Democratizing Biomanufacturing using Perfusion Fermentation

Laura Crowell, PhD
Director R&D, Sunflower Tx
DCVMN Webinar
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Contact: laura@sunflowertx.com
Sunflower is an early-stage company delivering next-generation biomanufacturing solutions to global innovators for protein medicines, vaccines and food.

Just like anyone can grow sunflowers anywhere, our solutions are easy to use and empower anyone to efficiently make proteins anywhere.
## Diverse Industries Are Producing Proteins & Need Manufacturing Solutions

<table>
<thead>
<tr>
<th>Biopharma Companies</th>
<th>Research Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Discovery</td>
<td>Protein Design &amp; Eng.</td>
</tr>
<tr>
<td>Drug Development</td>
<td>Synthetic Biology</td>
</tr>
<tr>
<td>Process Development</td>
<td>Metabolic Engineering</td>
</tr>
<tr>
<td>Manufacturing</td>
<td>Vaccine/Drug Discovery</td>
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<tr>
<th>Biotech Companies</th>
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<tr>
<td>Animal Health</td>
<td>Vaccine/Drug Security</td>
</tr>
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<td>Cosmetics</td>
<td>Biologic Defense</td>
</tr>
<tr>
<td>Foods &amp; Nutraceuticals</td>
<td>Pandemic Preparedness</td>
</tr>
<tr>
<td>Materials</td>
<td>Workforce Training</td>
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</tbody>
</table>

**BOLD** = Sunflower’s Existing Traction
Conventional Biomanufacturing Capacity Requires Significant Upfront Resources & Highly Skilled Workforce
Our Goal: Make Protein Manufacturing More Accessible

Accessibility = Easy to obtain + Easy to use

- Lower Cost
- Small Footprint
- Deployable

- Simple
- Automated
- Efficient
- Flexible

Host organism and manufacturing approach impact cost, simplicity, efficiency and flexibility
Eukaryotic Microbes are Simple and Efficient Hosts for Protein Manufacturing
# Eukaryotic Microbes Enable Process Simplicity

<table>
<thead>
<tr>
<th></th>
<th>Bacteria (E. coli)</th>
<th>Eukaryotic Microbes (P. pastoris)</th>
<th>Mammalian Cells (CHO)</th>
</tr>
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<tbody>
<tr>
<td>Protein Secretion</td>
<td>&lt;&lt;50%</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Initial Purity</td>
<td></td>
<td>&gt;70%</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>No Endotoxin</td>
<td>✔</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>No Adventitious Agents</td>
<td>✔</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Process Complexity</td>
<td>HIGH</td>
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# Eukaryotic Microbes Enable Lower Cost

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<td>HIGH</td>
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<td>Cost of Goods Manufactured</td>
<td>MED</td>
<td>LOW</td>
<td>HIGH</td>
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Continuous Manufacturing Enables Efficiency, Quality and Productivity
Continuous Manufacturing Enables **Efficiency**

Bioreactor volume required to produce 1 kg of IgG1 in CHO in 30 days*

Continuous manufacturing reduces footprint by 7-fold

*Based on data from Walther et. al., Biotechnology Journal. 2018
Continuous Manufacturing Enables Quality

Consistent cell environment leads to higher cell viability and product quality

*Based on data from Walther et al., Biotechnology Journal. 2018
**Continuous Manufacturing Enables Productivity**

**Instantaneous protein titer** is not a complete representation of protein produced for continuous processes.

**Continuous processes run longer** and produce more total protein.

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**Instantaneous Protein Titer (mg/L)**

![Graph showing instantaneous protein titer over time with Fed-Batch and Continuous processes compared.]

**Total Protein (mg)**

![Graph showing cumulative total protein over time with Fed-Batch and Continuous processes compared.]

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Continuous Manufacturing Enables Productivity

**Space-Time Yield** is a metric for normalized comparison

\[
\text{Space-Time Yield} = \frac{\text{Mass of Protein}}{\text{Bioreactor Volume}} \times \frac{1}{\text{Cultivation Day}}
\]

Continuous manufacturing enables significantly higher space-time yields.
Perfusion Fermentation: Continuous Manufacturing for Eukaryotic Microbes
Historical Challenges To Continuous Manufacturing for Microbes

Legacy Method: Chemostat

Feed → Harvest (Contains cells & spent media)

Limited achievable cell mass due to constant cell harvest

Conventional Perfusion (used with CHO)

Feed → FILTER → Cells → Cell-Free Harvest

Removes cells from the bioreactor environment leading to starvation for microbes
Sunflower’s Technology Enables In-Vessel Perfusion for Eukaryotic Microbes

Benefits of In-Vessel Perfusion

- Cells never removed from bioreactor environment
- Healthier, more consistent cell culture
- Longer campaigns
- Ultra high cell mass achievable and maintainable
- Continuous harvest of secreted protein

