Sunflower

Application Note

Equipment Highlight

Keywords

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Advanced Media Control Strategies Using the Daisy Petal® System

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Abstract

Supporting the growth and productivity of fermentative organisms requires proper feeding of carbon and other critical nutrients. In biomanufacturing, many feeding strategies are employed across different process modes - including batch, fed-batch, and continuous operations - providing both advantages and disadvantages for cell-based systems. Utilizing the appropriate feeding strategies for a given organism is advantageous because it enables the capacity to adequately match nutrient supply to the organism's metabolic needs. In return, this allows for more consistent growth, increased biomass production, and improved product yields. The Sunflower Daisy Petal® Perfusion Bioreactor System is equipped with a unique dual pump setup that enables the configuration of advanced media control strategies. In this application note, we explain in-depth the media control strategies of the Daisy Petal® and describe its capabilities for optimizing feeding during perfusion fermentation across different use cases.

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Introduction

In biomanufacturing, microbes often are used to produce high-quality recombinant proteins or biologically-derived products for use in therapeutics and bioindustrial applications. Appropriate nutrient feeding is crucial for controlling cellular growth and metabolism, maximizing product yields, and minimizing the expression of fermentative byproducts. Cells require media containing many components to remain productive, with some needs varying over time and/or in response to cellular behavior. Additionally, process-specific additives such as lipids, growth factors, and transcriptional inducers are often employed in certain applications.

Many feeding strategies are used by fermentation experts to deliver adequate nutrient supply and meet cellular requirements, which vary depending on the process type. In batch and fed-batch processes the feeding strategies available are often inadequate to keep up with the metabolic demand that cells bring forth. Appropriate feeding practices are important to maximize biomass and process outputs, however, this also leads to high production of metabolic wastes and eventually culture crash.[1] Continuous bioprocesses are more effective at maintaining cultures for extended periods of time thanks to the constant removal of spent media,[1] but this can come with increased complexity as feeding needs to be optimized for longer durations.

One of the biggest challenges of current approaches to feeding is the lack of modularity. Typically, supplying media or substrate to a bioreactor involves the use of a single feed pump. This configuration makes it difficult to control nutrient concentrations and their respective ratios within the reactor. Usually, a concentrated solution of the main growth substrate (i.e., carbon source) is fed into the reactor along with a few other key nutrients (Figure 1A). These low-volume and high-concentration additions often lead to variable concentrations of other nutrients in the bioreactor. Some vendors offer modular hardware configurations that can add extra pumps to a pre-configured system, but these are often costly and complicated for users to implement on their own, requiring expert knowledge to control and design experiments.[2]

The Daisy Petal® offers a new approach for users via perfusion fermentation with a pre-programmed dual-pump feeding system. The Daisy Petal® overcomes the limitations to fermentation longevity and space-time yields experienced in traditional batch, fed batch, and chemostat fermentors through perfusion fermentation operations, enabled by in-vessel cell retention devices (CRDs), which allow for the continuous removal of waste products and secreted proteins without cells ever leaving the reactor (Figure 1B). The dual pump feeding system supports advanced media control strategies, enabling enhanced control and delivery of media, substrate, and key nutrients, all without the need for coding or programming skills. In contrast to other available hardware configurations, this setup allows for modular control of each pump, which can deliver specific

