Sunflower

Application Note

Equipment Highlight

Keywords

Bioprocess automation, End-to-end manufacturing, Microfactories

Dahlia System™: A microfactory for protein biologics

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Abstract

Conventional biomanufacturing of recombinant proteins is expensive, inflexible, and concentrated in a few regions, severely limiting global access to life-saving treatments. To address this, we developed the Dahlia System™, a flexible, fully automated, and continuous microfactory designed for microbial bioprocessing. The Dahlia System™ drastically reduces the barrier to entry for biomanufacturing, requiring only 200 square feet, 90% less capital expenditure, and non-expert labor. We demonstrate the system's ability to operate in an uncontrolled warehouse environment to produce clinical-quality bulk drug substance (BDS) for two different biopharmaceutical products: a SARS-CoV-2 subunit vaccine candidate (RBD) and the radiation toxicity treatment G-CSF. The Dahlia System™ continuously manufactured >50,000 RBD dose equivalents in 5 days, and >1,000 G-CSF dose equivalents in under a week, with quality metrics (identity, purity, safety) consistent with clinical benchmarks, and critically, no bioburden detected. **The Dahlia System™ revolutionizes biomanufacturing by enabling decentralized, on-demand production, enhancing regional autonomy, improving global access to therapeutics, and fostering the growth of bioeconomies in new geographies.**

Introduction

Manufacturing of recombinant proteins is increasingly important for global health, economic development, and national security. Recombinant protein products include cancer, diabetes and rare disease treatments, vaccines, alternative foods, sustainable materials, and medical countermeasures. The National Security Commission on Emerging Biotechnology estimates that by 2030, most people on the planet will have consumed, used, worn, or been treated by a product of emerging biotechnology,[1] such as recombinant proteins.

Conventional biomanufacturing of recombinant proteins is expensive, inflexible, and highly manual. A standard large-scale fixed biomanufacturing facility takes 3 - 5 years to build, costs >$200M, requires hundreds of expert operators, and is generally designed for only one product.[2] The significant upfront resources required to establish this kind of facility severely limits the number of market participants that can build one and has resulted in very uneven global distribution of manufacturing sites. More than 79% of all biopharmaceutical manufacturing facilities are located in North America (37%), Europe (24%), and China (18%).[3] This regional concentration of biomanufacturing facilities restricts global access to life saving treatments, particularly in low- and middle-income countries (LMICs).

In addition to health impacts, the uneven distribution of biomanufacturing capacity leads to significant economic impacts. For example, Africa imports >70% of its pharmaceuticals, including 99% of its vaccines, leading to massive trade deficits.[2] Even within the US the economic impacts of the biopharmaceutical industry are highly concentrated. Only five states – California, Massachusetts, New York, New Jersey, and Illinois – benefit from more than 40% of the total U.S. biopharmaceutical economic impact.[4] **Reducing the barriers to entry for new participants in biomanufacturing is critical to improve global access to recombinant proteins and to catalyze the growth of bioeconomies in new regions.**

In contrast to the "mega facilities" traditionally used in biomanufacturing, other consumer goods, such as home appliances, textiles, batteries and electric vehicles, rely on microfactories to disperse manufacturing capacity. These distributed, small-footprint manufacturing facilities bring manufacturing closer to consumers and build local economies.[5] Microfactories are based around small, tractable, automated manufacturing units that can be deployed anywhere with trained non-expert labor. Given the need for user-friendly biomanufacturing capacity, **Sunflower has built a microfactory for protein manufacturing called the Dahlia System™–a flexible, automated and continuous facility for microbial bioprocessing.** Here, we describe the Dahlia System™ and demonstrate its fully-automated operation to produce two different clinical-quality biologics in an uncontrolled warehouse.

Dahlia System™ Overview

We have developed the fully automated small footprint Dahlia System™ for on-demand, turnkey production of protein bulk drug substance (BDS) in diverse environments (Figure 1). Each Dahlia System™ campaign (1 - 2 weeks) supports gram to kilogram outputs through host cultivation by perfusion fermentation, protein purification by straight-through chromatography, and protein concentration and formulation using tangential flow filtration (TFF). All process steps deployed on Dahlia™ are controlled similarly to conventional approaches to Chemistry, Manufacturing, and Controls (CMC) for regulated biomanufacturing. **The Dahlia System™ can be deployed in significantly smaller facilities with non-expert labor, greatly reducing the barriers to entry for biomanufacturing.** Key attributes of the Dahlia System™ and the improvement realized compared to legacy biomanufacturing approaches are detailed in Table 1.

