Development of a Space Compatible Biomanufacturing System

Synthetic Biology Project, Game Changing Development Program, Space Technology Mission Directorate

Frances Donovan, Ph. D. | NASA Ames Research Center

Image Credit: NASA

What is Synthetic Biology?

"Synthetic biology is a field of science that involves **redesigning organisms** for **useful purposes** by **engineering them** to have **new abilities**."

-National Human Genome Research Institute

Biological systems are:

- Scalable
- Programmable
- Precise (pure isomers)
- The only route of production in some cases (protein therapeutics)
- Low temperature and pressure
- Regenerable

Synthetic Biology in Space

Mission resources will need to be produced on-site for mission sustainability/cost effectiveness

- Fuels, foods/nutrients, chemicals, plastics, binders, medicines, enzymes
- Biomanufacturing can provide compounds abiotic systems cannot

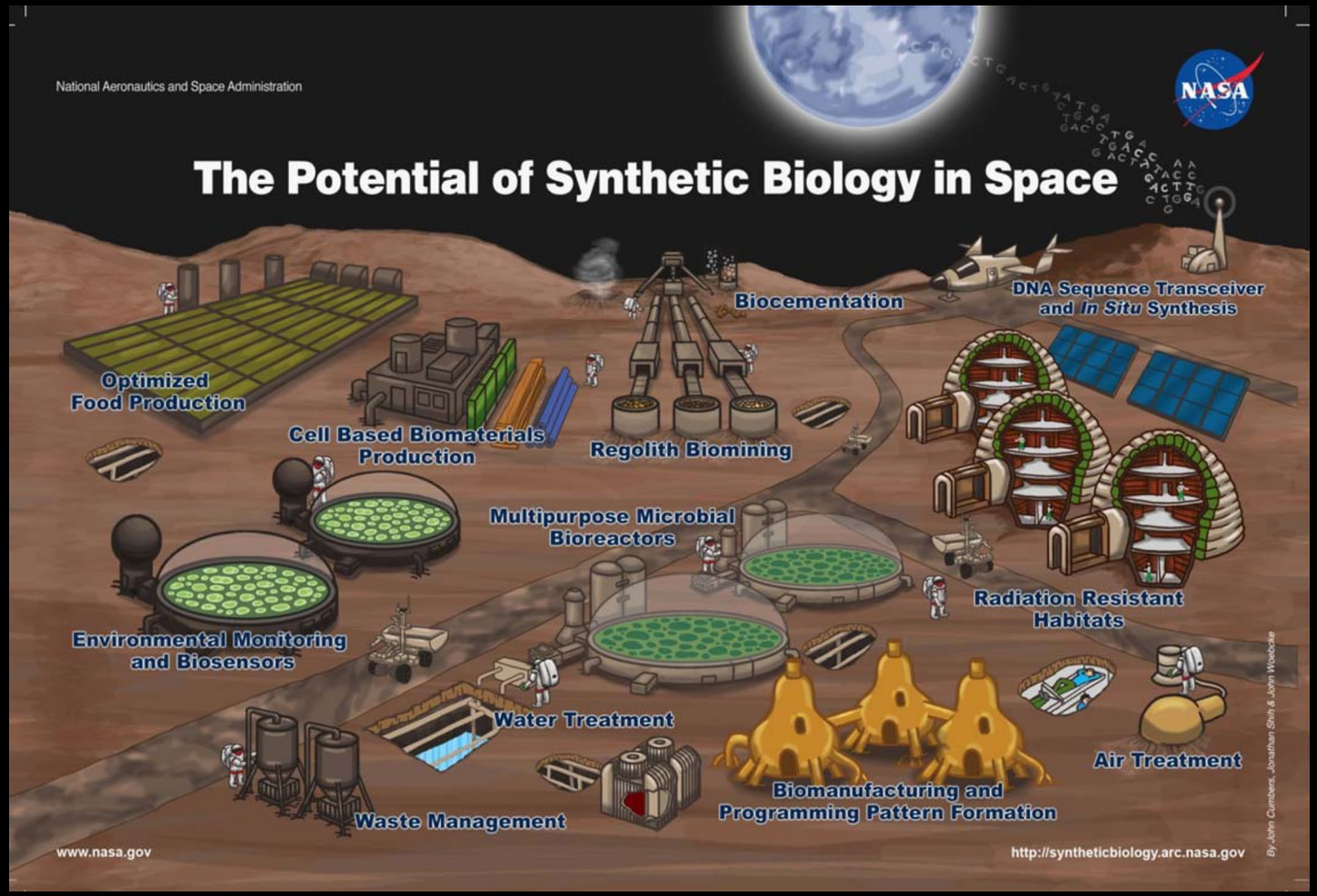
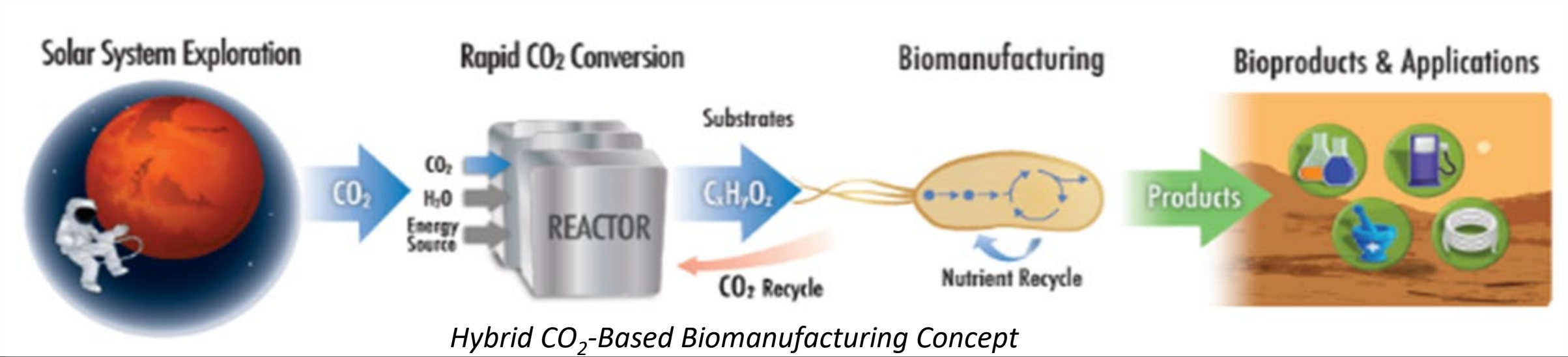


Image Credit: NASA

CO_2-Based Manufacturing- from CO_2 to products that support sustainable space exploration