*Cell Retention Device
Daisy Petal™ Single-Use Bioreactor System: Perfusion fermentation for eukaryotic microbes

EFFICIENT PRODUCTION IN A SMALL FOOTPRINT

- Bench-top sized but **produces like larger reactors** (up to grams protein in 1–2 weeks from 1L working vol.)
- Unique single-use structured consumable for **intuitive installation by non-expert users** and rapid **flexibility**
- Custom software enables **fully automated** operation
- **Immediatly deployable** - just add cells & process fluids
Daisy Petal™ Single-Use Bioreactor System: Perfusion fermentation for eukaryotic microbes

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EARLY ACCESS UNITS DELIVERED SUMMER 2022

“The Daisy Petal is really simple to use. I knew exactly how to get started with the system and consumables.”
- Customer in Petal Early Access Program with no previous bioprocess experience
Perfusion Fermentation Sustains Production of High-Quality Proteins

**CONTINUOUS MICROBIAL PRODUCTION IS MORE EFFICIENT THAN BATCH**

- **>1.2 grams** (4,200 dose equivalents) of unpurified G-CSF produced in **9 days** in 1 L working volume reactor
- Space-Time Yield: **>130 mg/L/day** (**>500 mg/L** fed-batch titer)
- Process ran **4 days longer** than conventional batch cultivation
- Perfusion fermentation enabled by proprietary in-vessel cell retention device
Daisy Petal™ Simplifies Fermentation Optimization

A 3 LEVEL, 2 FACTOR FULL FACTORIAL DOE REQUIRES 9 CONDITIONS

- **Standard Fed-Batch**: 9 Experiments
  - 1 vessel x 36+ days
  - 9 vessels x ~5 days

- **Continuous Perfusion**: 1 Experiment
  - 1 vessel x 10 – 11 days
Continuous Operation Links Parameter Testing

Variable Process Parameters
- pH
- Dissolved oxygen (DO)
- Temperature
- Perfusion Rate (Feeding)
- Carbon input (Feeding)
- Cell Bleed Rate

A single experiment can test multiple process conditions

![Diagram showing process steps]

- **Start New Recipe Step**: Controllers automatically adjust to new set points
- **Perfusion operation turns over fluid in reactor**
- **Cells adapt to new steady state**
- **Collect** samples at new steady state
- **Repeat** for new conditions

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>24</td>
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</table>
Early Access Customers Demonstrate Benefits of Perfusion

MAB PRODUCTION TESTED WITH HIGH BIOMASS (~600 WCW) AT MULTIPLE GROWTH RATES

<table>
<thead>
<tr>
<th>mAb STD</th>
<th>mAb STD</th>
<th>ProA Purified Samples from Daily Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

- Growth Rate 1
- Growth Rate 2

HC
LC
Diverse Proteins Produced Using Daisy Petal™

**ANTIBODIES & MAB-LIKE PRODUCTS**
- Monoclonal antibodies
- Single-domain antibodies (sdAb) & multimers
- Fragment antigen-binding regions (Fabs)

**CYTOKINES & HORMONES**
- G-CSF
- IFNα-2b
- hGH

**VACCINE ANTIGENS**
- COVID subunit vaccine candidates
- Rotavirus subunit vaccine candidates
- Virus-like particles (VLPs)

**ENZYMES & OTHER PROTEINS**
- DNA / RNA editing enzymes
- Bacterial toxins

**Dahlia Petal™ is Equivalent to 200L Batch Bioreactor**

**15L Working Volume**
Can produce **100 grams** protein per week in <20 m²

**SCALE-UP TO DAHLIA PETAL™ RESULTS IN CONSISTENT SPACE-TIME YIELDS**

- G-CSF successfully produced during hardware testing
- **>5 grams** G-CSF produced in only **4 days**
- Space-Time Yield: **>70 mg/L/day**
- *Space-Time Yield from Daisy Petal™ @ 4 days: 77 mg/L/day*

![Image of equipment and graph](image)

- Std @ 0.05, 0.1 mg/mL
- M

<table>
<thead>
<tr>
<th>kDa</th>
<th>100</th>
<th>75</th>
<th>50</th>
<th>25</th>
<th>15</th>
<th>10</th>
<th>5</th>
<th>3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
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Unpurified perfusate (Day #)

- 3
- 4

- G-CSF successfully produced during hardware testing
- >5 grams G-CSF produced in only 4 days
- Space-Time Yield: >70 mg/L/day*
- *Space-Time Yield from Daisy Petal™ @ 4 days: 77 mg/L/day*
Benefits of Perfusion Fermentation with Sunflower Systems