The Dahlia System™ operates in a manufacturing footprint of only 200 square feet, and requires 90% less capital expenditure than traditional approaches. The modular design of the Dahlia

Table 1. Key attributes of Dahlia System™ compared to legacy biomanufacturing.

Manufacturing Attribute	Legacy Biomanufacturing	Dahlia System™	Improvement Realized
Facility capital cost	>$100M	<$10M	90% less
Manufacturing Footprint	8,000-20,000+ sq ft	200 sq ft	>10X smaller
Facility controls and readiness	High classification required	Turnkey; Functionally closed	Brownfield compatible
Facility operators required	>100	~10 (only 2 per system)	90% fewer
Operator skill level	Highly skilled	Trained non-expert	Reduced operator dependency
# of unit operations to BDS	18 for G-CSF	7 for G-CSF	60% fewer and fully integrated
Energy requirements	>10 MW continuously	<<1 MW continuously	>>90% less
Turnover validation requirements	Sterilize in place; Test for contaminants	Single-use componentry removal, no testing	90% less water required, immediate turnover
Turnover time	Days to weeks	24 h	3-10X less
Product flexibility	Single product/class	Demonstrated processes for >15 products across 9 classes	>5X more agile

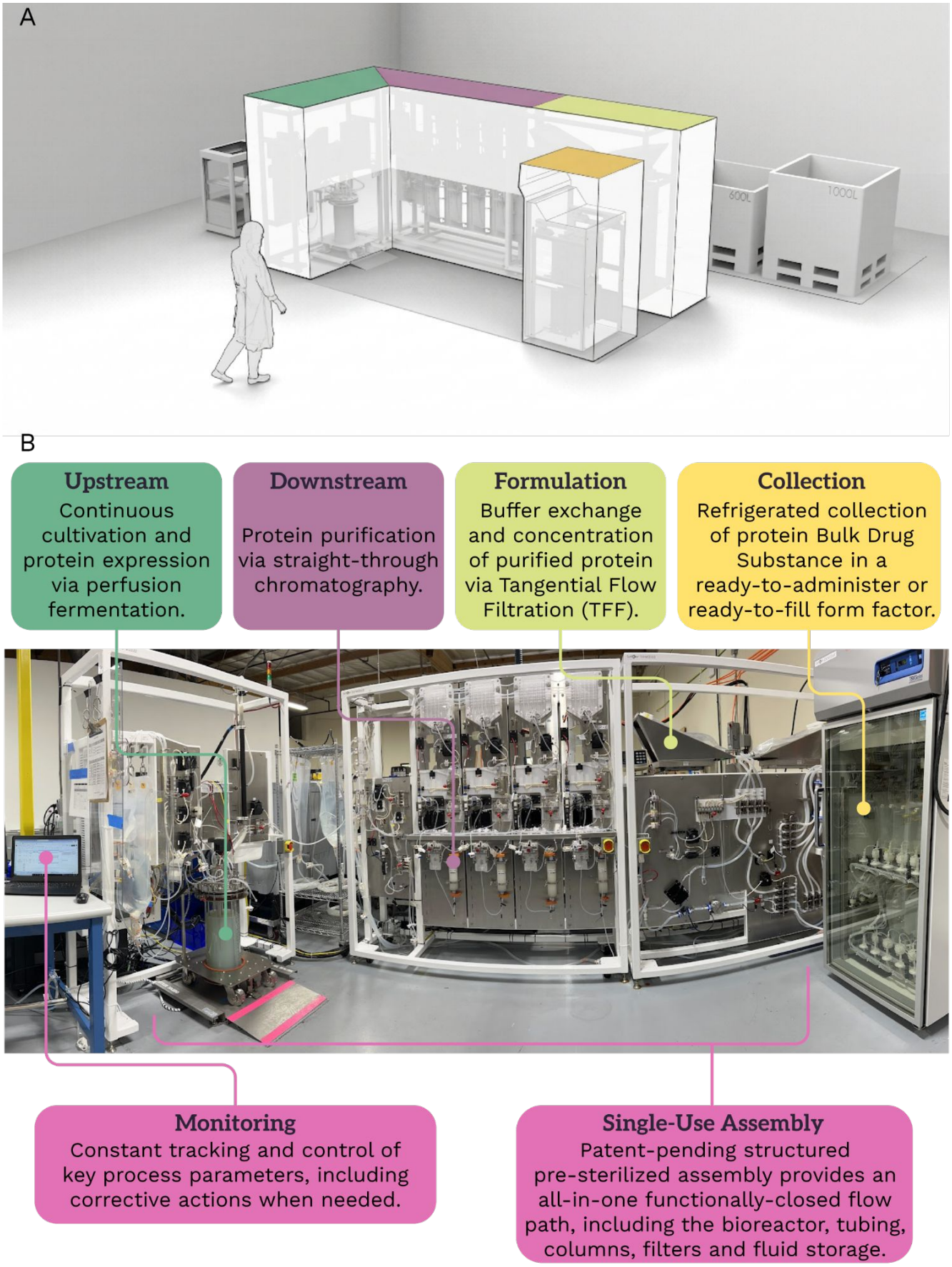

Figure 1. Rendering (A) and photo (B) of Sunflower's Dahlia System™ in operation.

System™ provides direct and functionally closed fluidic interfacing of unit operations without holding tanks by using single-use assemblies for all product contact surfaces. **This design eliminates the need for decontamination or cleaning between campaigns, removing the potential for cross contamination or cleaning related process failures while enabling rapid turnaround between production campaigns of different protein products.** The Dahlia System™ also includes all necessary auxiliary equipment, such as an air compressor, oxygen generator, and chiller, making it immediately deployable in diverse environments. All a user needs to get started is power, a single-use assembly, process fluids, and a cellular host.