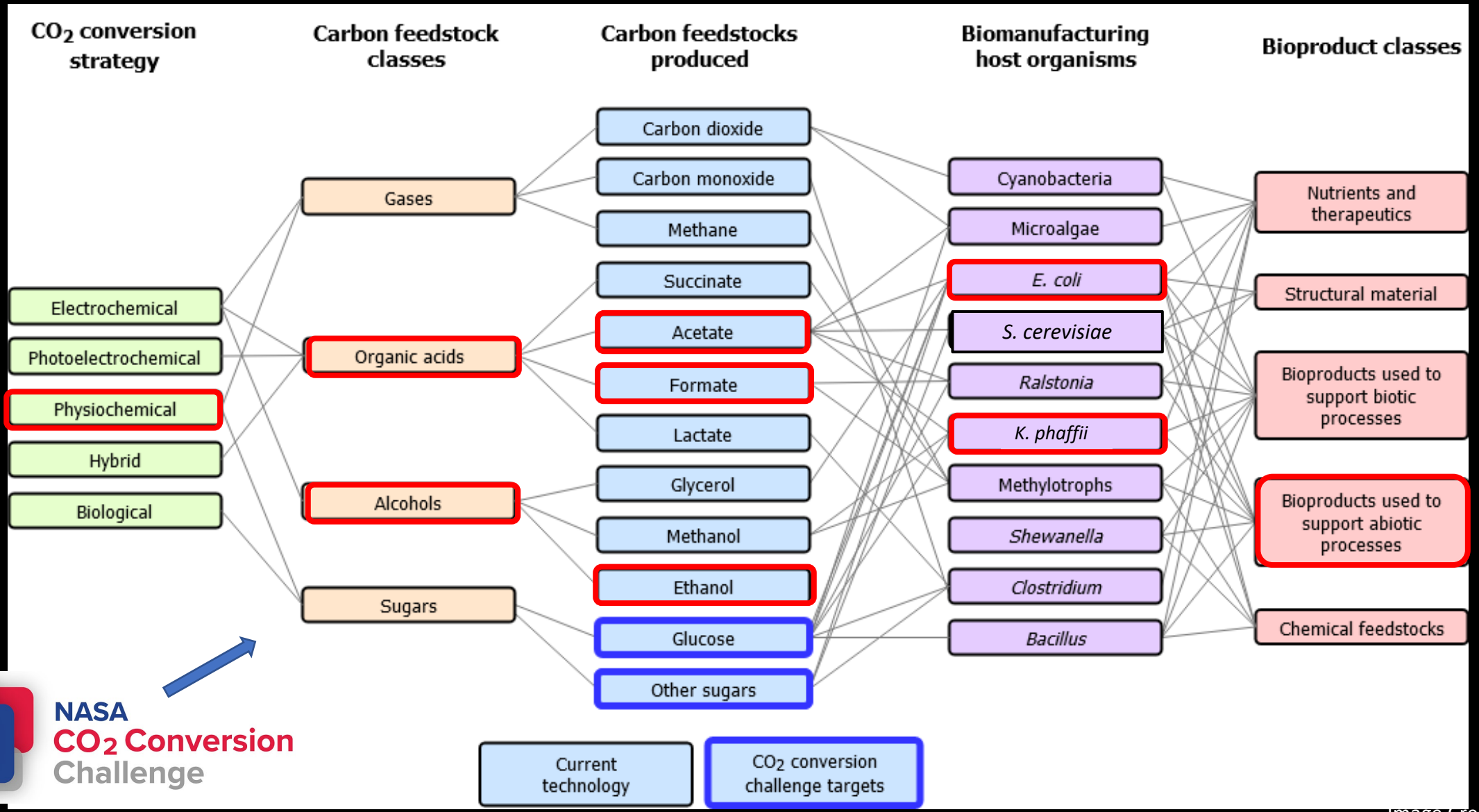


Hybrid CO_2-Based Biomanufacturing Concept

Project Goal: develop and demonstrate a prototype system that enables microbial manufacturing via abiotic CO_2 conversion to products that drive biomanufacturing for future long-duration missions.

Image Credit: NASA

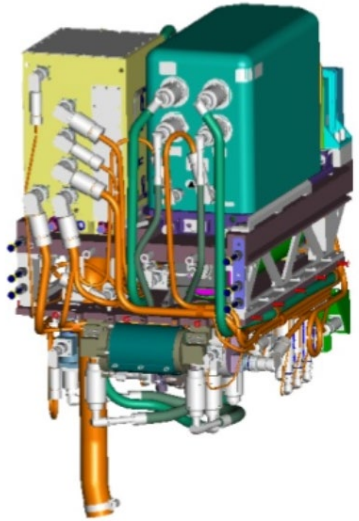
CO_2 -Derived Products, Feedstocks and Organisms



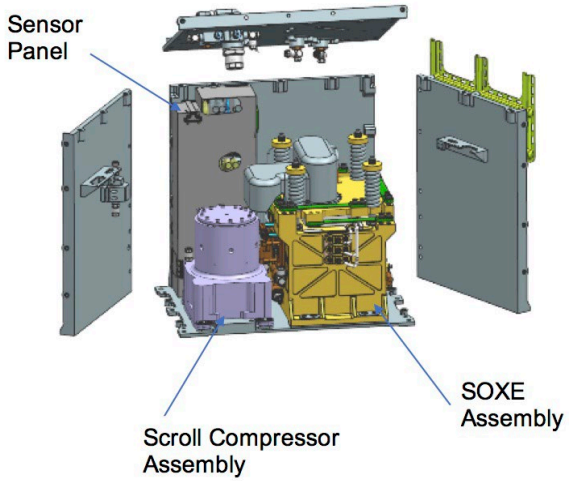
NASA CO_2 Conversion Challenge

Image Credit: NASA

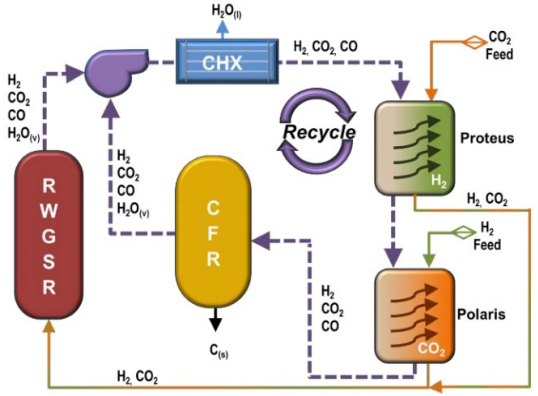
Current Extra-terrestrial Technologies that Support CO_2 Conversion



Sabatier reactor
Input: CO_2
Output: **CH_4**, H_2O
Technology maturity: Flight qualified hardware

