CO_2-Based Manufacturing System for Recombinant Protein Production

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Introduction

- Space biomanufacturing is an In-Situ Resource Utilization (ISRU) strategy to provide critical products while minimizing launch mass for long-duration, deep space missions.
- Electrochemical conversion of CO_2 to organic molecules is highly efficient
- We are designing a biomanufacturing platform to use electrochemical CO_2 conversion products for carbon substrates to support recombinant protein production

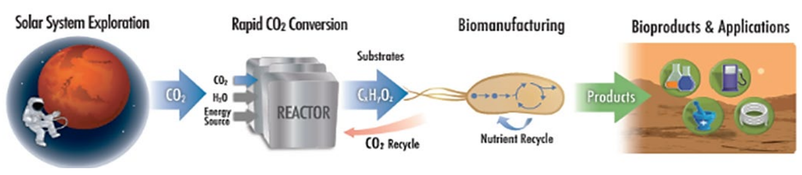

Figure 1: CO_2-Based Manufacturing ConOps

Materials & Methods

Goal: Develop and demonstrate a prototype, semi- autonomous system that enables microbial manufacturing via abiotic CO_2 conversion to products that drive biomanufacturing for future long-duration.

Materials:

Major System Components:

- Arduino Mega 2560 r3
- CHEMcell Bioreactor Rocker System (Chemglass)
- CHEMcell CLS-1200-2HC Temperature Controller (Chemglass)
- EZO PMP Peristaltic Pump (Atlas Scientific)
- PressureMAT Pressure Monitor (PendoTECH)
- Luer Fitting Single Use Pressure Sensors (PendoTECH)
- ValveLink8.3 Digital/Manual Controller (Automate Scientific)
- CBIO Custom Miltilyser (Claremont Bio)
- 1/8” SwitchEX Solenoid Pinch Values
- Vivaflow 200 (Satorius)
- Capturem His-Tag Purification Membrane 50 mm (Takara Bio)
- Custom Developed Membrane Bioreactor Bag
- Custom Developed Dried Media

Table 1: Recombinant Microorganisms

Microorganism	Products	Carbon Source(s)
Escherichia coli	- Carbonic Anhydrase - emGFP	- Acetate
Komagataella phaffii (*Pichia Pastoris*)	- Carbonic Anhydrase - Cutinase	- Acetate - Ethanol

Methods:

- Systematically investigated and selected system architecture for a semi-autonomous bioprocessing system (Soundararajan, et al, 2023, Figure 2)
- Constructed and tested prototype system with single-use components that meet biological material, and reusable components to modulate single-use components

