Sunflower

Application Note

Upstream Process

Keywords

Bioreactor, continuous bioprocess, perfusion fermentation, protein manufacturing, yeast

Space-Time Yield

A critical metric for comparison of upstream biomanufacturing processes

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Abstract

Space-time yield is a metric to analyze upstream process yields throughout a production campaign. This metric enables distinct methods for protein production, such as batch, fed-batch, or continuous fermentation, to be directly compared to one another. This approach of direct comparison provides researchers with the capability to consider process yields, input costs, production time, and facility constraints within their evaluation of potential unit operations for the design of a goal-oriented manufacturing strategy. In this application note, we explore how to calculate space-time yield using *Pichia pastoris* and Chinese hamster ovary cells (CHO) as model organisms, and we highlight the unique differences between fed-batch and continuous fermentation processes that result in divergent space-time yields. Since higher space-time yields ultimately result in more protein for less space and time, considering space-time yield when choosing a manufacturing plan can be a useful tool for lowering future commercial production costs.

Introduction

Titer is not a good metric for comparing fed-batch and continuous fermentations

Product concentration, or titer, is often used to describe the productivity of fed-batch fermentations, where the goal is to achieve a large quantity of a heterologous protein for further downstream processing. Titer is the amount of protein present in the bioreactor at any given time reported as a concentration (e.g., g/L). The titer reached at the end of a fed-batch fermentation is essentially the total quantity of protein made throughout the process, which can be used to compare the productivity of processes performed in a similar format (time, volume, media, host organism, cultivation type, etc). In continuous processes, however, nutrients are constantly fed to the bioreactor while secreted protein and waste products are continuously removed from the bioreactor. This results in an extremely dense biomass that can maintain protein expression at high levels throughout an extended fermentation campaign. For example, in perfusion fermentation, secreted protein is continuously harvested from the healthy biomass throughout the fermentation campaign, which typically runs for time periods twice as long as similar fed-batch fermentations. In such continuous processes, the reactor titer at a given time point is not an appropriate representation of the overall harvested protein yield.

Space-time yield is a measure of efficiency and productivity for continuous processes

Space-time yield is a normalized metric for productivity that can be used for any cultivation mode or protein expressing host organism. Space-time yield is defined as the total mass of protein produced per bioreactor volume per cultivation day irrespective of the method of cultivation, whether batch-based or continuous. This versatile metric normalizes the quantity of protein per cultivation scale and cultivation length, allowing for convenient comparison across scales or process lengths, which often differ between cultivation methods. For example, continuous fermentation campaigns are typically lengthy and can often be extended with minimal additional effort. Fed-batch campaigns, however, are usually shorter than continuous ones and cannot be significantly lengthened due to fundamental limitations in nutrient availability as the campaign extends in time. This leads to increasingly unhealthy biomass over time, which ultimately causes a culture to stop producing heterologous protein in fed-batch processes. Given these differences in cultivation operations, space-time yield provides researchers with a tool to select the optimal process for their production needs based on a daily yield comparison.

Here, we describe how to calculate the space-time yield for different processes and demonstrate the utility in using this metric for comparison across fermentation modes, cultivation lengths, and scales.

Methods

The mathematical framework presented in Bausch et al., was used to determine the space-time yield of several cultivations using different manufacturing strategies and hosts[1]. The key characteristics for the model cultivations are shown in Table 1.

Fed-Batch and Continuous Fermentation for *Pichia pastoris*

Comparison of a traditional fed-batch fermentation (Process 1) to a perfusion fermentation process representative of a process executed using Sunflower's Daisy Petal™ Perfusion Bioreactor System (Process 2) was performed using assumptions for key process parameters relevant for the fermentation of *Pichia pastoris*. For both *P. pastoris* fermentation modes, a two stage process was assumed, including a biomass accumulation phase at the maximum growth rate, followed by a production phase after the maximum viable cell density is met. Fermentation lengths of 6 days for fed-batch and 12 days for perfusion were assumed based on literature reports and operational experience. The specific productivity, defined as micrograms of product produced per gram of cells per hour during the production phase of fermentation, is assumed to be the same in both types of operation. The maximum viable wet cell weight was assumed to be higher for the

TABLE 1: Key model parameters and assumptions used to compare cultivation processes.