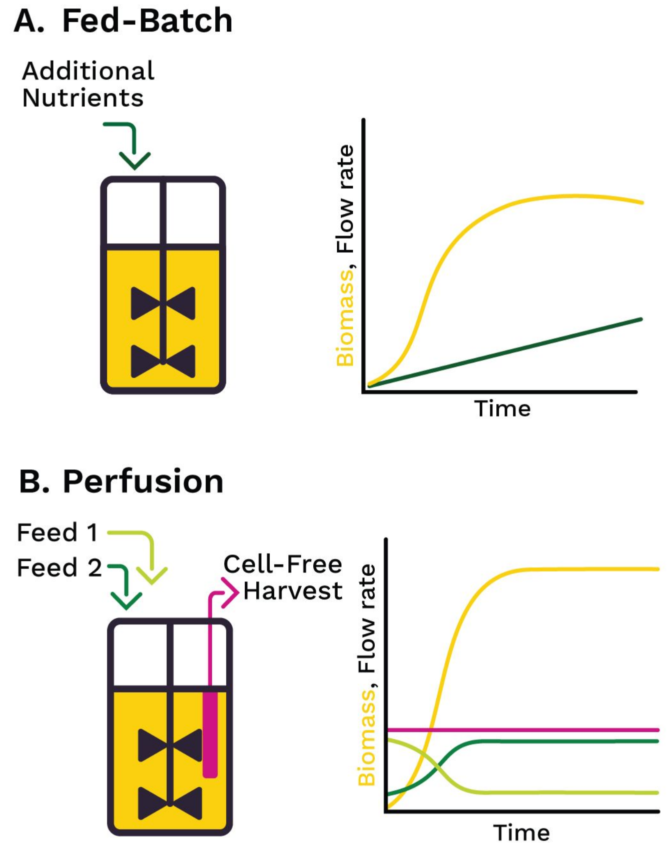

Figure 1. Comparison of feeding strategies during (A) fed-batch fermentation in legacy equipment and (B) perfusion fermentation in the Daisy Petal®.

combinations of key nutrients and substrate tailored to adapt to various process demands (Figure 1B).

In this application note, we review current feeding strategies employed in upstream bioprocessing and discuss the limitations of these approaches. Then, we highlight the unique dual feeding pump setup deployed in the Daisy Petal® and how its automated fluid delivery can be utilized to optimize feeding in fermentation processes.

Current Practices for Nutrient Delivery and Feed Control in Biomanufacturing

Constant Media Feeding

A constant media feed is the most basic feeding mode and involves delivering a single media to the bioreactor at a fixed flow rate for the duration of the fermentation process (Figure 2A). The simplicity of this mode is advantageous due to the low complexity of implementation and scale-up. However, constant feeding gives poor control over substrate concentration, particularly as it relates to the quantity of biomass in the bioreactor. This makes it difficult to maintain a constant and stable growth rate using this operating mode, and brings risks of substrate accumulation or starvation. Since feeding is usually controlled via a single pump, other key nutrients are also fed at the same fixed rate, which may not match cellular demand and can lead to inconsistent nutrient delivery and metabolic stress.

Discontinuous Feeding

Discontinuous feeding methods, such as bolus or pulse feeding, are often used to overcome the limitations of a constant feed strategy. Bolus feeding delivers a concentrated volume of substrate at fixed time intervals (Figure 2B), or in response to a significant process change or an unexpected substrate depletion, as indicated by metabolic indicators like a DO spike or pH drift. This method quickly restores critical nutrients, such as carbon, but can cause substrate inhibition and catabolite repression.[1] It also leads to dilution of the culture and increased fluctuations in substrate concentration that can lower productivity and be inefficient for some organisms.[3]

Linear Feeding

Linear feeding often is considered to be an improvement over a constant media feed due to its capability to better match nutrient delivery with increasing biomass, which is practical when supporting high density fermentation. In this mode, the feed rate increases linearly with time (Figure 2C), as defined by Equation 1, where the feed rate at time t, $F(t)$, is determined by the initial feed rate, F_0, and the feed constant, K.

$$F(t) = F_0 + Kt \qquad (1)$$

The linear increase in nutrient supply leads to improved substrate utilization, which usually results in increased growth and productivity.[1,3] The feed constant can be empirically determined, enabling process predictability and tech transfer between fermentation scales. Cell growth is exponential, however, so linear feeding cannot exactly match the increasing metabolic demand experienced during normal biomass accumulation. Often, linear feeding leads to overfeeding at the start of a process, resulting in substrate inhibition or catabolite repression, and underfeeding in later stages, resulting in nutrient limitation and starvation.