- **Continuous** - Overcomes limits of batch production for lower costs (both capital & operational)
- **Flexible** - Accommodates a range of operations and proteins
- **Automated** - Custom software for ‘hands-free’ operations
- **Scalable** - Systems designed to transition from idea to manufacturing
- **Intuitive** - Designed for users with diverse backgrounds in production
- **Deployable** - Ready for installation and immediate use in many environments
- **Sustainable** - Reduced waste (particularly cleaning waste)
- **Efficient** - More protein for lower cost and less space
Perfusion Fermentation Enables End-to-End Automated Biomanufacturing
Steps Needed for End-to-End Biomanufacturing

1. Expression
   - Protein is produced by cells

2. Purification
   - Protein is separated from impurities

3. Formulation
   - Protein is put into a buffer safe for administration
Steps Needed for End-to-End Biomanufacturing

1. Expression
   - Perfusion Fermentation

2. Purification
   - Straight-through Chromatography

3. Formulation
   - Tangential Flow Filtration
Straight-through Chromatography Enables Intensified Manufacturing

Straight-through chromatography

Eliminate buffer conditioning (pH / salt changes) between chromatography steps

- # of hold tanks
- buffer usage
- processing time
- manufacturing footprint
- operator touch points
Straight-through Chromatography: 2-column example

Conventional Chromatographic Staging

4 Buffers
1 Hold Tank and/or Buffer Exchange
Straight-through Chromatography: 2-column example

Conventional Chromatographic Staging

- Buffer: Column 1 Capture
- Column 1 Elution
- Column 2 Capture
- Column 2 Elution

4 Buffers
1 Hold Tank and/or Buffer Exchange

Straight-through Integrated Staging

- Buffer: Column 1 Capture
- Column Transition (Bridging)
- Column 2 Elution

3 Buffers
NO Hold Tank and/or Buffer Exchange
Conventional Processes are Lengthy

G-CSF in *E. coli* (18 Step conventional process)

Expression in *E. coli* (inclusion body) → Resuspension → Lysis → Centrifugation → Extraction → Centrifugation → Resuspension

Centrifugation → Resuspension → Oxidation → Centrifugation → Dilution → Dowex Resin → Filtration → Anion Exchange Chrom.

pH adjustment → Cation Exchange Chrom. → Formulation

Sunflower Integrated Processes are Efficient

G-CSF in *E. coli* (18 Step conventional process)

Expression in *E. coli* (inclusion body) → Resuspension → Lysis → Centrifugation → Extraction → Centrifugation → Resuspension

Centrifugation → Resuspension → Oxidation → Centrifugation → Dilution → Dowex Resin → Filtration → Anion Exchange Chrom.

pH adjustment → Cation Exchange Chrom → Formulation

G-CSF in Sunflower Process (7 Steps)

Expression in *P. pastoris* (secreted) → In-vessel Filtration → pH adjustment → Cation Exchange Chrom. → Anion Exchange Chrom. → Hydrophobic Induction Chrom. → Formulation

Perfusion fermentation and straight-through chromatography lead to a 60% reduction in the number of manufacturing steps

Crowell et al., Nat. Biotechnol., 2018

Sunflower Systems Use Similar Processes for Many Proteins

**Human growth hormone**
- Expression in *P. pastoris* (secreted)
- In-vessel Filtration
- pH adjustment
- Cation Exchange Chrom.
- Anion Exchange Chrom.
- Formulation

*Crowell et al., Nat. Biotechnol., 2018*

**Recombinant protein vaccines**
- Expression in *P. pastoris* (secreted)
- In-vessel Filtration
- pH adjustment
- Cation Exchange Chrom.
- Anion Exchange Chrom.
- Formulation

*Dalvie et al., PNAS 2021; Dalvie et al., Microb. Cell Factories 2021*

**Single-domain antibody (nanobody)**
- Expression in *P. pastoris* (secreted)
- In-vessel Filtration
- pH adjustment
- Cation Exchange Chrom.
- Anion Exchange Chrom.
- Formulation

*Crowell et al., Biotechnol. Bioeng. 2021*

**Monoclonal antibody (mAb)**
- Expression in *P. pastoris* (secreted)
- In-vessel Filtration
- pH adjustment
- Chrom. Step 1
- Chrom. Step 2
- Formulation

DCVMN Webinar - August 8, 2023
Sunflower’s End-to-End Manufacturing Systems:
Simple hardware to enable capacity for anyone

**Daisy™ System**
- **R&D/Process Development**
  - Automated Protein Bulk Production
  - (up to grams per week)

**Dahlia™ System**
- **Efficient Commercial Production**
  - Automated Protein Bulk Production
  - (up to 10 kg annually in <50m²)

Immediate Process Transfer
Sunflower End-to-End Systems: Breakthrough automated small footprint commercial protein manufacturing