	Process 1	Process 2	Process 3
Host Organism	*Pichia pastoris*	*Pichia pastoris*	CHO
Fermentation Type	Fed-Batch	Continuous	Fed-Batch
Bioreactor Volume (L)	1	1	1
Cultivation Length (days)	6	12	14
Specific Productivity ($\mu g/g_{cells}/hr$)	100	100	500
Maximum Cell Growth Rate (hr^{-1})	0.15	0.15	0.03
Maximum Viable Wet Cell Weight (g_{cells}/L)	350	550	40
Perfusion Rate (vvd)	N/A	1.8	N/A
Cell Bleed Rate (vvd)	N/A	0.14	N/A

perfusion process based on the healthier cell environment provided by continuous fermentation and Sunflower's routine maintenance of >600 g/L wet cell weights during perfusion campaigns executed with the Daisy Petal™. The perfusion rate and cell bleed rate were reported in vessel volumes per day (vvd). For the continuous process, protein removed through the cell bleed line is not considered in the product yield, since it is directed to waste and not typically recovered.

Fed-Batch Cultivation for CHO

Parameters for the CHO fed-batch cultivation were taken from Bausch et al[1]. Production and growth were assumed to happen simultaneously. For the CHO fed-batch process, the maximum viable cell density (20 x 10^6 cells/mL) and specific productivity (25 pg/cell/day) were converted assuming an average mass of 2 ng per cell[1,2].

Results & Discussion

In this study, we compare the productivity of cultivation processes with different host organisms and manufacturing approaches. The wet cell weight, instantaneous protein titer, cumulative protein yield, and space-time yield were calculated for each process.

Fed-Batch and Continuous Fermentation for *Pichia pastoris*

A fed-batch and a continuous fermentation process were compared using the same host – *P. pastoris* (FIG 1). Two key differences in these fermentation

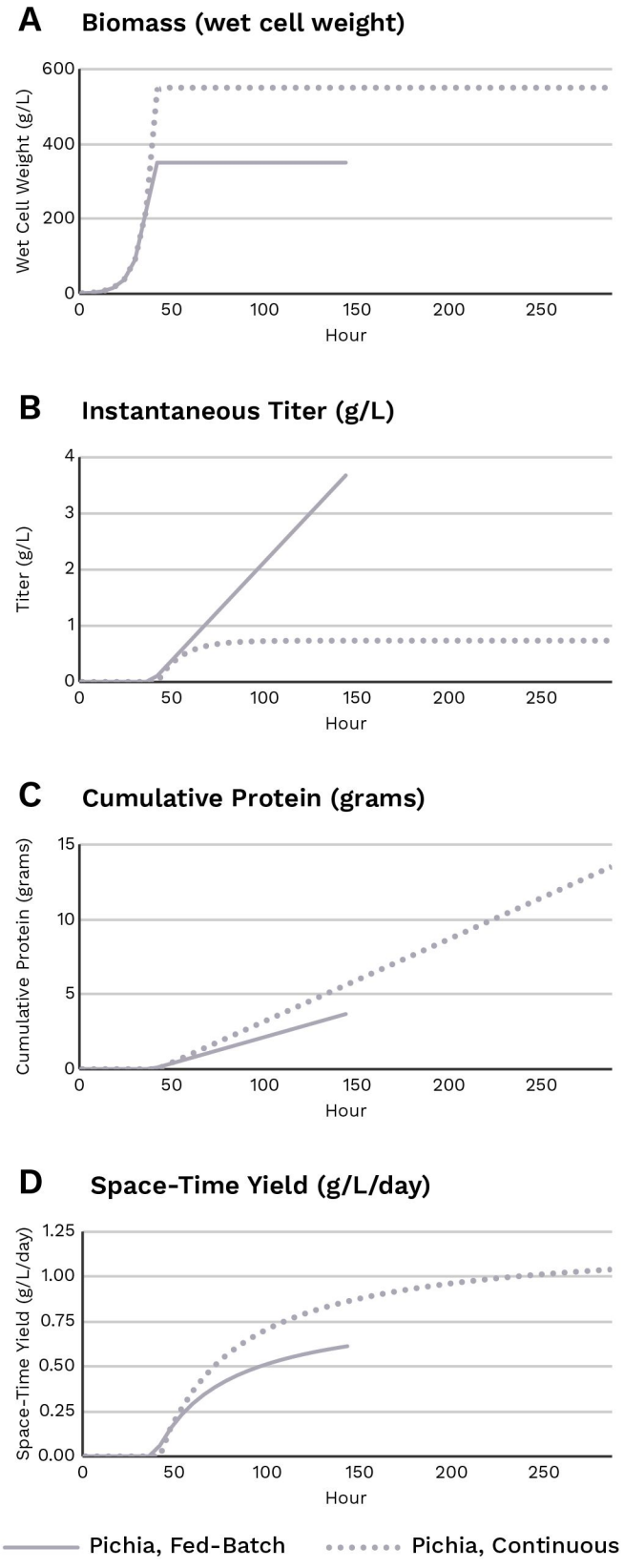

FIGURE 1: Process and productivity comparisons between two P. pastoris fermentation modes.

