibility as well as fast and efficient handling of new projects upon transition from one campaign to the next, (b) low qualification and validation efforts, since no cleaning in place (CIP) and steaming in place (SIP) processes are needed, (c) simple facility design avoiding complicated hard-piping, since steel and glass equipment such as fermenters and columns are used and connected to the bags via plastic tubes using tube welder or quick connector assemblies, and (d) low investment and maintenance



Multi-step front end purification

- Cell removal
- Concentration
- Diafiltration
- Capture chromatography

Integration of space and cycle time

- Optimal use of space
- Minimal piping volumes
- Fully automated (incl. buffer preparation, integrity testing, CIP/SIP, documentation)

Fig. 8: Integration of unit operations: Integrated recovery