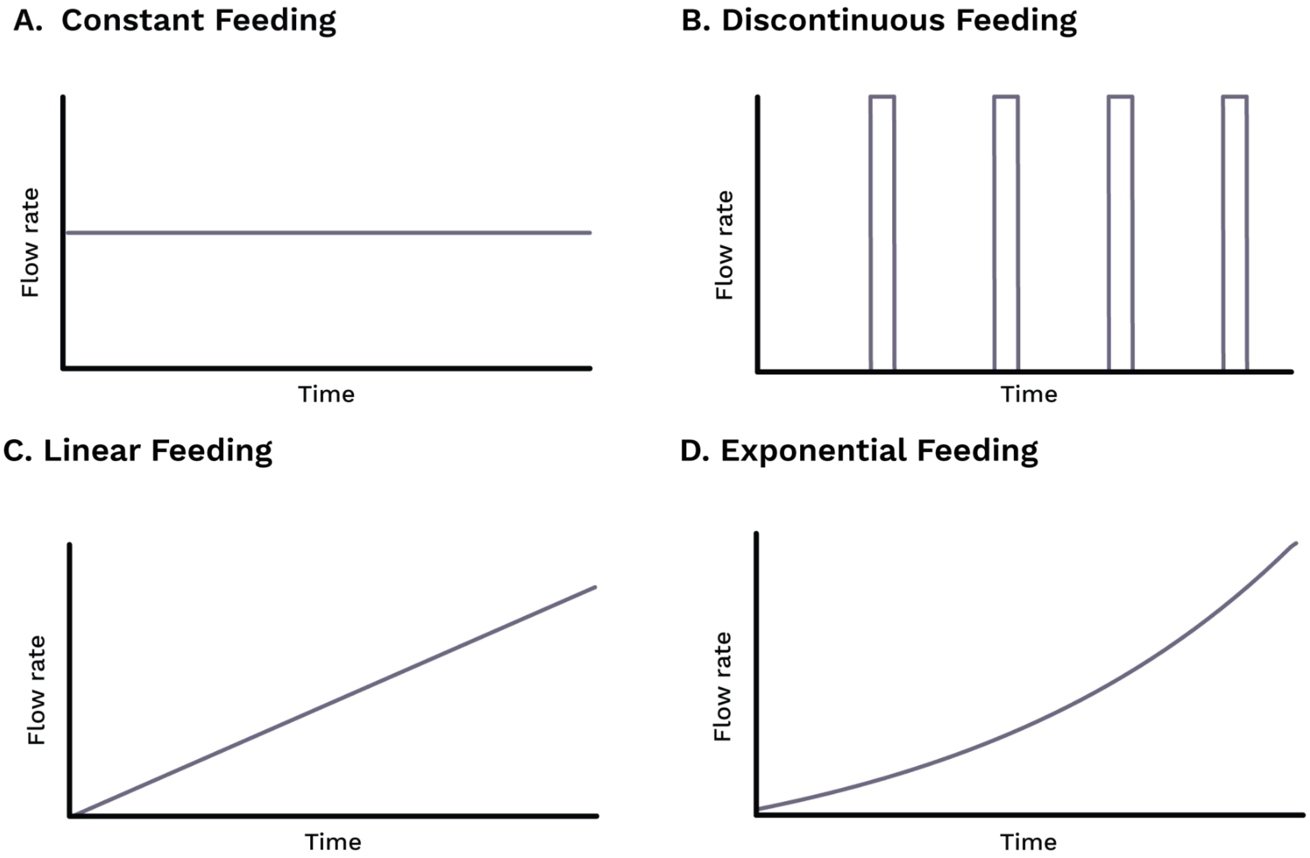

Figure 2. Comparison of current practices for nutrient delivery and feed control in biomanufacturing. (A) Constant Media Feeding, (B) Discontinuous Feeding, (C) Linear Feeding, and (D) Exponential Feeding.

Exponential Feeding

Exponential feeding is usually the most efficient strategy for supplying nutrients to a bioreactor during a growth phase. With this mode, media is fed increasingly at an exponential rate (Figure 2D) as defined by Equation 2, where $F(t)$ is the feed rate at time t, F_0 is the initial feed rate, and μ is the specific growth rate.

$$F(t) = F_0 \, e^{\mu t} \tag{2}$$

Since growth of fermentative organisms is exponential, the feed rate can be mathematically determined based on growth kinetics. This enables better control of nutrient delivery and results in more efficient substrate utilization, which translates into maximal biomass, reduced byproduct formation, and increased product yields.[3] Feeding exponentially limits accumulation of highly concentrated media at the beginning of a process, and reduces the potential for nutrient limitation in the later stages.

