

High Productivity Harvest: A Novel Approach to Fed-batch Process Intensification

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Introduction

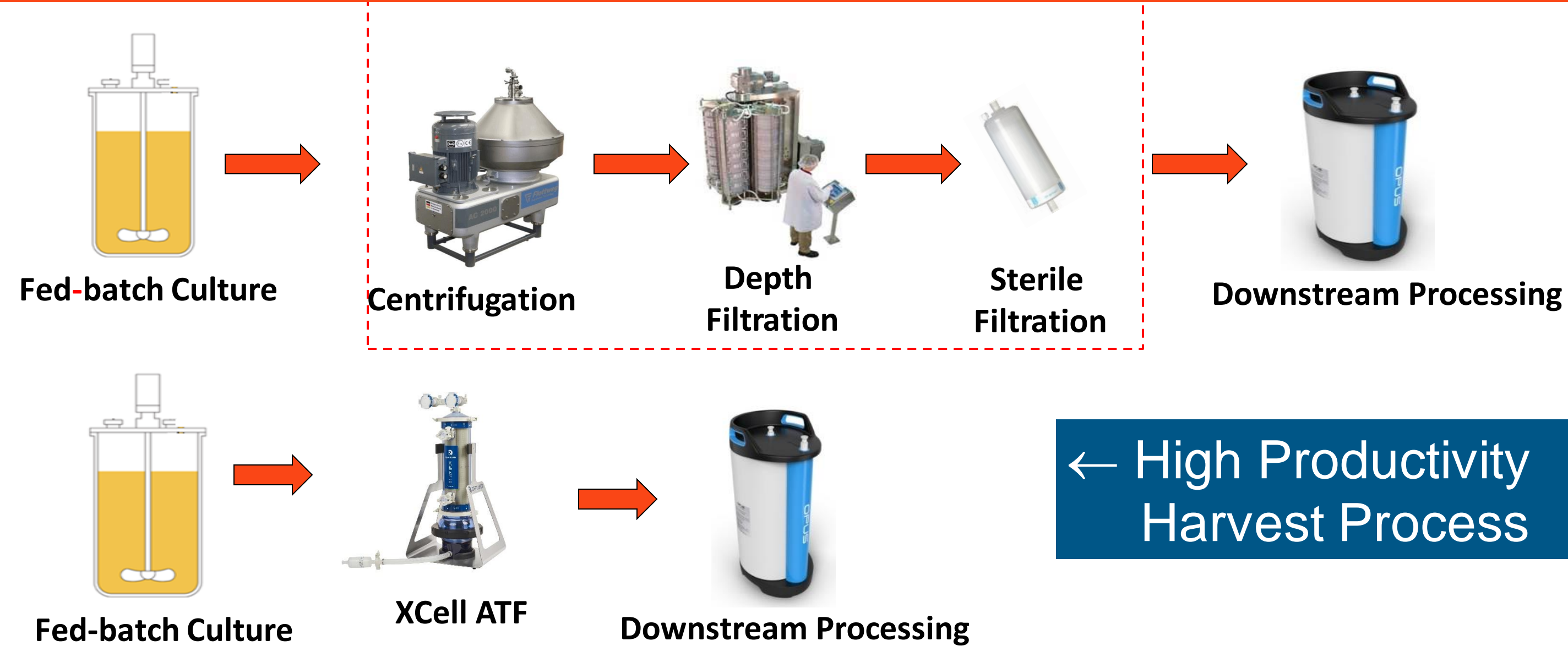
Fed-batch cell culture processes require a clarification step to recover product without altering the product quality. It is important to remove cell debris and solids prior to further downstream processing and loading on expensive capture columns to increase column lifetime and product purity. The current standard clarification technology of centrifugation and depth filtration often requires open processing and a large footprint. This multi-stage process is cumbersome, difficult to scale, and regularly impacts protein yield. We present a scalable single-step novel clarification method, High Productivity Harvest (HPH), for fed-batch cultures using the XCell™ ATF System. Unlike centrifugation and depth filtration, HPH allows for an integrated clarification process while maintaining cell viability to minimize protein degradation. HPH has been shown to elevate protein production compared to a traditional process.