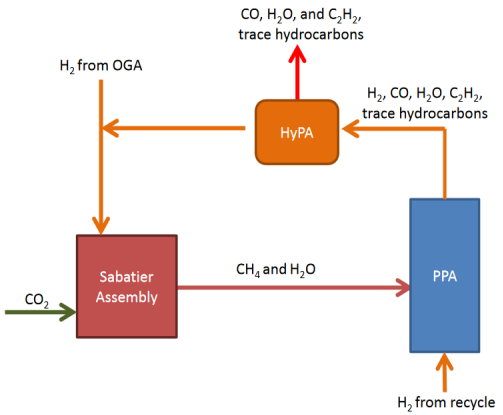


MOXIE [1]
Input: CO_2
Output: O_2, **CO**
Technology maturity: *in situ* demo testing on Mars



RWGSR: Reverse water gas shift reactor
CFR: Carbon formation (Bosch) reactor
Proteus: H_2 extraction assembly
Polaris: CO_2 extraction assembly

Bosch reactor [2]
Input: CO_2
Output: O_2, C
Technology maturity: In development



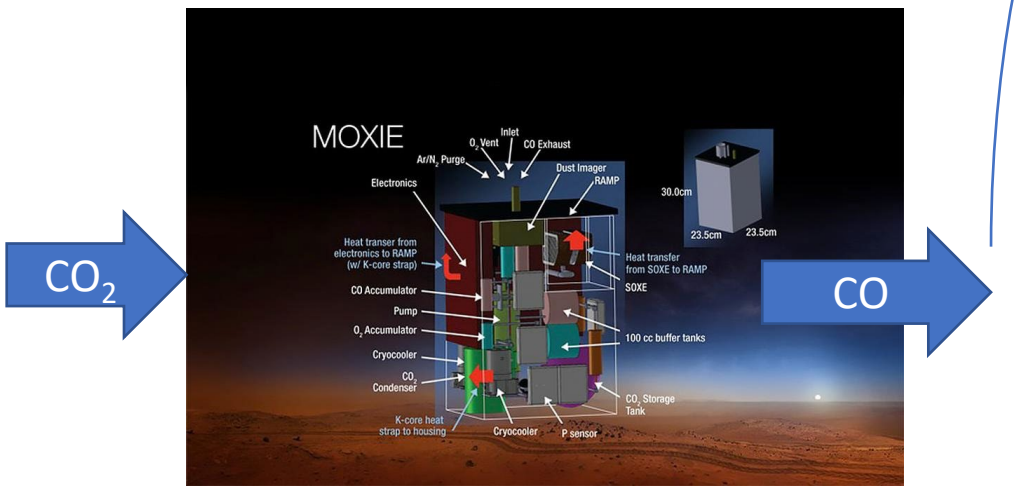
HyPA: Hydrogen purification assembly
PPA: Plasma pyrolysis assembly
OGA: Oxygen generation assembly

Plasma pyrolysis [2]
Input: CH_4
Output: H_2, C_2H_2, maybe C
Technology maturity: In development

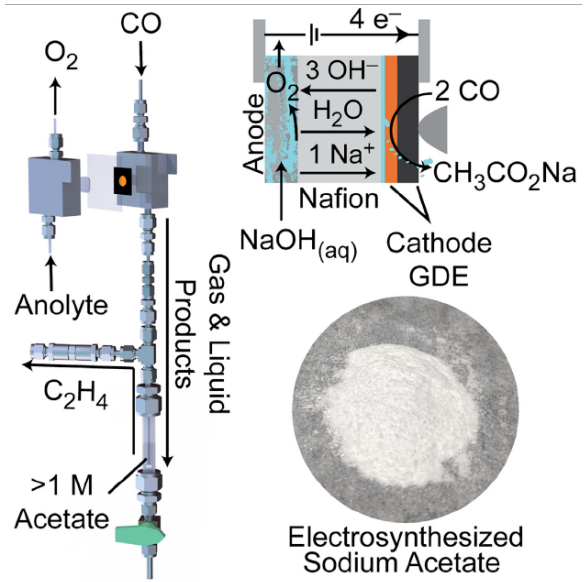
Of the existing conversion technologies Sabatier and MOXIE produce compounds that can be used as feedstock for biomanufacturing (CH_4 and CO respectively, acetylene is a potential feedstock).

Image Credit: NASA

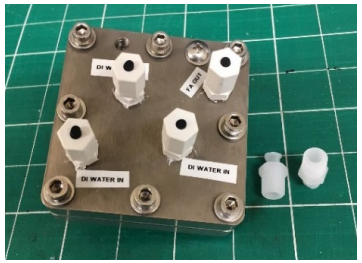
CO_2 Conversion Methods Produce Acetate, Formic Acid, Ethanol, and potentially Sugars



Acetate



Formic Acid



Commercially available reactor

- Formic acid reactor. Selectivity (Faraday efficiency) above 95%.

Sugars

NASA CO_2 Conversion Challenge

- $1M NASA Centennial Challenge
- Completely abiotic conversion of CO_2 to sugars – glucose was main target
- 3 Phase 2 awards distributed
- *One contestant, the **Air Co. also makes ethanol,** a viable substrate tested in our system*

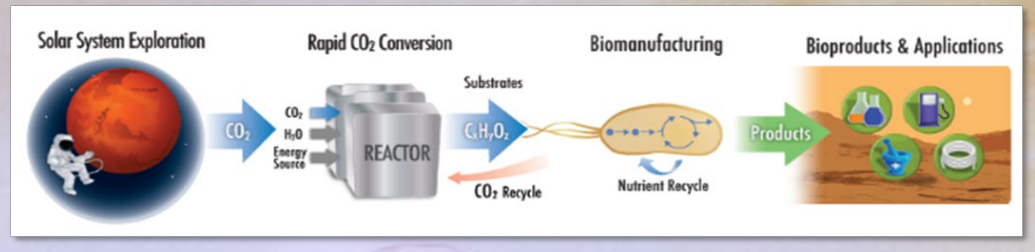
MOXIE is a solid oxide electrolysis system that can be used to produce O_2 and CO from CO_2 [1] and has now demonstrated oxygen production on Mars. Acetate production from CO has been demonstrated at Stanford University to produce 1M acetate using gas diffusion electrolysis [2]. Theoretically, these two systems could be connected to each other to produce acetate for biomanufacturing.