Advanced Feeding Control Strategies on the Daisy Petal® Perfusion Bioreactor System

The Daisy Petal® is designed to offer users the ability to configure various advanced media control strategies during perfusion fermentation using a unique dual pump setup. The system allows for the mixing of two independent media from up to six different sources at any given time. Media components, such as carbon, nitrogen or media additives, can be controlled independently from a complete media formulation. This approach offers more flexibility and modularity for process development and optimization, without compromising on consistent delivery of key nutrients.

The Daisy Petal® comes with a software suite including Nursery™, a remote recipe editor and data visualization tool, where users can design and configure process recipes and HelianthOS™, the central operating system for all of Sunflower's bioprocessing equipment. Via Nursery™, users can create step-by-step recipes choosing either the standard media control strategy, allowing up to three media inputs via a single pump, or Dual Pump Mode, which supports six media inputs controlled by two pumps. With Dual Pump Mode activated, users can choose from several control strategies for media delivery in each operational step of a recipe. HelianthOS™ enables the deployment of configured recipes while providing reliable automation for control of all fermentation parameters, such as pH, DO, temperature, fluid level, and feeding.

Single Media Control

Single Media Control is a feeding strategy designed to deliver a single medium to the bioreactor during a specific step of a recipe (Figure 3A). This feed mode can operate using a single pump or both system pumps when configured in Dual Pump Mode.

In its standard configuration, Single Media Control supports up to three different media, each delivered sequentially, one at a time, across distinct recipe steps. When Dual Pump Mode is employed in combination with Single Media Control, up to six independent media can be supplied to the bioreactor over the course of a campaign. Because only one medium is delivered at a time, each formulation prepared for this control strategy must contain all required nutrients, including the primary carbon source or substrate. Single Media Control is therefore most suitable for processes in which nutrient composition remains constant within a given step, such as cultivation steps where biomass is maintained and nutrient variation is not required.

Constant Ratio Control

Constant Ratio Control is a feeding strategy used to deliver two independent media simultaneously at a fixed ratio during a single process step (Figure 3B). In this configuration, each medium is assigned to a dedicated pump (Pump A or Pump B), enabling consistent and homogenous mixing of both fluids.

Using Constant Ratio Control, the relative proportions of Media A and Media B remain constant throughout the step. To maintain a consistent perfusion rate, these ratios are defined such that the combined contribution of both media always equals 100%. This approach is particularly suited for applications where the required nutrient concentrations remain stable within a step but are