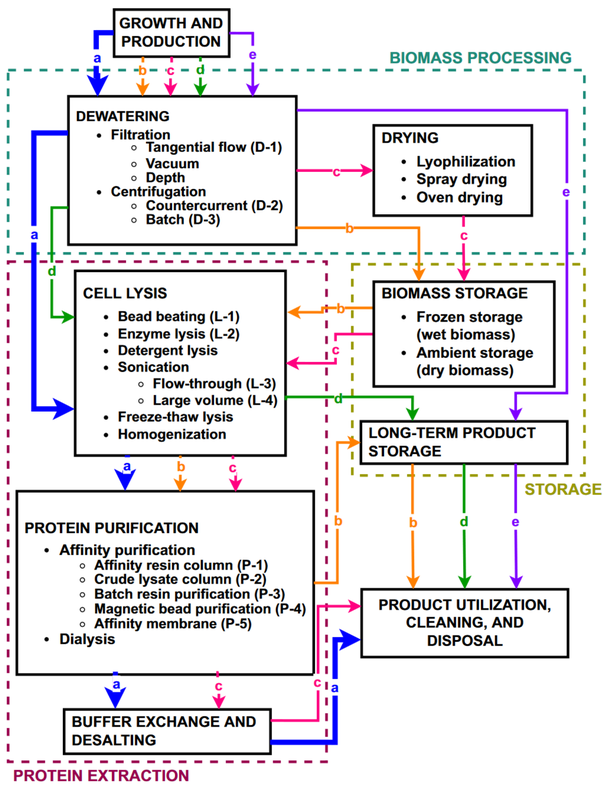

Figure 2: CO_2-Based Manufacturing System Architecture

Results

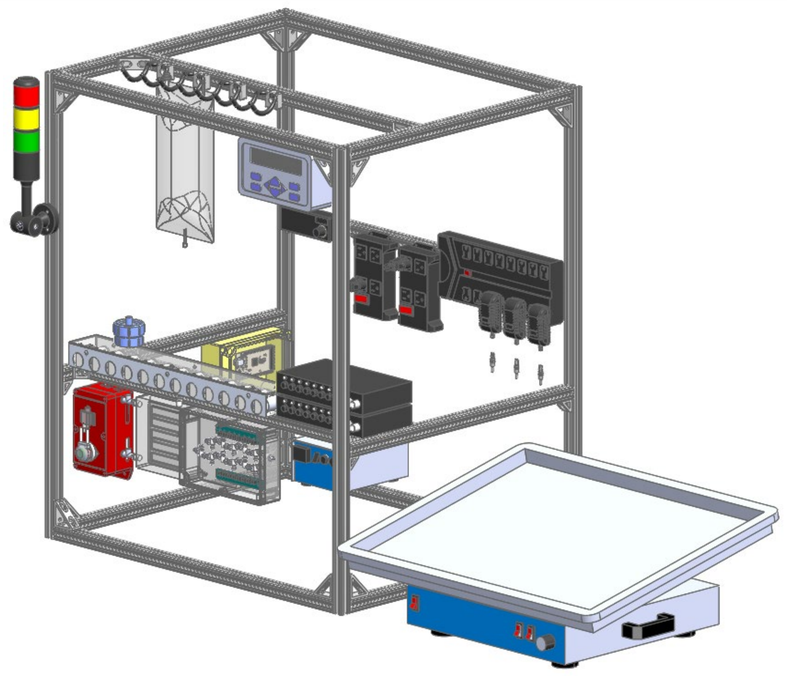

Figure 3: CO_2-Based Manufacturing Prototype CAD
A CAD model combining all subsystems for system development. Note system has not been optimized for size

The final platform was developed (Figure 3), constructed (Figure 4) and is currently undergoing testing for its ability to purify recombinant proteins (Figure 5).

Figure 4: Constructed CO_2-Based Manufacturing Prototype Platform
Actual system developed based on CAD model

Figure 5: CO_2-Based Manufacturing Prototype Platform Being Tested with *E. coli* emGFP
Testing of system using the visually distinguishable emGFP as an indicator of performance prior to testing with other products.

Results

- Multiple runs of the platform were performed to measure various run parameters of the system.
  - Data from a run of 3 independent replicates of *K. phaffii* is shown in Table 1.
  - The effectiveness of the system to concentrate and purify cutinase is shown in Figure 6).

Table 2: Collected Run Data Using *K. phaffii*

Parameter	Run 1 1/29/2024	Run 2 2/1/2024	Run 3 2/2/2024	Average
Total System Run Time (hrs)	73.2	72.4	73.4	73.0 ± 0.5
Bag set up Time (hrs)	0.6	0.5	0.5	0.5 ± 0.1
Downstream Processing Time (hrs)	0.9	1.4	1.0	1.1 ± 0.3
Growth Time (hrs)	71.6	70.5	72.0	71.4 ± 0.8
Initial (OD 600 nm)	0.025	0.020	0.019	0.021 ± 0.003
Harvest OD (600 nm)	4.896	4.896	5.832	5.208 ± 0.540
Power Consumed (kW)	0.064	0.062	0.062	0.063 ± 0.001
Energy (kWh)	4.630	4.300	4.520	4.483 ± 0.168
Elution Volume (ml)	25	29	45	33 ± 11

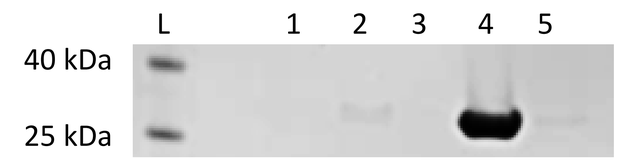

Figure 6: SDS-Page Showing Cutinase Purification from CO_2-Based Manufacturing
1. Supernatent, 2. Effluent from His-Tag Filter, 3. Wash Effluent, 4. Elution, 5. Post Wash Effluent

Conclusion & Future Work

Conclusion: This prototype platform demonstrates the potential for recombinant protein production using CO_2-derived carbon sources while reducing mass, power, volume, heating, cooling, and crew time over conventional systems.

Future Work:

- Further test and optimize the current system
- Look to improve and further reduce volume of system
- Begin testing the system with other recombinant organisms

References & Acknowledgements

- Soundararajan, et al. "Theoretical design of a space bioprocessing system to produce recombinant proteins." *npj Microgravity* 9.1 (2023): 78.

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