Dr. Matthew Kanan
Stanford University

NOTE: References in notes section below

Assumptions, Design Drivers, and ConOps

- Assumes platform is inside a temperature/pressure/radiation shielded structure.
- Assumes 100 batch runs (~6 days/run) for purposes of trade studies and systems analyses.
- **Design drivers include consideration of mass, volume, power, crew interfaces and time, repeated use/growth cycles, and flexibility.**



Application

Concept Example:
$CO_2 + H_2$

P/C Conversion System

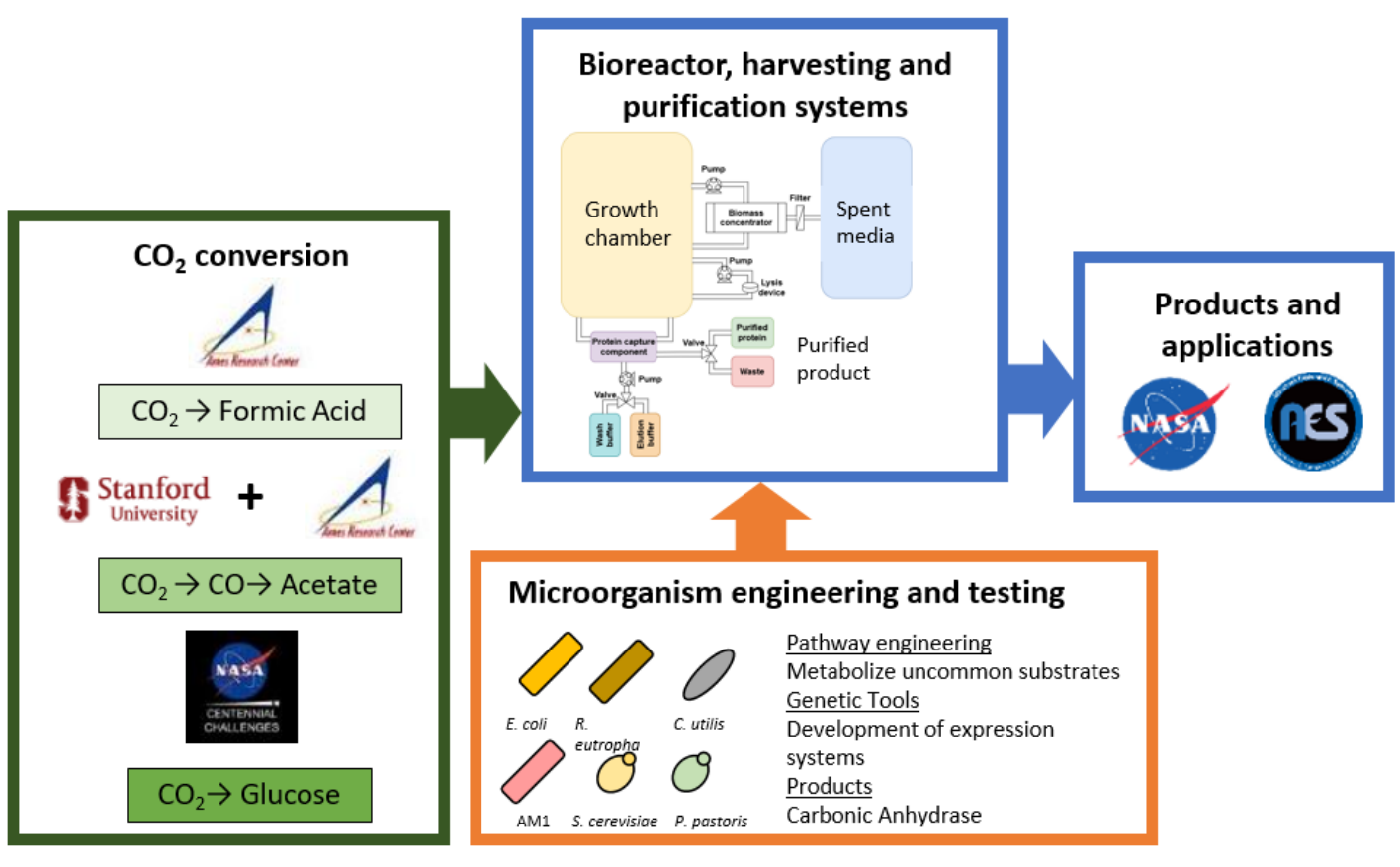
Microbial Medium

Production platform

Product

Image Credit: NASA

CO_2-based Manufacturing: Acetate as carbon source and Carbonic Anhydrase production as test case



Test Case:

- CO_2 conversion to acetate
- *E. coli* to produce Carbonic Anhydrase
- Carbonic Anhydrase addition to liquid Amines CO_2 removal systems -- improved CO_2 removal in life support systems

Image Credit: NASA

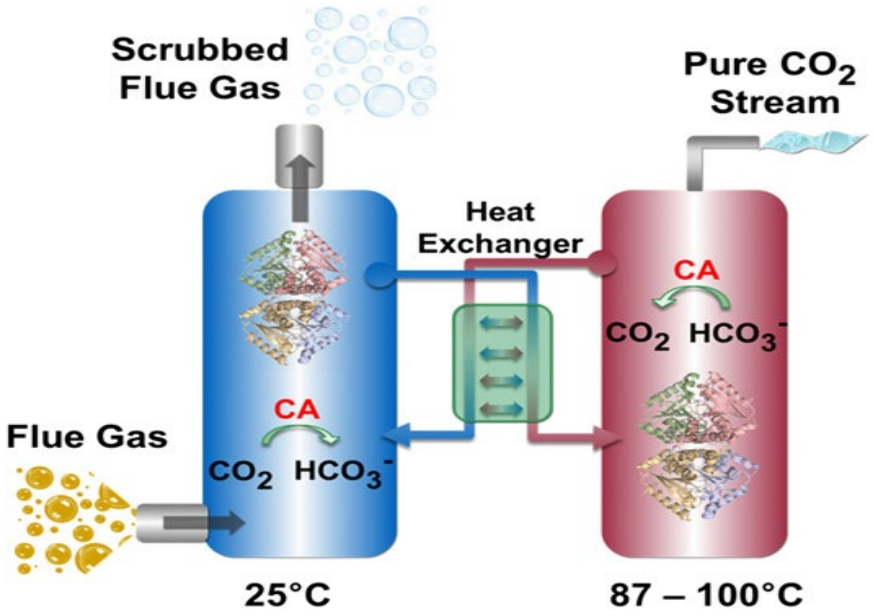
Carbonic Anhydrase as catalyst in liquid Amines CO_2 capture Systems

Collaboration with AES LSS Liquid Amines project

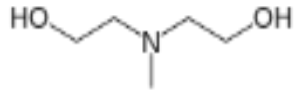
Carbonic Anhydrase & Tertiary Amines for CO_2 Capture

- Tertiary amines (MDEA) absorb CO_2 by stabilizing CO_3^- (bicarbonate) (**A**).
- Conversion of CO_2 to CO_3^- is rate limiting (**A**).
- Carbonic anhydrase catalyzes the hydration of CO_2 to bicarbonate (**B**).
- Carbonic anhydrase could improve performance of tertiary amine systems (**C**).

C.