In the case study presented here, HPH was initiated at 0.25, 0.5 and 1.0 vessel volumes per day (vvd) four days prior to the end of fed-batch cultivation using an XCell™ ATF System. During this period, perfusion methodologies increased viable cell density (VCD), and maintained cell viability at >90% due to the replacement of nutrients and removal of toxins. The same XCell™ ATF system was then used to harvest the remaining protein from the bioreactor on the final day of cultivation. These results indicate that protein output can be doubled and the cell suspension clarified without increasing the original fed-batch process time. The entire process is performed as a single step in an integrated, enclosed, bioburden free environment, yielding clarified product ready for downstream purification.

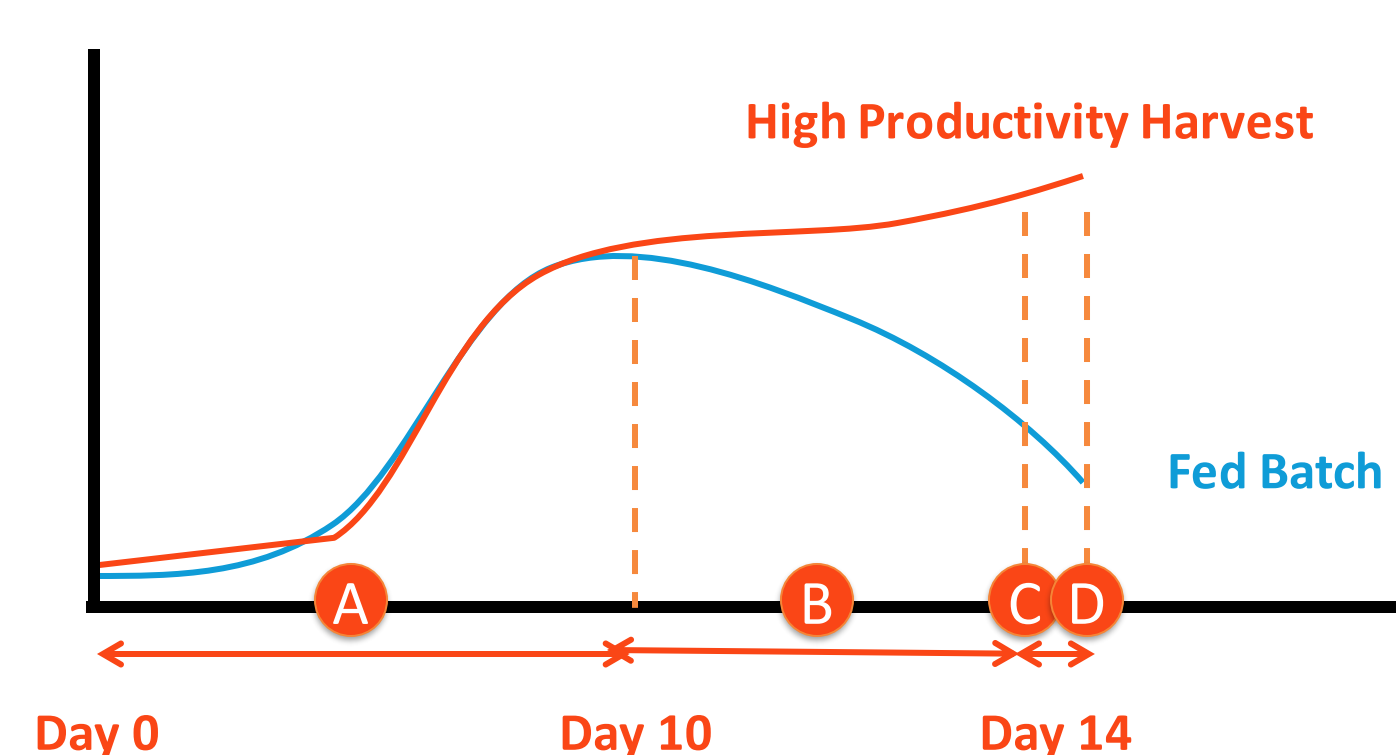
Method