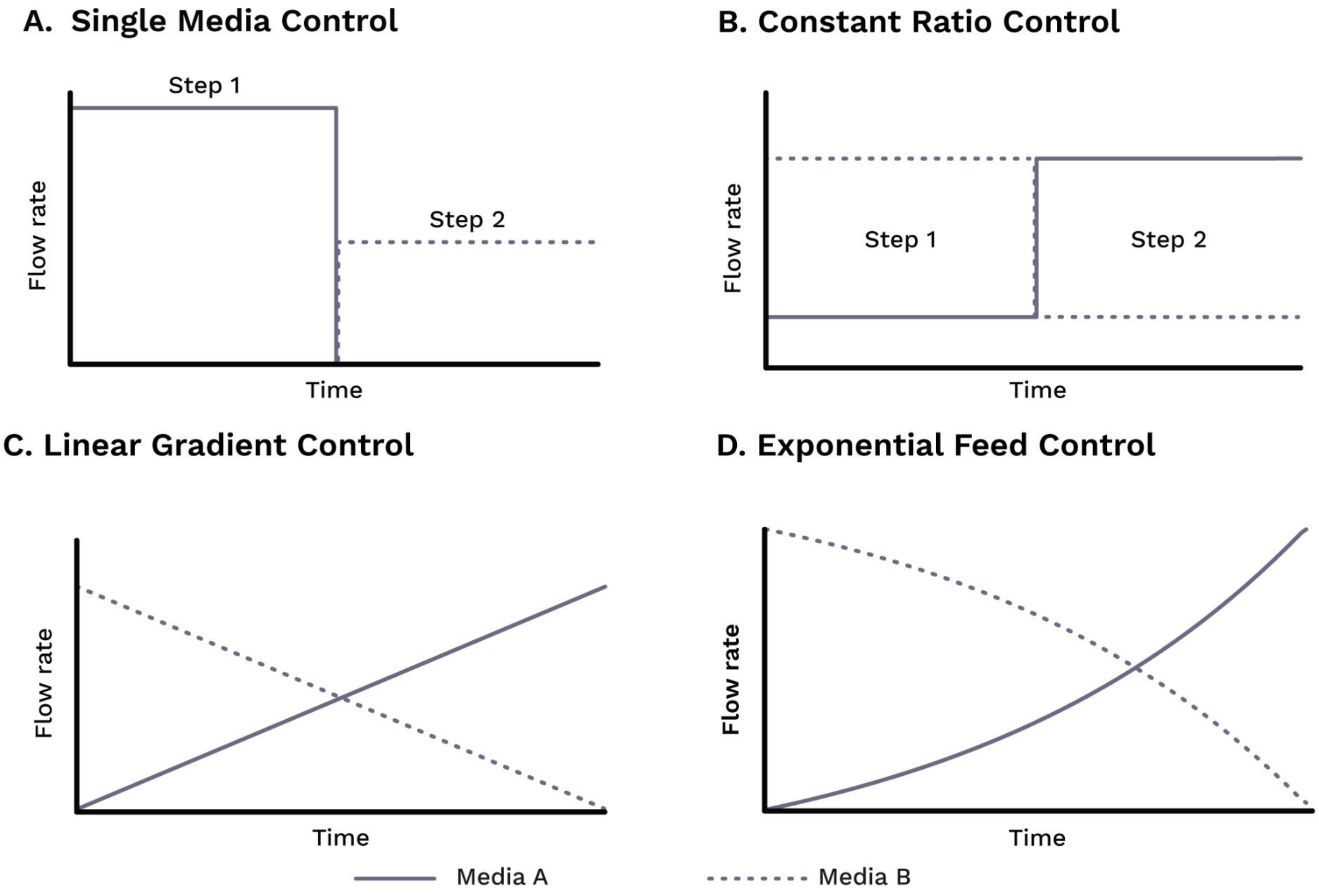

Figure 3. Representation of advanced feeding control strategies with the Daisy Petal® Perfusion Bioreactor System. (A) Single Media Control, (B) Constant Ratio Control, (C) Linear Gradient Control, and (D) Exponential Feed Control.

adjusted between successive steps. For example, a stepwise increase in carbon source concentration can be achieved by designating Pump A to deliver a complete medium with a high carbon concentration, while Pump B delivers a complete medium without any carbon. To achieve a stepwise increase, discrete recipe steps can be programmed where in Step 1 the ratio is 25% A : 75% B, and in Step 2 the ratio is 75% A : 25% B (Figure 3B).

Compared to the delivery of a single media, the Constant Ratio feeding mode provides greater flexibility, allowing a gradual delivery of nutrients across recipe steps to match increasing metabolic demand associated with biomass growth and protein expression. This capability also supports process development activities, enabling systematic evaluation of varying nutrient concentration across steps to optimize culture performance.

Linear Gradient Control

Linear Gradient Control is a feeding strategy designed to deliver a controlled mixture of two media to the bioreactor, in which the relative ratio of each medium changes linearly over the course of a process step (Figure 3C). This approach contrasts with traditional linear feeding, where the feed rate of a single complete medium increases linearly over time. With a linear gradient, a single key component (e.g., carbon) can be supplied increasingly while base nutrients in the medium remain constant.

When configuring a recipe in Nursery™, users define the starting and ending concentrations of the medium connected to Pump A. The recipe builder then automatically calculates the respective starting and ending concentrations for Pump B such that the combined contribution of both media equals 100% to maintain a constant perfusion rate throughout the process step.