A. Tertiary amines



MDEA

$R_2NCH_3 + H_2O <-> R_2NH^+CH_3 + OH^-$

$CO_2 + H_2O <-> HCO_3^- + H^+$ (rate limiting)

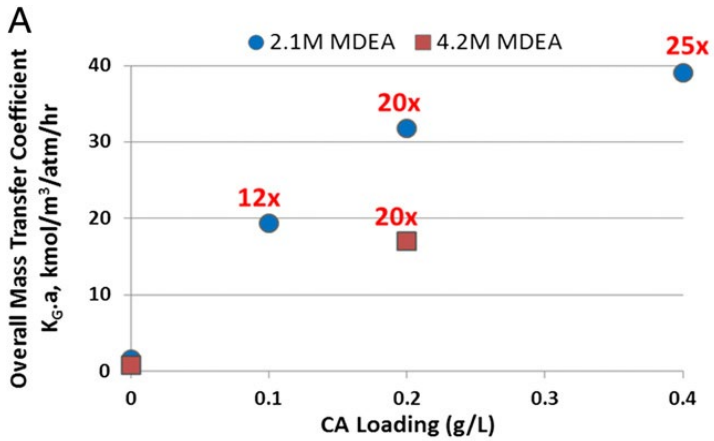
B. Carbonic anhydrase

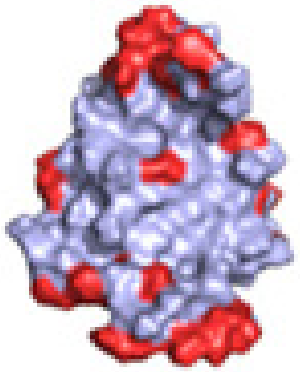
$CO_2 + H_2O <--> HCO_3^- + H^+$

Spontaneous: 1.5^{-1} s^{-1}

With CA: $10^4 – 10^6$ s^{-1}

$10^5 – 10^7$ fold rate improvement



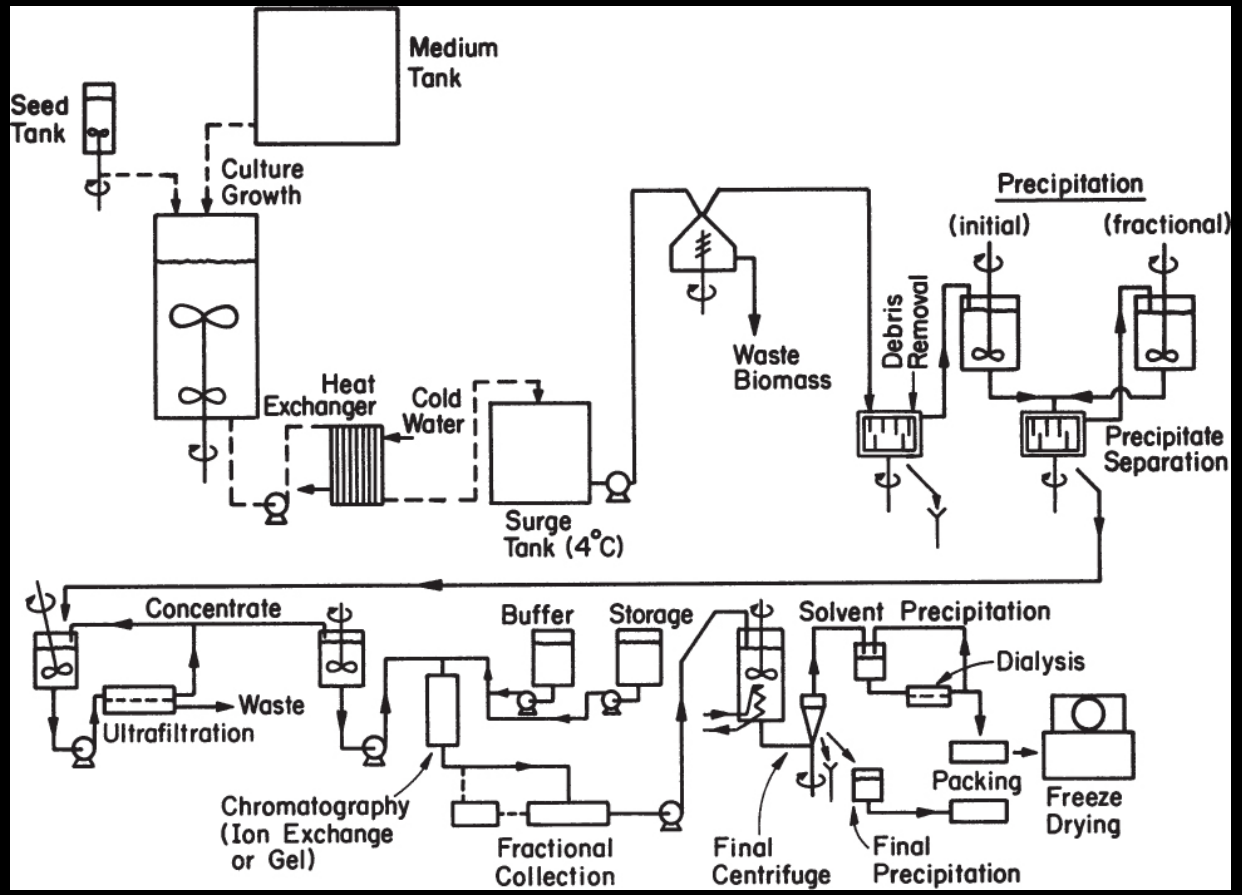


Alvizo et al 2015

Commercial Biomanufacturing on Earth

- Industrial system for Recombinant Enzyme Production

10L Bioreactor and Dewatering System



Process Flow for Industrial Production of Enzymes

Source: https://www.repligen.com/application/files/2015/3961/3373/Continuous_Processing_Book_Volume_1_2014.pdf
Bioprocess Engineering: Basic Concepts, 3rd Edition

How to make this work in space? Process and Options Analysis

CHOICE: Mostly single use components (especially where in contact with biomass) with some reusable components.

RATIONALE: Reusable components have strict cleaning/sterilization requirements which adds to crew-time and complexity of design.

CHOICE: Filter sterilization using single-use filters.

RATIONALE: Cost analysis of different sterilization methods indicate that it has the least impact on mass and cost for the scale considered.

CHOICE: Single-use bag bioreactor with necessary sensors (Final bioreactor configuration, sensor list – TBD).

RATIONALE: The bag bioreactor has low ESM, and preliminary experiments indicate it may work with E. coli. Sensors would be helpful in tracking growth and integrating automation in future iterations.

CHOICE: Affinity chromatography.