processes are the biomass achieved during production and the overall fermentation length. Continuous fermentation provides a healthier cell environment in the bioreactor since nutrients are continuously replenished and waste products are continuously removed. This results in higher achievable biomass that can be sustained for significantly longer as compared to fed-batch processes (FIG 1A).

Figure 1B shows the comparison of protein titers between the two fermentation modes. In the fed-batch process, the titer increases over time until the fermentation is stopped due to declining cell health after day 6. The final titer reached in the bioreactor is 3.7 g/L. In the continuous process, a steady state titer of 0.73 g/L is reached after about 5 days. This titer is maintained for the next 7 days until the end of the campaign (12 days total fermentation length) as the biological process is held in a steady state for continuous feeding and continuous removal of cell waste.

In Process 1 (fed-batch), the titer of protein in the bioreactor at harvest is representative of the cumulative amount of protein made during the cultivation (FIG 2). In contrast, the titer observed upon sampling Process 2 (continuous) is not

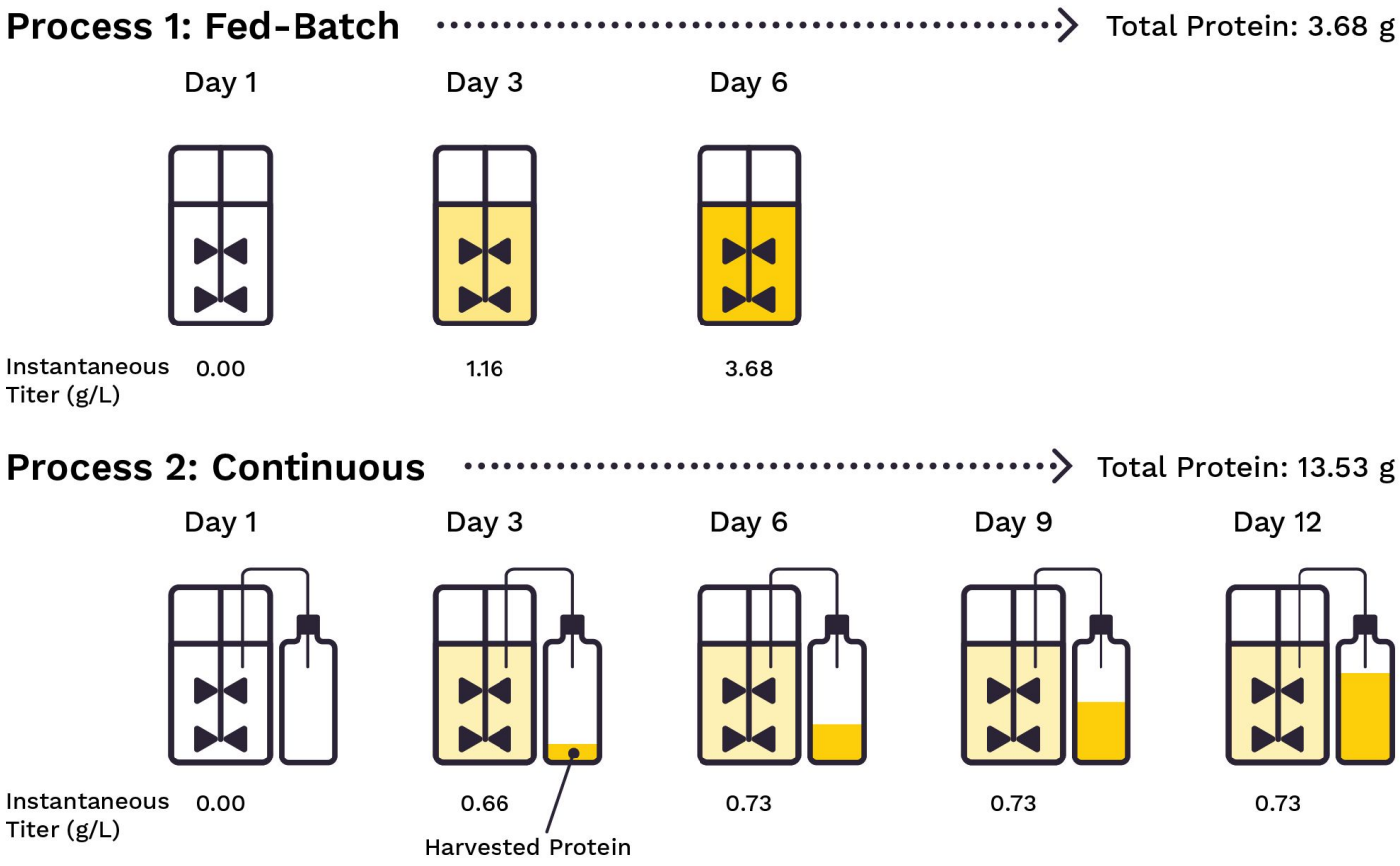

FIGURE 2: Representation of two fermentation types and differences in cumulative protein harvested.

representative of the cumulative protein expressed, since protein is continuously harvested during the production process. As shown in Figure 1C, the cumulative protein in the continuous process is immediately higher than that of the fed-batch process due to the higher achievable cell mass at the time of induction. After 6 days, the continuous process has produced 5.6 grams of product. This is over 40% more protein than expressed during the entire fed-batch process (3.7 grams). Furthermore, the continuous process extends for twice as long as the fed-batch process, ultimately resulting in over 13 grams of harvested protein, more than 3-fold more protein than the fed-batch process (FIG 2).