Components /Conditions	Cell Culture	Components /Conditions	Traditional Fed-batch	HP Harvest Fed-batch
Cell Line	CHO-DP12	XCell™ ATF System	XCell™ ATF2 system with C24 v2.5 controller	
Antibody	Anti-IL8 mAb	Hollow Fiber	PES 0.2um hollow fiber cartridge	
Media Composition	CD Opti CHO media + 100ng/mL LR3 + 4mM Glutamax	Fed-Batch Duration	14 days	
Bioreactor	Applikon and Eppendorf glass bioreactors	Feed/Perfusion Rate	Day 3: Feed 10% Day 6: Feed 10% Day 9: Feed 10%	Day 3: Feed 10% Day 6: Feed 10% Day 9: Feed 10% Day 10: Continuous Harvest
Bioreactor w.v	1-1.2L	Drawdown Clarification (Flux)	N/A	20LMH
Temp	37C°	Shear Rate	N/A	2000s ⁻¹
pH	6.8 – 7.2			
Agitation	400 rpm			
DO Set point	40%			
Air	500 mL/min through drilled hole sparger			
Seeding Density	~0.4E6 Cells/mL			

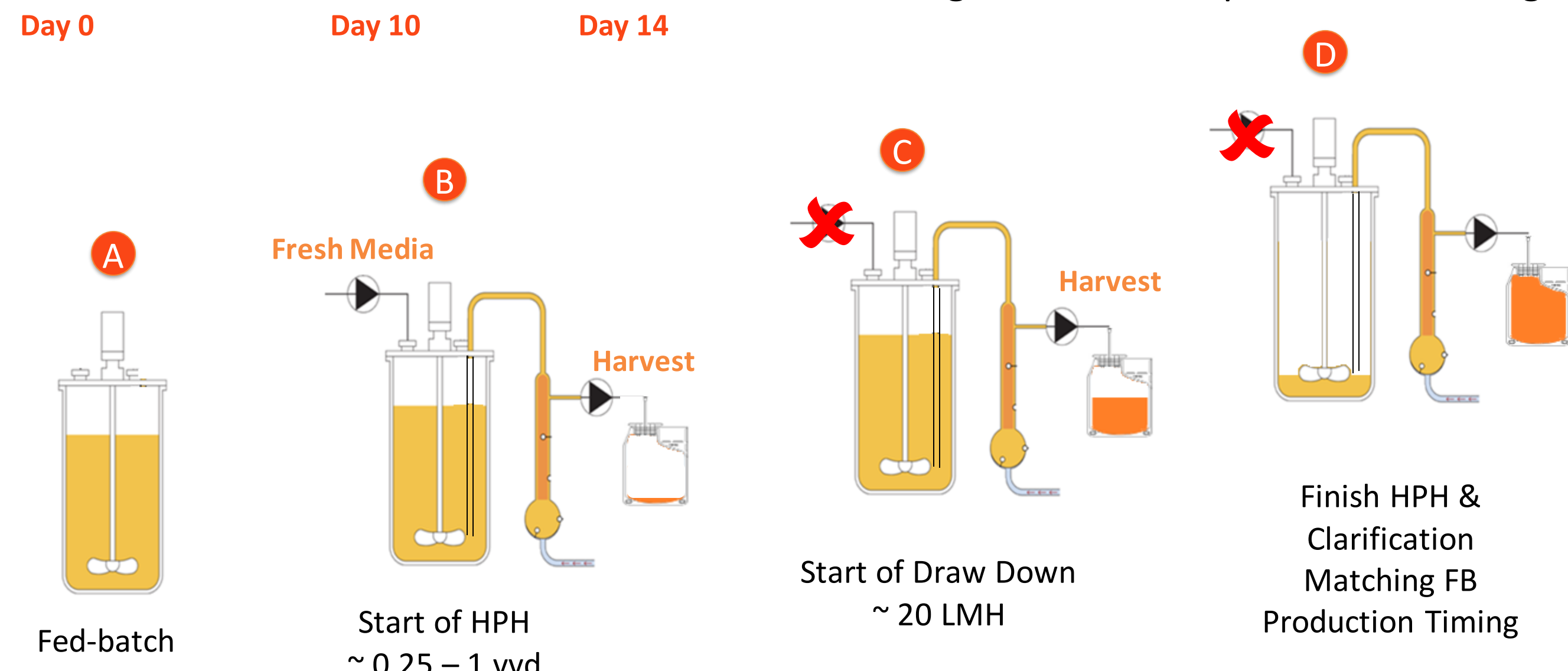
Conventional vs. High Productivity Harvest Process