Sunflower's 21 CFR Part 11 compliant software suite empowers walk-away automation of the Dahlia System™, with user interface and system controls designed to support consistent successful deployment of biomanufacturing processes by individuals with limited training and no prior experience in bioprocessing. Operators load a pre-developed bioprocess 'recipe' onto the system operating software, HelianthOS™, and are then directed by the software how to install process-specific consumables, including a single-use assembly and bioprocess fluids. The system automation performs readiness checks of the hardware, software, and consumables, and then directs the operator to start the process by inoculating the bioreactor. No coding or chemical engineering skills are needed to supervise bioprocesses executed on the Dahlia System™. The system's automated corrective actions, which massively reduce risks to operator safety and product quality, are deployed through HelianthOS™ as needed to maintain process controls and respond to process deviations like flow imbalances between unit operations, filter fouling or flow path pressure elevations.

Production of Clinical-Quality Biopharmaceutical Proteins Using the Dahlia System™

We have demonstrated operation of the Dahlia System™ in a warehouse environment to produce thousands of formulated doses of clinical-quality BDS for multiple products, including the radiation toxicity treatment G-CSF and a SARS-CoV-2 subunit vaccine candidate. Bioprocesses were developed for each protein product before deployment on the system, including designing a *Pichia pastoris* (*Komagataella phaffii*) strain secreting the protein active ingredient, and developing processes for expression using perfusion fermentation, purification via straight-through chromatography, and formulation through tangential flow filtration. Bioprocess parameters for each modular unit operation (such as cultivation pH, temperature, and dissolved oxygen (DO), purification flow rates and column volumes, and concentration and diafiltration factors) were used to build a product-specific process 'recipe' for deployment of each end-to-end process on the Dahlia System™.

The process timeline for the production of the SARS-CoV-2 vaccine candidate (RBD)