INTEGRATED SYSTEM FOR CONTINUOUS PRODUCTION OF PROTEIN BULK

End-To-End: Integrated operations for expression, purification, formulation and collection

Fully Automated: Add cells & process fluids, push start, & collect protein bulk for a product in just days

Multi-Product: Closed single-use design for agility and flexibility

“Move-In” Ready: Operates in many different types of spaces and environments
UP TO 10 KG ANNUAL PROTEIN OUTPUT IN <50m² WITH MULTI-PRODUCT FLEXIBILITY
High-Quality Protein Generated on Both Systems

DAISY & DAHLIA SYSTEMS DEMONSTRATE MULTI-PRODUCT CAPABILITIES USING PROCESSES TRANSFERRED FROM ONE TO THE OTHER

G-CSF (filgrastim)

SARS-CoV-2 RBD (subunit vaccine)

*Processes deployed for both molecules were not optimized for yield (Drafted for equipment demonstrations only)
**Bulk Drug Substances Produced on Systems are Similar**

**DAISY & DAHLIA SYSTEMS PRODUCED CLINICAL QUALITY BULK DRUG SUBSTANCES IN SIMPLE MANUFACTURING ENVIRONMENTS IN < 1 WEEK**

<table>
<thead>
<tr>
<th>Protein</th>
<th>System Used</th>
<th>Dose Equivalents</th>
<th>Host-cell DNA (ng DNA/mL)</th>
<th>Host-cell Protein (total protein)</th>
<th>Bioburden* (CFU/plate)</th>
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</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td></td>
<td>~100</td>
<td>&lt;10</td>
<td>&lt; 0.1%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;1,000</td>
<td>&lt;10</td>
<td>&lt; 0.1%</td>
<td>0</td>
</tr>
<tr>
<td>COVID-19 Vax Subunit</td>
<td></td>
<td>&gt;2,000</td>
<td>&lt;10</td>
<td>&lt; 0.1%</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>&gt;50,000</td>
<td>&lt;10</td>
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*Performed by third-party contractor according to the Compendial method USP 61 microbiological examination of Non-Sterile Products (Microbial enumeration test).
Sunflower Systems Maximize Facility Utilization

STATE-OF-THE-ART SINGLE-USE PILOT PLANT

SUNFLOWER (Many Product Classes)

MULTI-PRODUCT CAPABILITY AND AGILITY ENABLES LOWEST MANUFACTURING COSTS
## Projected Annual Product Output From Our Approach

<table>
<thead>
<tr>
<th>Location</th>
<th>VACCINE DOSES* (50 ug / dose)</th>
<th>MAB (Trastuzumab) DOSES** (1 g / dose)</th>
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<tbody>
<tr>
<td>Puerto Rico</td>
<td>2.9 Million</td>
<td>580</td>
</tr>
<tr>
<td>California</td>
<td>40 Million</td>
<td>9,000</td>
</tr>
<tr>
<td>Argentina</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>South Africa</strong></td>
<td>160 Million</td>
<td>36,000</td>
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*Medium titer process (1 g/L); 50 – 70% population vaccinated; 2 doses per person

**High titer process (4 g/L); HER2+ breast cancer patients; 18 doses per person per year; 30–50% market penetration
Sunflower’s Vision for Democratization of Protein Supply

We believe enabling regional manufacturing capacity for protein products can promote prosperity everywhere.

ACCESSIBLE SOLUTIONS POWER NEW PRODUCTS & BUSINESSES

NEW INNOVATIONS FUEL REGIONAL MARKET EXPANSION

MANUFACTURING CAPACITY GROWS TO MEET DEMAND
Sunflower Can Help You Explore the Benefits of Integrated Manufacturing using Eukaryotic Microbes

Hardware Access

- **Daisy Petal™** perfusion fermentation system commercial release in early 2024! **Accepting Pre-Orders Now**

- Seeking **early access customers** for evaluation of **Dahlia Petal™**

- Seeking **early access customers** for evaluation of end-to-end **Dahlia™**

Contract Research Services

- Strain, media and process development in eukaryotic microbes

- Demonstrations on Daisy Petal™, Dahlia Petal™, or end-to-end Dahlia™
Acknowledgements

Sunflower Team

Alexandra Bonnyman  Devin Morrison
Jodie Crowley  Martin Rochefort
Benjamin Fagin  Michael Sheets
Adrian Foell  Akshada Shinde
Minh Le  Patrick Spooner
Sophie Lee  Mary Kate Tracey
Tim Lorgeree  Nikhil Unde
Kerry Love  

Talk to Kerry and Alex at DCVMN Annual Meeting in Cape Town, South Africa!
Visit us at: www.sunflowertx.com

Contact: laura@sunflowertx.com