Novel High Productivity Harvest Process using XCell™ ATF

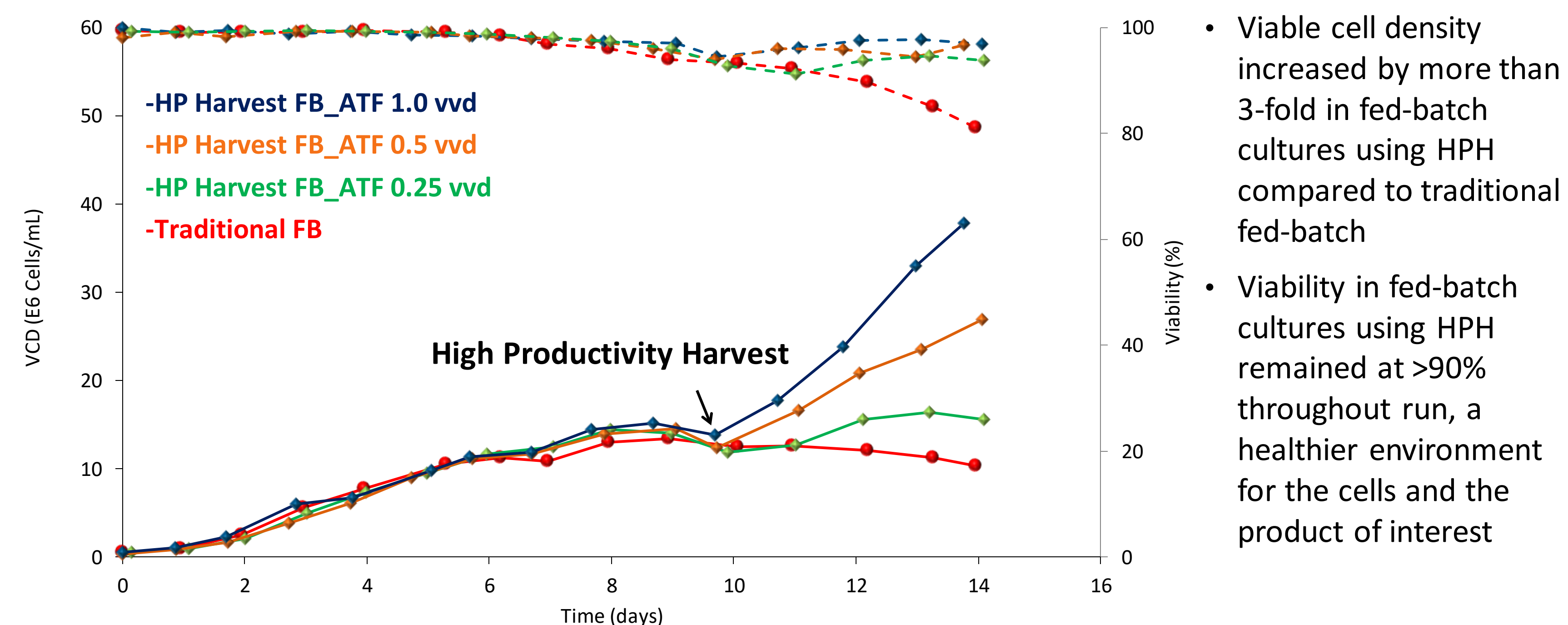


The fed-batch process is started per typical procedure (Step A). Once the peak cell density is achieved, Day 10 in this example, fresh media is pumped in at a set flow rate (Step B). As fresh media is pumped in, product harvesting is initiated. On the last day of the run, media addition ends and draw down of the volume in the reactor begins at 1-2 vvd (Step C, ~4-8 hours). The HPH process is completed to match the original fed-batch production timing.