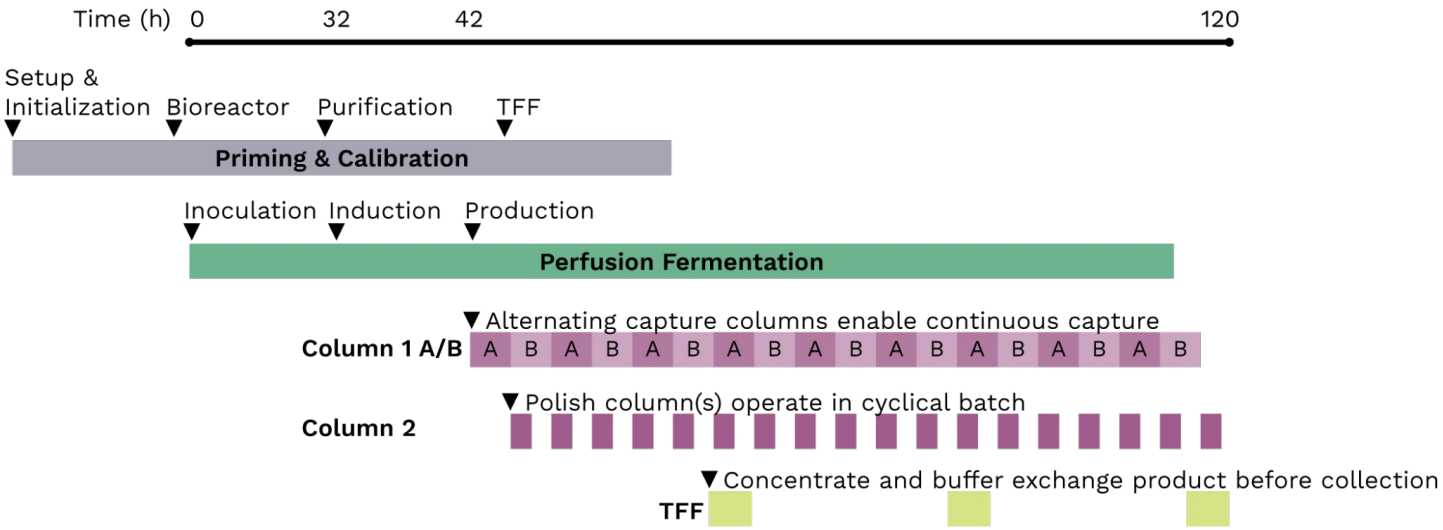

Figure 2. Process timeline for automated production of a SARS-CoV-2 vaccine candidate on the Dahlia System™.

is shown in Figure 2. Following initialization, HelianthOS™ guided the operator through installation of the pre-built single-use assembly, including the bioreactor, tubing, columns, and filters, and performed automated checks for proper installation and communication. Priming and calibration of each module was then automatically performed by HelianthOS™. The operator was notified when the bioreactor was fully charged and ready for inoculation. **Following inoculation, no operator intervention was required for the remainder of the campaign.**

The perfusion fermentation process started with a 32 hour biomass accumulation phase, followed by induction of RBD production by automatically feeding a media containing methanol, the inductive carbon source for heterologous protein expression. After an additional 10 hour transition period, perfusate from the stirred tank bioreactor was automatically directed to the purification module for the remainder of the 5 day campaign. The pH of the perfusate was adjusted to a predefined setpoint through in-line dilution prior to loading on the capture column.

The purification module contained two identical capture columns (Column 1 A/B), which were operated in an alternating fashion so that perfusate containing the expressed RBD antigen was continuously loaded onto a column. After a pre-specified time based on the loading capacity of the capture column, the perfusate stream was automatically switched from Column 1A to Column 1B. While Column 1B was loading, Column 1A was re-equilibrated, washed, eluted, cleaned, and equilibrated again. Each elution from Column 1 A/B flowed directly through Column 2, which was operated in flow-through mode. Flow-through from Column 2 was directed to the TFF. Following each elution, Column 2 was cleaned and re-equilibrated prior to the next elution cycle from Columns 1 A/B.

Table 2. Key metrics of a SARS-Cov-2 vaccine candidate and G-CSF manufactured on the automated Dahlia System™, including yield, purify, safety, and functionality.

Protein Bulk Produced	Yield	Purity		Safety	Function
	Dose equivalents in 1 week	Host-Cell Protein	Host-Cell DNA (ng DNA/mL)	Bioburden (CFU/plate)	Biolayer Interferometry
SARS-CoV-2 Vaccine Candidate	>50,000	<0.1%	<10	0	Binds to ACE2 and mAb CR3022
G-CSF	>1,000	<0.1%	<10	0	Not Tested*
Clinical Benchmarks	*N/A*	*<0.1%*	*<10*	*0*	*Varied*

**G-CSF produced using the same process on an earlier prototype showed expected function both* in vitro *in a cell-based proliferation assay and* in vivo *in rats.*[6]

The TFF operated automatically approximately once per day to concentrate and diafilter the purified RBD antigen. Formulated RBD antigen was then directed to the refrigerated collection module. A small amount of the formulated antigen was collected in a representative sample bag to enable quality testing without breaking the sterility of the bulk collection container.