Linear Gradient Control is particularly useful when a key nutrient must be varied gradually within a process step (e.g., implementing a linear increase in carbon feed concentration). In this setup, both media are complete formulations; however, one typically lacks a key nutrient (such as carbon), while the other contains this nutrient at a high concentration. This feeding mode is especially advantageous in processes where increasing the supply of a specific nutrient (e.g., carbon) is beneficial, while maintaining other nutrient concentrations is critical to avoid inhibitory effects on growth or productivity.

Exponential Control

Exponential Control is a feeding strategy used to deliver two media to the bioreactor during the same process step, in which the ratio of each medium changes exponentially over time (Figure 3D). This approach enables precise control of nutrient composition to mimic biological growth kinetics. When configuring a recipe in Nursery™, users specify the starting concentration and the desired exponential rate (or specific growth rate, μ, in h^{-1}) for the medium connected to Pump A. The end concentration is then automatically calculated using Equation 2.

The corresponding concentrations for the medium connected to Pump B are automatically determined such that the sum of Media A and Media B equals 100% at any given time to maintain a constant perfusion rate throughout the step. As the process step progresses, the proportion of each medium changes exponentially from the defined starting concentration according to the specified growth rate. This configuration allows users to vary the concentration of a key nutrient, such as a carbon source, exponentially throughout a single process step while maintaining all other key nutrient concentrations. Exponential Control is particularly valuable for feeding to match microbial or cell culture growth kinetics, supporting metabolic optimization, or maintaining substrate-limited conditions in advanced process development and production applications.

Case Study: Using Multiple Media Delivery Strategies in a Single Fermentation

Here, we demonstrate the application of Exponential, Single Media, and Constant Ratio Control schemes with the Daisy Petal® in a perfusion fermentation process for an AI-designed therapeutic protein candidate. We used an exponential feed to provide nutrients for biomass accumulation at a rate consistent with cellular growth, then a combination of Single and Constant Ratio media feed strategies to test the impact of various carbon and total nutrient feed rates on growth and productivity throughout the production phase.

Method and Results

A *Pichia pastoris (K. phaffii)* strain engineered to express an AI-designed protein[4] was cultivated via perfusion fermentation in the Daisy Petal®.[5] A fermentation process recipe was designed in Nursery™ and deployed on the Daisy Petal® using HelianthOS™. The bioreactor was charged to a 1 L working volume with Sunflower's chemically defined growth media[6] containing glycerol. A frozen stock of cells was used to inoculate the bioreactor at an OD/mL of 0.15.

In the first recipe step, which was designed to build biomass, defined growth media containing glycerol was delivered to the bioreactor as controlled by the HelianthOS™ software using Exponential Control via two distinct pumps. Pump A was connected to a 2x concentrated media with 10% (w/v) glycerol, and Pump B was connected to glycerol-free media containing all other essential media components at a 1x concentration. To ensure a consistent perfusion rate, the total delivery rate from pumps A and B was automatically maintained at 1.8 vessel volumes per day (VVD) during this step. The ratio of fluid delivery from pumps A and B was automatically varied to achieve an exponential glycerol feeding profile based on the preset specific growth rate. Feeding was initiated at a glycerol concentration of 1.25% (12.5% Pump A : 87.5% Pump B) and the ratio was increased exponentially for a duration of 42 hours, resulting in a final glycerol feed of 10% (100% Pump A : 0% Pump B) (Figure 4A). This strategy achieved a biomass concentration of 265 g/L wet cell weight (WCW) at the start of methanol-based heterologous protein induction (42h) without the use of a seed train prior to inoculation (Figure 4B).

Notably, the Exponential Control was performed without the need to set defined mathematical functions in the software or perform any coding operations. The only user inputs required were the starting percentage concentration of Pump A, a predetermined exponential rate, and the desired step length.

Using both Single Media Control and Constant Ratio Control, we designed several steps in the fermentation process to evaluate the impact of increasing either methanol input, total nutrient input, or both. During these recipe steps, chemically defined media containing methanol was delivered to the bioreactor as controlled by the HelianthOS™ software using two distinct pumps. Pump A was connected to media with a low concentration of methanol and all other necessary nutrients, and Pump B was connected to media with a high concentration of methanol and all other necessary nutrients. The recipe setpoints for each step are shown in Table 1. Through four process steps, we evaluated two overall nutrient feed rates and four methanol feed rates at two effective methanol concentrations. Each feeding scheme was maintained for 24 hours and samples were taken at the end of each step to measure productivity.