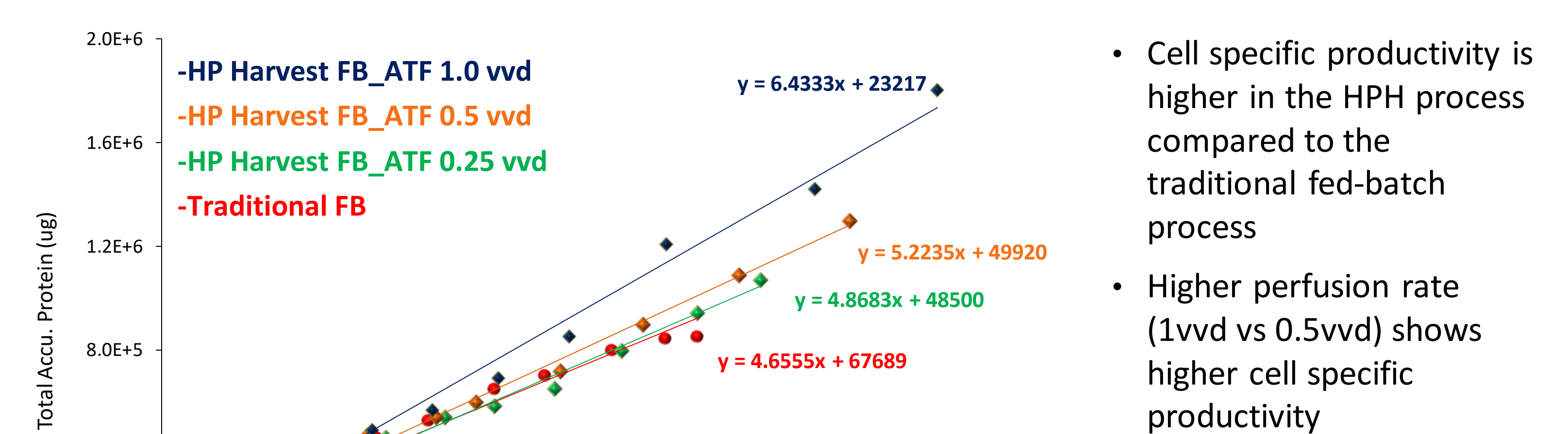


Results

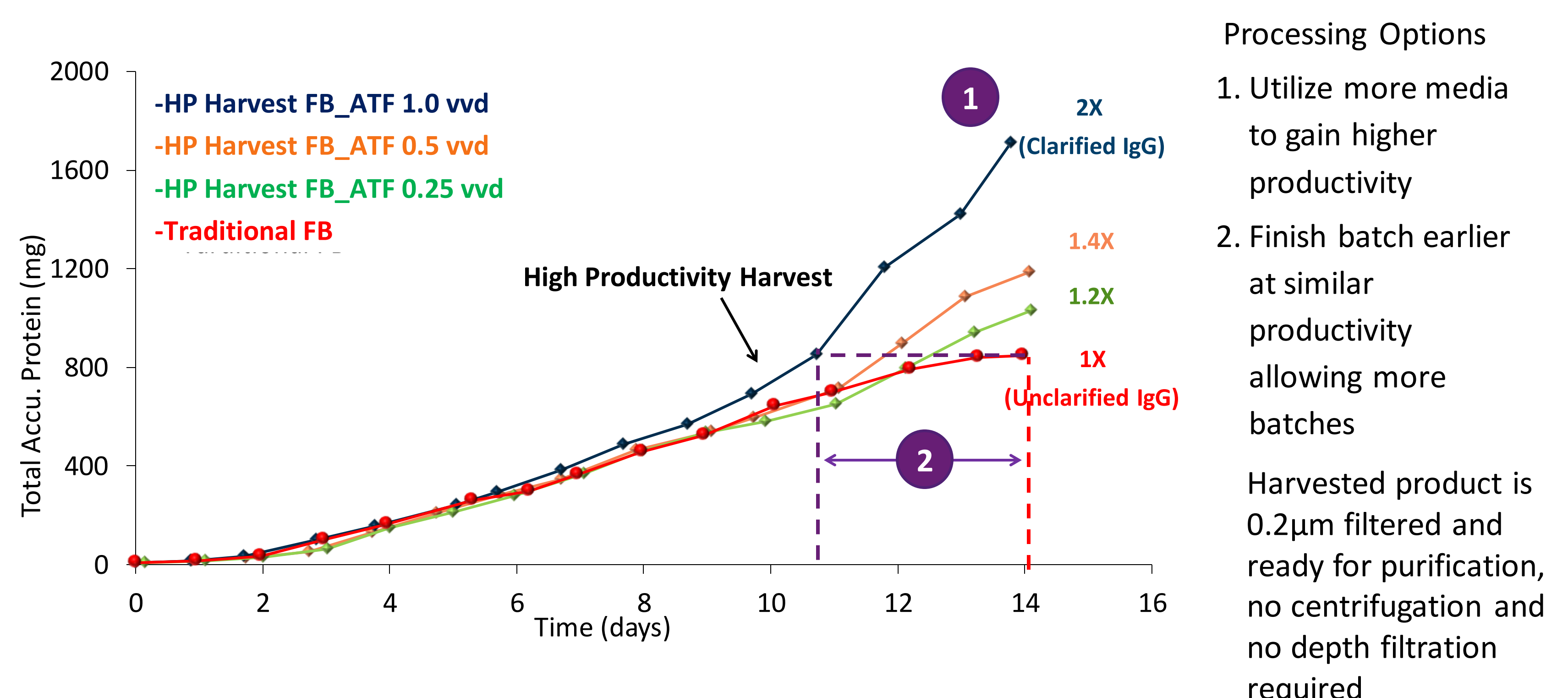
Viable Cell Density and Viability



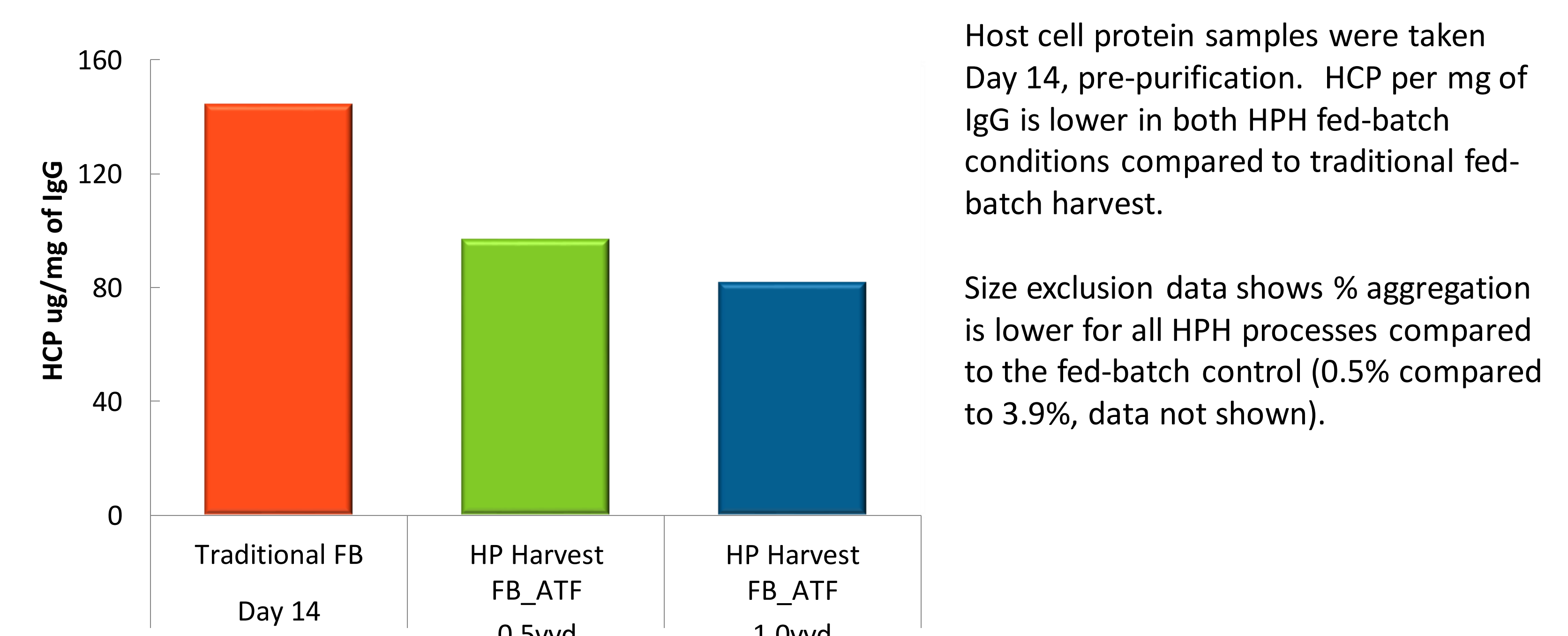
Cell-specific Protein Productivity



Total Accumulated Protein



Product Quality



Conclusions

HPH is well aligned with industry trends towards process intensification, process flexibility, and working within a single-use format in a less qualified working environment. The productivity and process benefits include:

- Up to 2X increase in protein production without additional time OR harvest days earlier at the same titer, increasing facility throughput
- Healthier culture = lower process related impurities
- Elimination of centrifugation and depth filtration equipment and operations
- 0.2µm filtered, closed system and single step process
- Harvested material is ready for purification