The collected RBD bulk drug substance was analyzed for typical quality metrics, including identity, purity and safety (Table 2, Figure 3). Over 50,000 dose equivalents of RBD were collected in only 5 days. Host-cell DNA and host-cell protein testing indicated levels consistent with clinical-quality benchmarks. Biolayer interferometry (BLI) was performed to evaluate *in vitro* potency and functional activity. The RBD antigen manufactured on the Dahlia System™ showed expected binding to ACE2, the human receptor for SARS-CoV-2, and mAb CR3022, a known neutralizing antibody. Bioburden was also measured by a third party contractor

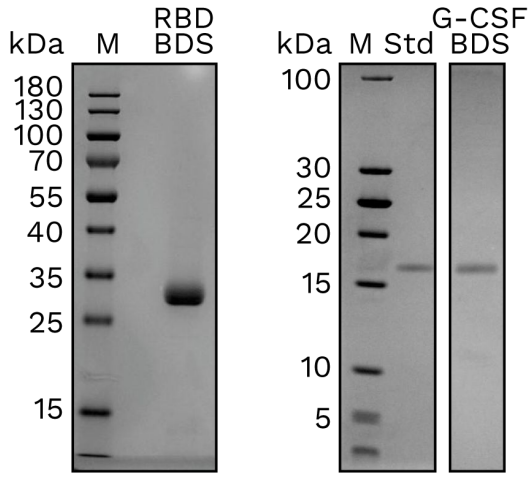

Figure 3. SDS-PAGE of a SARS-CoV-2 vaccine candidate (RBD) and G-CSF bulk drug substance produced on the Dahlia System™.

according to the compendial method USP <61> "Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests." Importantly, **despite operating the Dahlia System™ in an uncontrolled warehouse, no bioburden was detected in any of the formulated BDS samples.** A similar demonstration manufacturing G-CSF achieved the same purity and safety, producing over 1,000 dose equivalents in less than 1 week (Table 2, Figure 3).

Discussion

We have demonstrated the application of the Dahlia System™ as a microfactory for biomanufacturing through the fully automated production of two clinical-quality protein products in an uncontrolled warehouse. The benefits of the Dahlia System™ compared to legacy biomanufacturing are detailed in Table 1, including significant reductions in footprint, upfront capital cost, and the number of highly skilled operators required. These improvements substantially reduce the barrier to entry to biomanufacturing and encourage sustainability throughout a facility's operational lifecycle.

A key benefit of the Dahlia System™ compared to legacy biomanufacturing is its flexibility, enabling the production of diverse protein classes and reducing the turnaround time between products to only 1 day. This flexibility is critical to enable local manufacturers to support regional needs by producing a larger variety of products at lower volumes based on demand. To this end, Sunflower has demonstrated proof-of-concept for the production of a wide variety of therapeutic proteins using manufacturing processes compatible with the Dahlia System™, including monoclonal antibodies (mAbs), single-domain antibodies (sdAbs), antibody Fabs, Fc-fusions, cytokines, interferons, vaccine antigens and AI-designed proteins (Figure 4). We have also demonstrated additional host organisms compatible with deployment on the Dahlia System™, including the filamentous fungi C1,[7] and bacteria.

