Application Note

Equipment Highlight

Keywords

Perfusion Fermentation, Continuous Bioprocess, Bacteria

Perfusion fermentation of bacteria in the Daisy Petal®

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Abstract

In this application note, we demonstrate perfusion cultivation of a bacterial expression host using the Daisy Petal® Perfusion Bioreactor System. We describe the cultivation of a gram-negative bacteria engineered by Opera Bioscience to secrete a reagent protein through the type III bacterial secretion system (T3SS). The HelianthOS™ operating system on the Daisy Petal® controlled the reactor hardware and maintained process setpoints during the perfusion fermentation of Opera's bacterial strain, achieving an eightfold increase in biomass and a tenfold longer operating lifetime compared to a batch process cultivating the same strain. These results confirm the versatility of the perfusion fermentation approach deployed in the Daisy Petal® and its ability to enhance productivity and process control for numerous microorganisms, including bacterial hosts, and also validate the use of bacteria to secrete proteins in a continuous fermentation process.

Introduction

Since the first FDA-approved bioprocess for the manufacture of recombinant insulin in *E. coli*, bacterial expression systems have been a prominent platform within the biomanufacturing sector. Although *E. coli* remains the most prevalent bacterial host in the biotherapeutic production space, there is growing demand for alternative bacterial hosts that address specific challenges, like protein activity and high purification costs, across biopharmaceutical and bioindustrial applications.[1,2] This has driven the emergence of diverse microbial host organisms for biomanufacturing.

Simultaneously, the biotechnology industry is extending efforts to develop and institute continuous bioprocessing solutions due to its advantages relative to legacy manufacturing methods, including higher process space-time yield, reduced waste products, and better product consistency. This motivates a market for continuous manufacturing solutions designed to support a diverse catalog of microbial hosts.[3-6] Sunflower Therapeutics' technologies directly address this need, having repeatedly demonstrated the capability to support and enhance the continuous cultivation of various microbes, including the bacterial host demonstrated here.

In previous application notes, we have discussed the numerous advantages the Daisy Petal® offers for microbial fermentation compared with other platforms, including the ability to achieve and maintain significant biomass, longer cultivation times, higher space-time yield, and improved product quality.[7-9] This work evaluates the successful cultivation of an emerging bioproduction host based on an alternative gram-negative bacterial strain demonstrating that the bioprocessing advantages of the Daisy Petal® perfusion bioreactor translate to bacterial hosts. Opera Bioscience has engineered their proprietary gram-negative bacterial strain to secrete a reagent protein with high starting purity via its endogenous type III secretion system (T3SS). Cultivated in the Daisy Petal®, this strain demonstrated an eightfold improvement in biomass accumulation while the system enabled continuous harvest of its secreted protein product for tenfold longer than a legacy batch process. This success adds bacteria to an expanding catalog of compatible expression systems, building directly on our prior demonstrations of yeast- and fungal-based bioprocessing to further cement the Daisy Petal® as a true General Purpose Technology for microbial cultivation.

Materials & Methods

Opera Bioscience provided their gram-negative bacterial strain, engineered to express and secrete a reagent protein via its endogenous secretion system, T3SS. The strain was cultivated via perfusion fermentation in the Daisy Petal® bioreactor. Prior to cultivation in the Daisy Petal®, Opera's strain was tested for compatibility with Sunflower's in-vessel cell retention device (CRD) as described in a previous Sunflower application note.[10]

The bioreactor was charged to a 1 L working volume with STx002, Sunflower's proprietary chemically defined growth media, containing sugar and a chemical inducer to activate protein expression. The Daisy Petal® bioreactor was inoculated at an initial cell density of 0.05 OD_{600}/mL. STx002 containing sugar and the chemical inducer was continuously fed to the reactor, while spent cell culture fluid from the bioreactor was continuously harvested through two distinct output streams: the 'harvest' containing cell-free fluid from the in-vessel CRD, and the cell 'bleed' containing unfiltered fluid and cells. An average perfusion rate of 2.5 vessel volumes per day (VVD) was maintained throughout cultivation. The HelianthOS™ operating system actively maintained bioreactor setpoints, keeping the reactor volume at 1 L, the temperature at 37°C, and the pH and dissolved oxygen at predefined setpoints during cultivation. HelianthOS™ initiated an automated shutdown of the system when the desired fermentation time (102 hours) was reached.