Since the cumulative protein harvested will vary across cultivation scales and lengths, a normalized metric is needed for a direct comparison between fermentation operations. Space-time yield, which is the total protein harvested per bioreactor volume per cultivation day, is a normalized metric for productivity that can be used for any cultivation mode. As shown in Figure 1D, the space-time yield for a continuous process is typically also higher than that for a fed-batch process of the same length. Notably, the space-time yield of a continuous fermentation improves over longer cultivation times, since the initial cell growth period is amortized across the total cultivation length.

Comparison to CHO Fed-Batch Cultivation

Figure 3 shows the comparison of a fed-batch cultivation using CHO cells to produce heterologous proteins to both fed-batch and continuous fermentation processes using *P. pastoris* as described above. In this comparison, we assumed that the CHO cells had a 5-fold higher specific productivity (using parameters from Bausch et al.). The growth rate and maximum cell viability, however, are significantly lower for CHO cells. This results in slower biomass accumulation and significantly less biomass overall during cultivation (FIG 3A). Accumulated biomass is frequently ten times higher in *P. pastoris* fermentation processes compared to CHO, suggesting that researchers should consider protein composition, time constraints, and equipment usage when selecting an expression organism[3].

As shown in Figure 3B, the titer achieved in a fed-batch campaign executed with CHO cells is higher compared to either of the *P. pastoris* fermentation processes, but it takes substantially longer campaigns to achieve those titers, which has significant implications for facility utilization and process scheduling within a commercial operation. Using the normalized metric of space-time yield (Figure 3D), it is clear that the space-time yield achievable for Pichia-derived processes in fed-batch or continuous operations is significantly higher than that achievable for a comparable CHO-based process, even using the assumption that CHO cells are typically 5-fold more