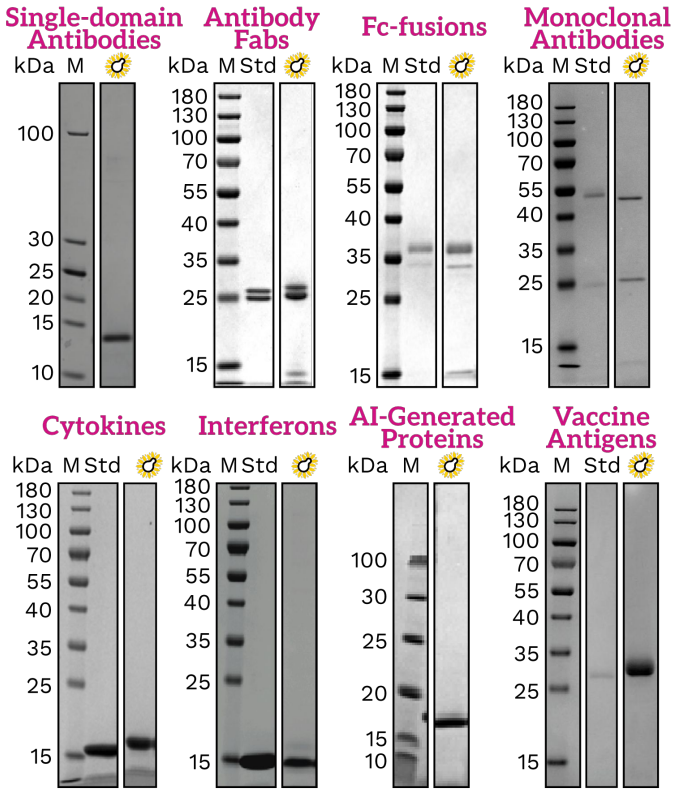

Figure 4. SDS-PAGE of varied protein classes manufactured using processes compatible with the Dahlia System™.

Many other microbes and protozoa are anticipated to be compatible with the Dahlia System™ including *Saccharomyces cerevisiae*, *Leishmania tarentolae*, *Bacillus subtilis*, *Trichoderma reesei*, and *Aspergillus niger*.

Based on initial (informal) feedback from global regulators, the Dahlia System™ is viewed as a piece of manufacturing equipment, similar to other bioreactors or purification systems used to deploy bioprocesses for the Chemistry, Manufacturing and Controls (CMC) of regulated therapeutics. Given that the Dahlia System™ is not a medical device, bulk drug substances produced on the system should undergo standard batch release testing prior to fill and finish operations. We do, however, foresee a

future state where parametric data collected during Dahlia System™ operation could be substituted for endpoint analytical data to speed batch release or even enable real-time release. We anticipate that this parametric release would be supported by the collection of substantial process analytical data for a given product based on its consistent process recipe execution many times on multiple systems. Sunflower is currently building the Quality Risk Management (QRM) documentation required for deployment of the Dahlia System™ hardware, consumables and software in regulated biomanufacturing facilities. Our Daisy Petal® Perfusion Bioreactor System, the commercially available bench-scale version of the first module in the Dahlia System™ for perfusion fermentation, already is 21 CFR 211 (Subpart D) and 21 CFR Part 11 compliant for use in regulated manufacturing and has been deployed in Good Manufacturing Practice-compliant space by a global vaccine manufacturer.[8]

Conclusion

The Dahlia System™ has the potential to revolutionize GMP biomanufacturing of protein-based therapeutics. It is the first fully automated, small footprint biomanufacturing system that deploys integrated and continuous bioprocesses for the production of protein bulk drug substance. **Deployment of the Dahlia System™ in microfactories would significantly reduce the barriers to entry for biomanufacturing and enable distributed manufacturing of biologics, enhancing regional production capabilities, eliminating vulnerabilities tied to single location manufacturing, improving global access to biologics, and building bioeconomies in new geographies, including LMICs and rural US communities.** Biomanufacturing microfactories based on the **Dahia System™ can also be used for expeditionary manufacturing of medical countermeasures for warfighters** to enable rapid response when and where it is needed. In short, the Dahlia System™ provides the agility and autonomy necessary to redefine global biopharmaceutical supply chains to the benefit of patients everywhere.

Materials & Methods

Process Development

Pichia pastoris (*Komagataella phaffii*) strains were engineered by Sunflower to secrete the protein active ingredient. Bench-scale equipment was used to develop protocols for the unit operations. Perfusion fermentation processes were developed on a Daisy Petal® Perfusion Bioreactor System. Chromatography processes were developed on an AKTA Pure™ and adapted for deployment on the straight-through chromatography module. Tangential flow filtration processes were developed on a KrosFlo® KR2i and adapted for the Dahlia TFF module. Bioprocess parameters for each modular unit operation (such as bioreactor pH, DO, and temperature, purification flow rates and column volumes, and concentration and diafiltration factors) were used to build process ‘recipes’ for deployment of specific end-to-end manufacturing processes on the Dahlia System™.