The total biomass in the bioreactor vessel was determined by collecting samples from inside the vessel throughout the cultivation period. Collected samples were diluted in PBS to an appropriate concentration, and the biomass density was determined by OD_{600}. Protein yield and quality were determined by SDS-PAGE using samples of the cell-free harvest fluid and the supernatant of material acquired from sampling the bioreactor directly.

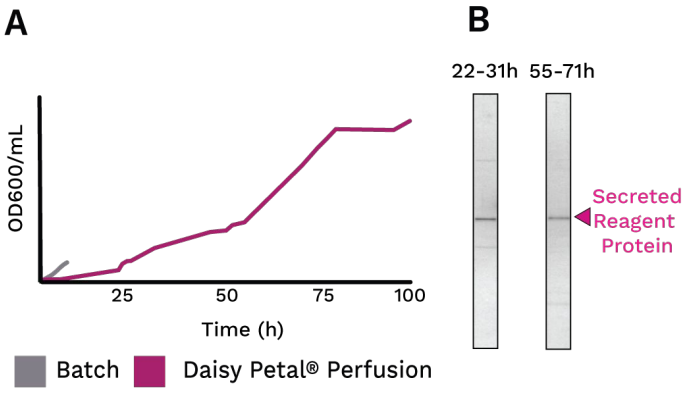

Figure 1. (A) Change in OD_{600}/mL over time of a batch (gray) and Daisy Petal® perfusion (magenta) fermentation of Opera's gram-negative bacterial strain. (B) SDS-PAGE of samples taken from pooled collections of the cell-free harvest fluid.

Results & Discussion

Perfusion fermentation of a bacterial strain using the Daisy Petal®

A reagent protein was produced through perfusion fermentation of Opera Bioscience's engineered bacterial strain in the Daisy Petal®. The perfusion fermentation of the bacterial strain sustained eightfold higher biomass for a duration tenfold longer than established batch processes cultivating the same strain (Figure 1A).

The Daisy Petal® enabled higher biomass and extended cultivation length by continuously supplying fresh nutrients while flushing out waste byproducts to prevent the premature decline of cell state typical of legacy batch methods. Concurrently, the proprietary in-vessel CRD kept the biomass inside the highly

controlled reactor environment, providing the ideal conditions necessary to support ultra-high cell densities over extended continuous runs.

The successful cultivation of Opera's strain utilized Sunflower's defined growth media STx002, a derivative of the previously discussed STx001.[11] This media was designed and formulated to enhance the productivity of continuous microbial fermentations, effectively facilitating growth and product expression in Opera's bacterial strain with minimal modification to its composition.

While demonstrating impressive biomass gains, the Daisy Petal® also supported the expression and secretion of the desired reagent protein. SDS-PAGE analysis of samples taken from the perfusate stream, a cell-free output separated from the reactor bulk using the in-vessel CRD, demonstrates the successful separation of the secreted reagent protein from the cell bulk throughout peak productive phases of the cultivation (Figure 1B). This highlights the capacity of the CRD to combine cultivation and harvest into a single unit operation for bioprocesses that generate secreted products. The secreted reagent protein shown in Figure 1B was obtained directly from the perfusate stream, without additional processing prior to SDS-PAGE analysis, underscoring the synergistic pairing of Opera's secreting bacteria with Sunflower's continuous fermentation platform.

Conclusion

The successful cultivation of Opera Bioscience's engineered gram-negative bacterial strain using the Daisy Petal® perfusion system highlights the transformative potential of in-vessel perfusion for bacterial systems. The Daisy Petal® platform, powered by the HelianthOS™ operating system, demonstrates the capacity to meet the specific requirements of bacterial fermentation whilst enabling continuous bioprocessing. These results, alongside our growing catalog of successfully cultivated microbes, demonstrate that the Daisy Petal® is a host-agnostic solution built to replicate this exceptional performance across microbial systems. Sunflower Therapeutics' Daisy Petal® perfusion fermentation system provides the tools to overcome the limitations of legacy manufacturing, delivering higher productivity and superior process control for the next generation of biologics.

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Related Resources

Dahlia System™: A microfactory for protein biologics

Chemically Defined Media for Yeast