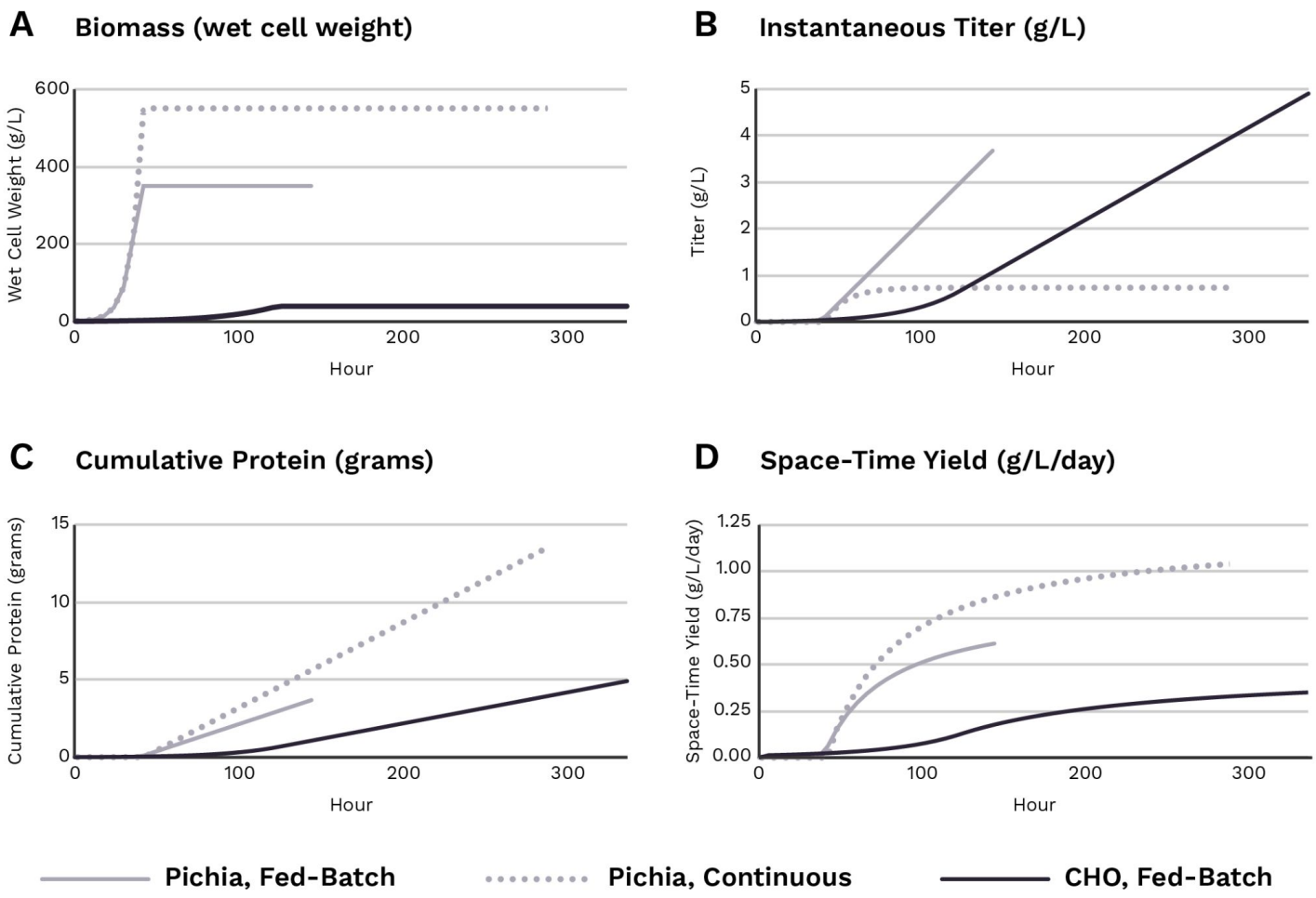

FIGURE 3: Process and productivity comparisons between model organisms and fermentation modes.

productive on a per cell mass basis. Overall, the continuous fermentation process results in a nearly 3-fold higher space-time yield, as compared to the campaign executed with CHO in fed-batch. We note here that our calculations model space-time yields, which are ultimately derived from cell-specific productivities. This fundamental metric should be calculated for each protein and strain based on growth rate and volumetric productivity[2-4].

Conclusion

Space-time yield is a normalized metric that can be used to directly compare productivity across cultivation modes and even different host organisms. Of the three processes modeled here, the continuous fermentation process using *P. pastoris* showed the highest achievable space-time yield, even with the assumption of a 5-fold lower specific productivity. High space-time yields ultimately result in more protein for less space and time, and therefore lower production costs. We advocate accordingly for space-time yields to be considered as a critical metric when assessing the potential of a draft process to achieve translational, clinical and commercial manufacturing goals for a given protein product.

References

1. Bausch, M., C. Schultheiss, and J.B. Sieck. Recommendations for comparison of productivity between fed-batch and perfusion processes. Biotechnol. J. 14, 1700721 (2019).
2. Kunert, R. and D. Reinhart. Advances in recombinant antibody manufacturing. Appl. Microbiol. Biotechnol. 100, 3451-3461 (2016).
3. Kunert, R., J. Gach, and H. Katinger. Expression of a Fab fragment in CHO and Pichia pastoris. A comparative case study. BioProcess Int. June Supplement, 34-40 (2008).
4. Maccani, A. et al. Pichia pastoris secretes recombinant proteins less efficiently than Chinese hamster ovary cells but allows higher space-time yields for less complex proteins. Biotechnol. J. 9, 526-537 (2014).

Related Resources

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Application Note: Perfusion fermentation in the Daisy Petal™ bioreactor