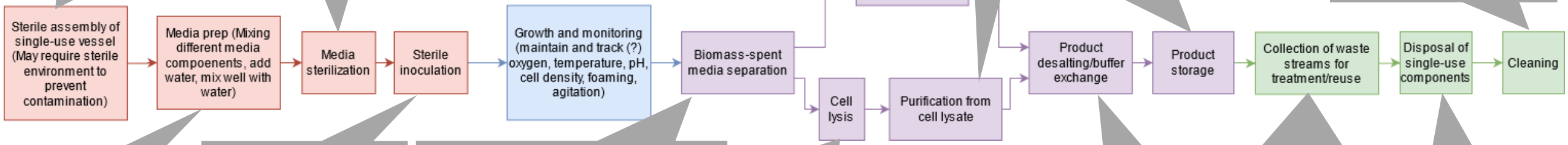
RATIONALE: The current demonstration product is a carbonic anhydrase protein optimized with an affinity tag (His-tag).

CHOICE: Ambient storage/immediate addition to CO_2 capture system (Choice of either process - TBD).

RATIONALE: The protein chosen is stable at ambient temperature, but depending on use case and yield immediate use may be optimal.

CHOICE: Include in process (Cleaning procedures - TBD).

RATIONALE: Some components may need to be reused due to mass or stability constraints. They need to be cleaned and prepared for reuse.



CHOICE: Pre-mixed media packets ready for use (Mixing procedure – TBD).

RATIONALE: There are many media components that would require significant crew-time to add individually.

CHOICE: Limit any contamination of growth vessel and associated components (Sterility requirements – TBD).

RATIONALE: Contaminating organisms can inhibit growth and yield, especially on low-energy carbon sources like acetate.

CHOICE: Include biomass concentration (Choice of filter/centrifuge - TBD).

RATIONALE: Preliminary cost and use case analyses indicate that there is a significant benefit to concentrating the biomass in case of an intracellular product. In case of an extracellular product, the removal of large particles (like cells and organic matter) will prevent clogging and biofilm formation of downstream processing components.

CHOICE: Include cell lysis (Choice of disposable bead beater/lysis enzyme/sonication – TBD)

RATIONALE: The current demonstration product is an intracellular protein which needs lysis. Also, inclusion of a removable lysis module provides for platform flexibility to process intra- and extracellular products.

CHOICE: (Inclusion in process flow – TBD).

RATIONALE: It is unclear if any of the buffer components will interfere with the final application/stability during storage. Until further tests are done, it is unclear if this step is necessary.

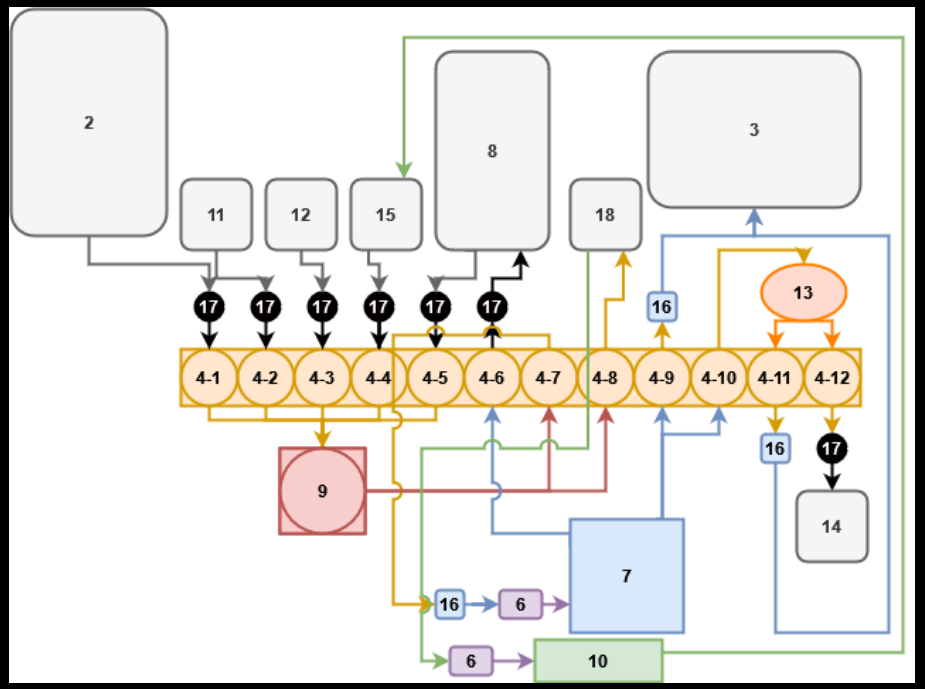
CHOICE: Spent media may be reused. All other waste needs to be disposed or treated by ECLSS (Method of reuse/disposal - TBD).

RATIONALE: The benefits of reusing organic matter are unclear, and there does not seem to be any benefit to reusing purification buffers. But there may be some advantages to reusing spent media. Experiments are required to better understand the value of reuse and recycling and optimize it.

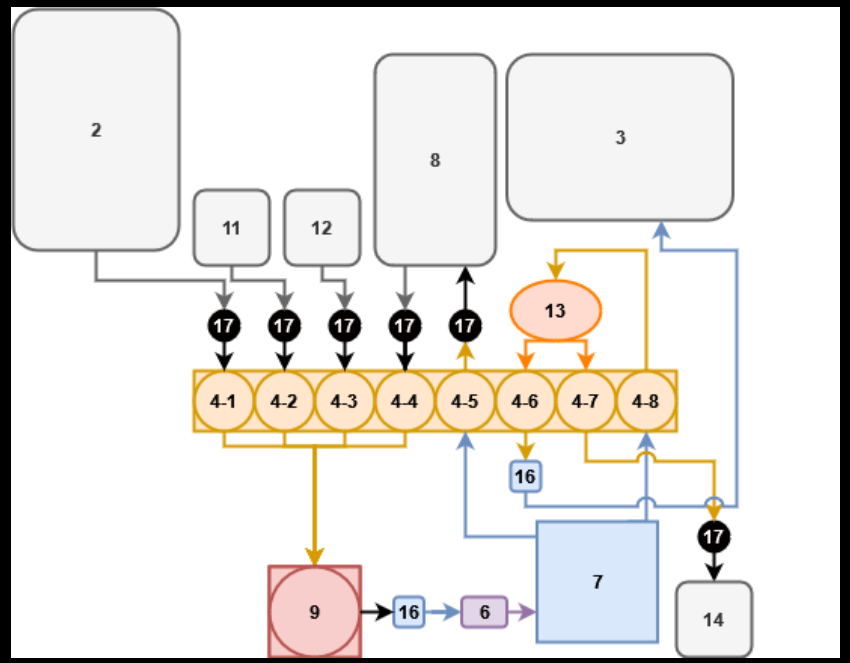
Flexible by Design. Platform Can be Configured for Intracellular Production and Lysis, or for Secreted Products

- Different design configurations for different organisms
  - *E. coli- non-secreted Carbonic Anhydrase production*
    - Lysis and additional biomass separations
  - *K. phaffii – secreted products, large biomass*
    - Secretion – lysis step removed, single separation step
    - Carbonic Anhydrase and Cutinase test products