Production of a SARS-CoV-2 vaccine

Heterologous SARS-CoV-2 receptor binding domain (RBD) was produced on the Dahlia System™ using strains of *Pichia pastoris* engineered by Sunflower. The system was operated in an uncontrolled warehouse. The bioreactor was charged with defined growth media containing glycerol to a 15 L working volume. Frozen cell stock was used to inoculate the bioreactor at a starting OD_{600}/mL of 0.10. Defined growth media containing glycerol was continuously fed to the reactor at a perfusion rate of 1.8 vessel volumes per day (VVD) for 32 hours to build biomass. Cell-free harvest fluid was directed to waste during this time. Next, the Dahlia System™ automatically induced heterologous protein production by switching to a defined production medium containing methanol. The production medium was fed at a perfusion rate of 1.8 VVD. After an initial media adaptation period (10 hours), the cell-free harvest fluid was directed to the downstream module for purification. The HelianthOS™ operating system actively maintained bioreactor level, pH, temperature, and dissolved oxygen at the predefined set points throughout the campaign.

The cell-free harvest fluid from the bioreactor was adjusted to a predefined pH via in-line dilution prior to loading onto the first chromatography column. For each purification cycle, ~25 column volumes (CV) of adjusted perfusate was loaded onto a 200 mL prepacked multimodal cation exchange (MMCEX) column. After completing the load on Column 1A, the perfusate was automatically loaded onto Column 1B, containing the same pre-packed capture resin. While Column 1B was loaded, Column 1A was re-equilibrated, washed, eluted, stripped and re-equilibrated again. Columns 1 A/B continued to alternate to enable continuous loading from the bioreactor. Eluate from Column 1 A/B was directed to a 200 mL prepacked anion exchange (AEX) column operated in flow-through mode. Flow-through from Column 2 was directed to the formulation module. Column 2 was then stripped and re-equilibrated prior to receiving another elution from Column 1 A/B.

The formulation module utilized hollow fiber 5 kDa membranes for concentration and diafiltration. The material was first concentrated 3X, then diafiltered into the formulation buffer using 6 diavolumes. The formulated RBD antigen was then over-concentrated 1.4X to account for expected dilution during the collection operation. The formulated RBD antigen was directed to the temperature controlled collection module and analytical sample bags. The formulation module was operated after every 6th elution from the chromatography module. Each cycle of the formulation module was collected in a separate collection bag with a paired analytical sample bag for batch release testing.

Production of G-CSF

Heterologous G-CSF was produced on the Dahlia System™ using strains of *Pichia pastoris* engineered by Sunflower as described above for RBD, except for the following modifications. The media

adaptation period after switching to methanol-containing media and before directing the cell-free harvest to the purification module was 8 hours. A three step purification process was used. For each purification cycle, ~40 column volumes (CV) of adjusted perfusate was loaded onto a 200 mL prepacked multimodal cation exchange (MMCEX) column (Column 1 A/B). Eluate from Column 1 A/B was directed to a 300 mL prepacked anion exchange (AEX) column (Column 2) operated in flow-through mode. Flow-through from Column 2 was directed to a 270 mL hydrophobic charge-induction chromatography (HCIC) column (Column 3) operated in bind-and-elute mode. Column 3 was then re-equilibrated, washed, and eluted. Eluate from Column 3 was directed to the formulation module. Columns 2 and 3 were stripped and re-equilibrated prior to receiving another elution from Column 1 A/B. The formulation module was operated after every 4th elution from the chromatography module.

Analytics

Samples of the formulated bulk drug substance from the collection module for both RBD and G-CSF were analyzed to determine protein yield, purity, and quality. Protein yield was determined by absorbance at A280, corrected for the product-specific extinction coefficient. Dose equivalents were calculated based on a dose size of 50 µg for RBD or 300 µg for G-CSF. SDS-PAGE was used to analyze protein quality and purity. Samples were analyzed for host-cell protein (HCP) content using the *Pichia pastoris* 2nd Generation HCP ELISA kit from Cygnus Technologies according to the manufacturer's recommended protocol. Samples were analyzed for residual host-cell DNA using the Quant-iT dsDNA high sensitivity assay kit from Invitrogen™ according to the manufacturer's recommended protocol. Biolayer interferometry was performed using the Octet Red96. Bioburden testing was performed by a third party contractor according to the compendial method USP <61> "Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests."

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Related Resources

Perfusion fermentation in the Daisy Petal™ bioreactor

Improving production of recombinant proteins through yeast strain engineering