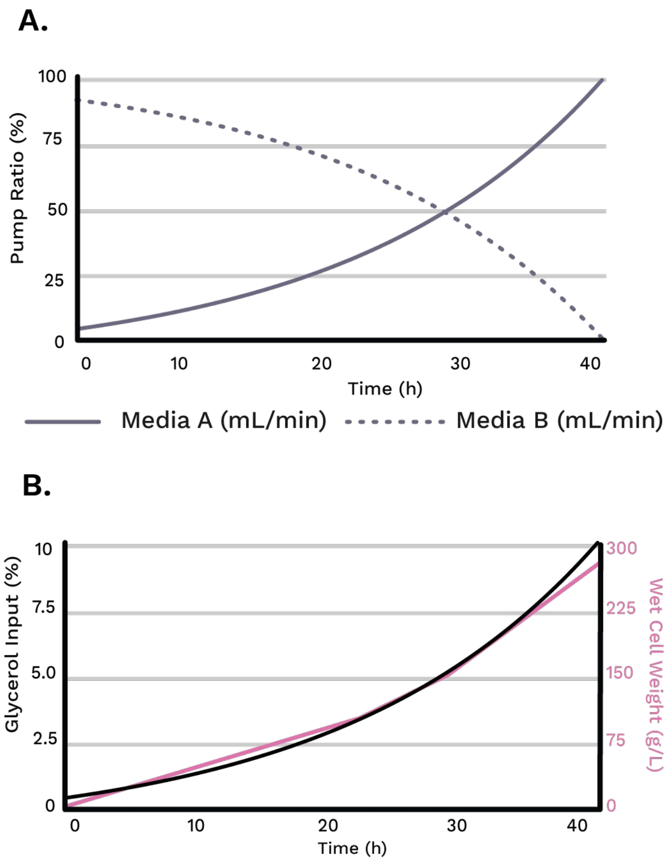

Figure 4. Exponential nutrient delivery during biomass accumulation. (A) Input pump ratios of 2X concentrated base media containing glycerol (solid line) and carbon-free base media (dashed line) as delivered to the stirred tank bioreactor. (B) Glycerol input as represented by effective percentage (black), and biomass accumulation as represented by wet cell weight (g/L) (pink).

Initially, we used Single Media Control to supply nutrients at a perfusion rate of 0.75 VVD and methanol at 35.6 g/L/day (6% MeOH). Next, Constant Ratio Control was used to increase the methanol feed to 47.7 g/L/day, while the total nutrient feed remained at 0.75 VVD (8% MeOH). The recipe then automatically switched back to Single Media Control to increase both the total nutrient feed and methanol feed rate to 1.5 VVD and 71.2 g/L/day, respectively (6% MeOH). Finally, a second

Table 1. Reactor setpoints used to control feed rates and their impact on performance.

Reactor Setpoints					Fermentation Inputs / Outputs			
Process Step	**Media Control Strategy**	**Pump A %**	**Pump B %**	**Perfusion Rate (VVD)**	**Methanol Feed Rate (g/L/day)**	**Effective Methanol (%)**	**Growth Rate (h^{-1})**	**Volumetric Productivity (mg/L/day)**
1	Exponential	Varied	Varied	1.8	-	-	-	-
2	Single Media	100	0	0.75	35.6	6	0.0026	135.1
3	Constant Ratio	33.33	66.67	0.75	47.7	8	0.0019	58.54
4	Single Media	100	0	1.50	71.2	6	0.0002	73.61
5	Constant Ratio	33.33	66.67	1.50	94.9	8	0.0000	66.10

instance of Constant Ratio Control was used to increase the methanol feed rate to 94.9 g/L/day, while keeping the total nutrient feed at 1.5 VVD (8% MeOH).