#	Component	#	Component
2	Bioreactor Bag	11	Wash Buffer Bag
3	Media Bag	12	Elution Buffer Bag
4	Pinch Valve Module	13	Protein Column
6	Pressure Sensor	14	Collection Bag
7	Tangential Flow Filter	15	Lysate Bag
8	Collection Bag	16	Luer Check Valve
9	Small Peristaltic Pump	17	Pinch Clamp
10	Lysis System	18	Lysis Bag



***E. coli* process flow**



***K. phaffii* process flow**

Image Credit: NASA

Modeling Subsystem Options to Drive Selection

npj | microgravity

www.nature.com/npjmgrav

ARTICLE OPEN

Check for updates

Theoretical design of a space bioprocessing system to produce recombinant proteins

Mathangi Soundararajan[1], Matthew B. Paddock[1], Michael Dougherty[1], Harry W. Jones[2], John A. Hogan[2], Frances M. Donovan[2], Jonathan M. Galazka[3] and A. Mark Settles[2]

- Equivalent system mass modeling was performed comparing design options for the **biomass dewatering, cell lysis, and product purification subsystems.**
- Additional considerations such as **storage condition requirements, reliability, crew interface requirements, and chemical or buffer requirements** were also weighed.
- Analysis helped derive **weighted options** for application in the platform.

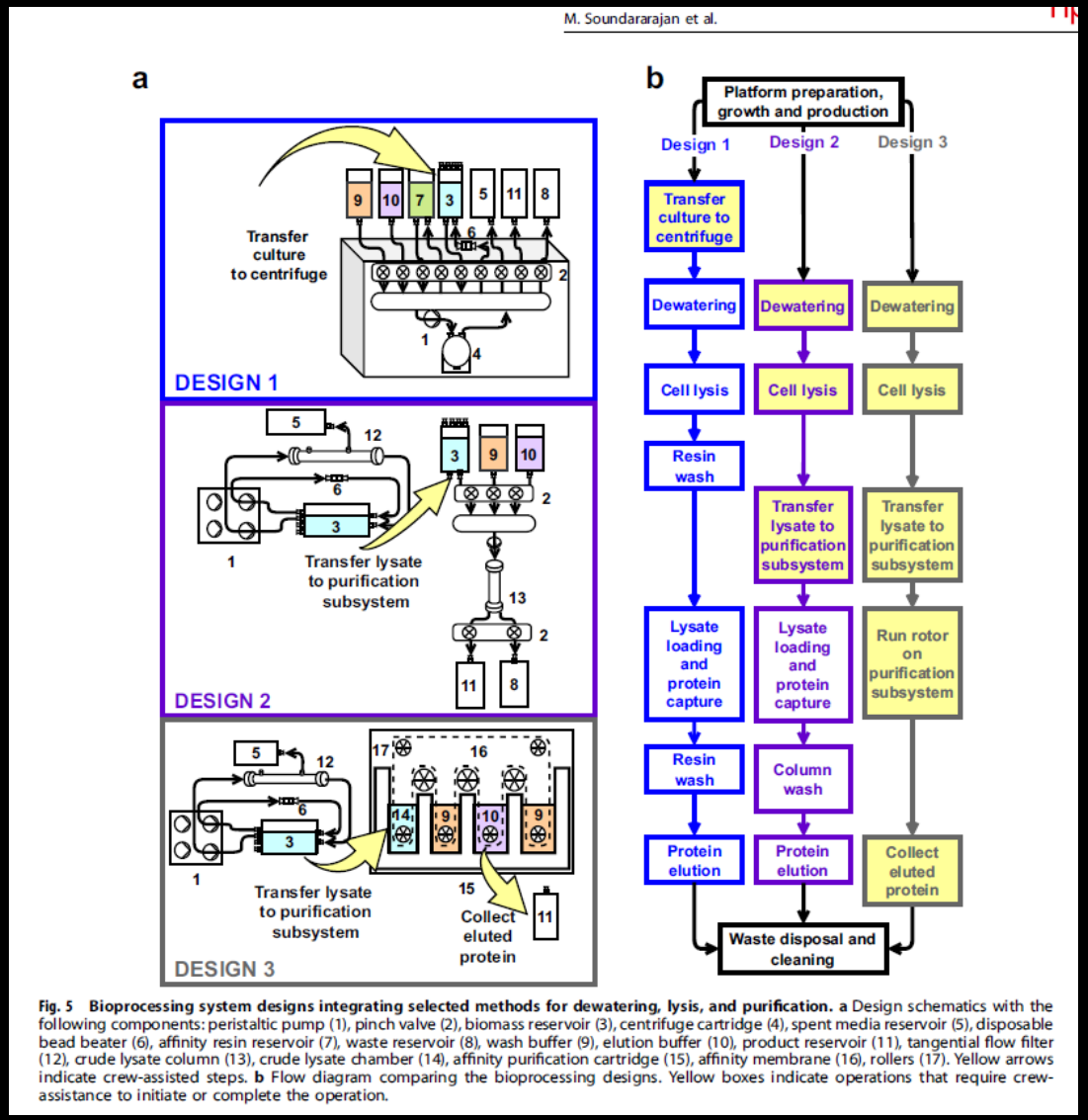


Fig. 5 Bioprocessing system designs integrating selected methods for dewatering, lysis, and purification. a Design schematics with the following components: peristaltic pump (1), pinch valve (2), biomass reservoir (3), centrifuge cartridge (4), spent media reservoir (5), disposable bead beater (6), affinity resin reservoir (7), waste reservoir (8), wash buffer (9), elution buffer (10), product reservoir (11), tangential flow filter (12), crude lysate column (13), crude lysate chamber (14), affinity purification cartridge (15), affinity membrane (16), rollers (17). Yellow arrows indicate crew-assisted steps. **b** Flow diagram comparing the bioprocessing designs. Yellow boxes indicate operations that require crew-assistance to initiate or complete the operation.