Increases in nutrient and methanol feeding drove fluctuations in growth rates and instantaneous volumetric productivity (IVP) across these process steps. In the first instance where only methanol was increased, the growth rate decreased from 0.0026 h^{-1} to 0.0019 h^{-1}, and the IVP decreased from 63.65 to 56.91 mg/L/day. When total nutrient feed was eventually increased, the growth rate decreased again, from 0.0019 h^{-1} to 0.0002 h^{-1}, however the IVP increased from 58.5 to 73.6 mg/L/day. When methanol was increased again, the growth rate and IVP both decreased, from 0.0002 h^{-1} to 0 h^{-1} and 73.6 to 66.1 mg/L/day, respectively.

Discussion

Perfusion operations with *Pichia pastoris* can quickly achieve high biomass.[7] To meet the escalating metabolic demands of large amounts of respiring cells, the Exponential Control scheme was utilized to deliver nutrients to the bioreactor during the growth phase of this fermentation, with an exponential factor that was determined empirically. The exponential feed is particularly advantageous because it effectively matches nutrient supply to growth kinetics, yielding healthier and highly productive cultures.[7,8] This rapid biomass accumulation is generally beneficial for maximizing volumetric productivity of recombinant proteins,[8] as growth typically slows down following heterologous gene induction when metabolic flux shifts toward heterologous protein expression. Starting the fermentation with a reduced glycerol concentration also prevents metabolic stress caused by overfeeding, and allows for a better transition to the induction phase.[9]

Once significant biomass was accumulated in the bioreactor, the process recipe was shifted to enable heterologous protein expression, which typically requires a stable stream of nutrients and inductive carbon to maintain protein expression. Constant Ratio Control was used to incorporate feeding step changes where only the carbon delivery was changed, while nutrients in the base media were kept constant. Productivity and growth rates decreased during steps where methanol alone was increased (Table 2), signaling diminishing returns for protein expression with increased methanol. This reduction in productivity is likely due to insufficient nutrient and carbon ratios for the given strain and product, as reflected by the increase in IVP after the total nutrient feed rate was increased (Table 1). Subsequently, when the methanol feed rate was further increased to 94.9 g/L/h, another loss in productivity was observed, indicating that the methanol feed rate alone was not the limiting factor, but rather the combination of both methanol and total nutrient availability.

Using both Single Media Control and Constant Ratio Control in this particular experiment served as a means to explore a wide range of nutrient and methanol feeding conditions within a single campaign. This experimental framework allowed us to identify precise feed rates for further investigation in future process optimization efforts. While traditional systems are often limited to rigid feeding profiles, this configuration allowed for a fully automated, multi-step fermentation where carbon to nutrient ratios shifted dynamically without a single manual adjustment. The hands-free execution of the complicated feeding strategy demonstrated in the case study described above showcases a capability unique to the Daisy Petal®, pushing the boundaries of what is possible in automated bioprocessing.

Conclusion

Modern bioprocessing requires equipment that can adapt to the dynamic needs of fermentative organisms. The Sunflower Daisy Petal® Perfusion Bioreactor System, powered by HelianthOS™ and Nursery™, represents a significant leap forward in feeding modularity with simple user interfaces. As described, traditional single-pump feeding strategies often force a compromise between meeting metabolic demand and maintaining stable nutrient concentrations during fermentation. The Daisy Petal® eliminates this trade-off through its integrated dual-pump architecture. By enabling advanced control modes, ranging from constant ratios to exponential feeds, the system allows users to decouple feeding of key nutrients from base media formulations. This flexibility frees the fermentation process from being bound to a single feeding strategy, allowing users to apply different strategies tailored to each phase of the fermentation. This modularity not only simplifies the control of complex fermentation kinetics, but also provides a scalable path toward maximizing biomass with superior product consistency and quality. By providing these capabilities coupled with walkaway automation via HelianthOS™, the Sunflower Daisy Petal® enables users to move beyond the limitations of legacy hardware and achieve new benchmarks in fermentation productivity and ease-of-use.

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Related Resources

Application Note: Perfusion fermentation in the Daisy Petal™ bioreactor sustains ultra-high biomass

Application Note: Daisy Petal™ simplifies fermentation optimization