Image Credit: NASA

Prototype Design and 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

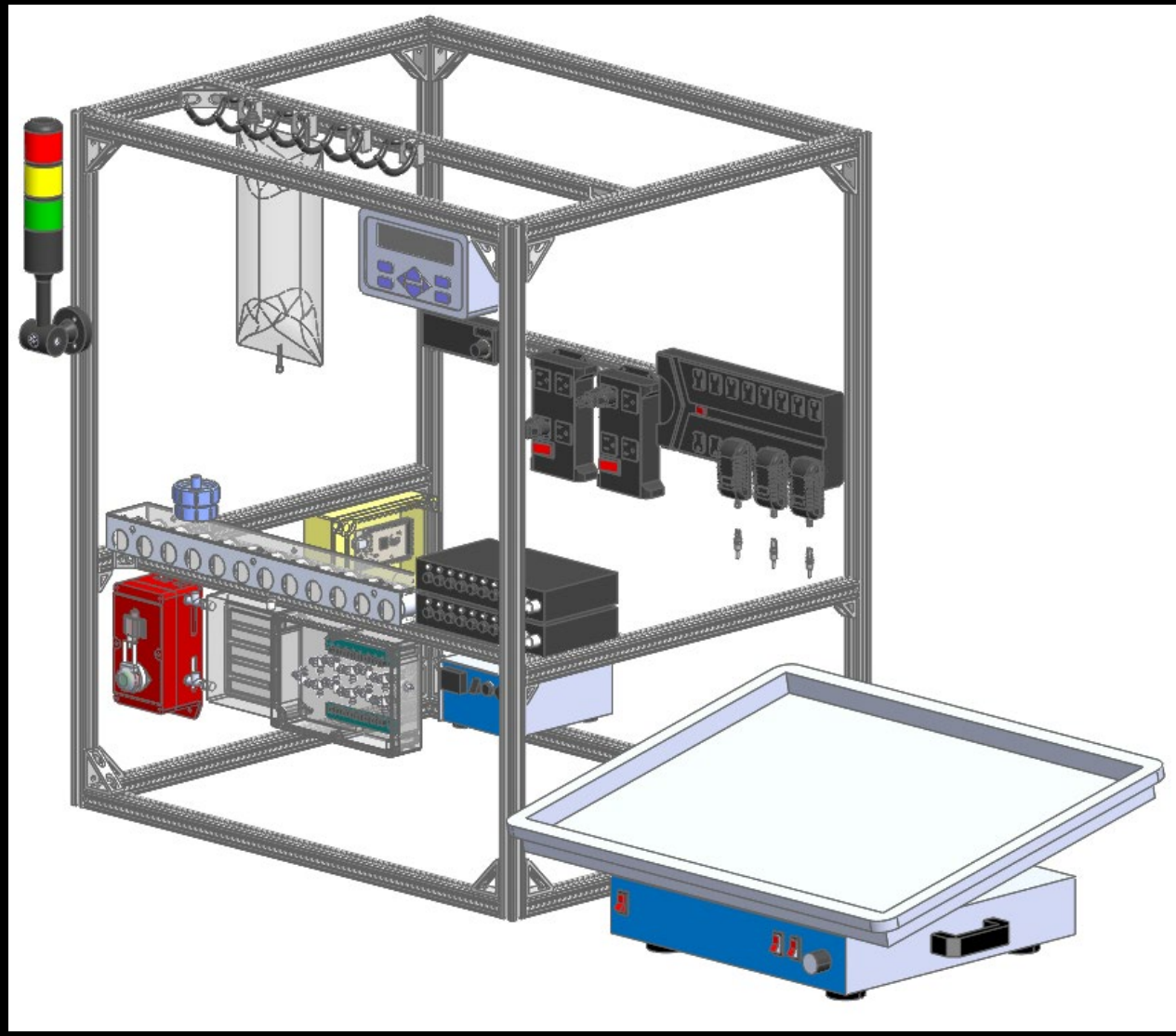


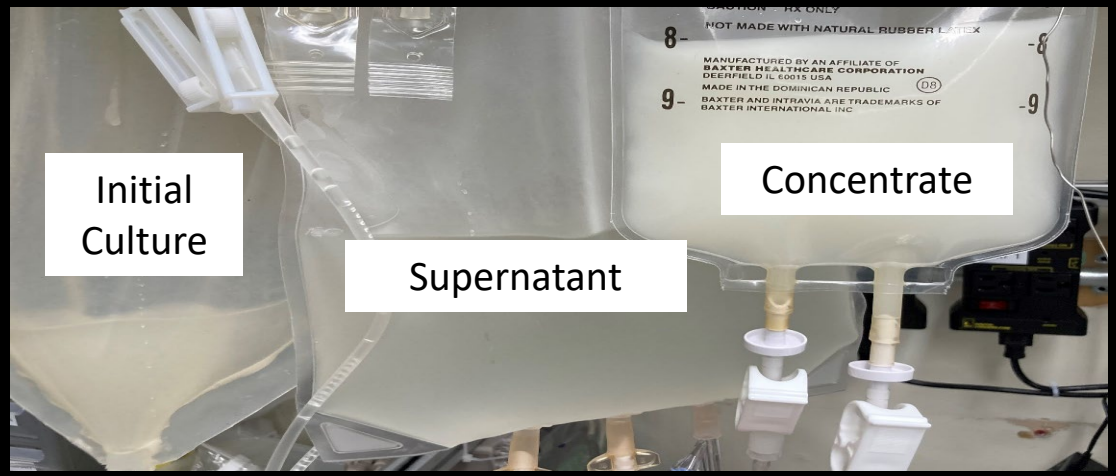
Image Credit: NASA

Results: Harvest Efficiency Surpasses KPP Threshold

- Tested and quantified harvest efficiency by measuring the % biomass/ media in the bioreactor bag as compared to after pumping through the tangential flow filter to assess recovery of either the biomass (needed for recovery of CA from *E.coli*) or the supernatant (recovery from *K.phaffii*).

- **KPP threshold =85%, goal = 97% recovery.**

Organism	Concentration Factor	Percent Recovery	Time (min)
E. coli	18x	93%	40
K. phaffii	- (supernatant)	94% - 98%	16-30



K. phaffii Dewatering Process images

Tangential Flow Filter

Lysis Test: Media dependent, limitations

- Developed and verified by ClaremontBio for LB-grown *E. coli*
- In-house tests for acetate grown *E. coli*
  - Did not perform well for acetate grown *E. coli*
    - *E. coli* grown on acetate media has morphological features that clogged lyser units
  - Performed well for LB (non-acetate) grown *E. coli*
    - Peptone-based media

Testing of lysis system in platform

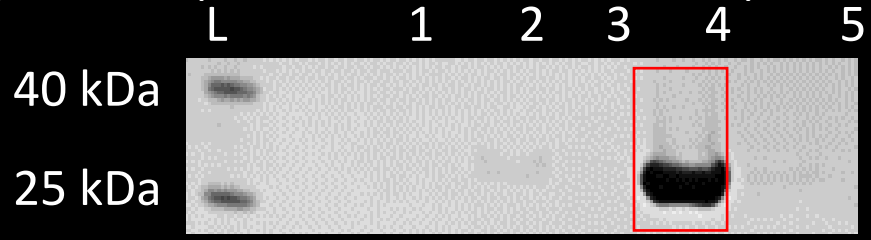
Reference: @OD=1 ======> 8.0E+08 cfu/mL						
	Vol (mL)	Cell conc. (cfu/mL)	Flow rate (mL/min)	Processing time (min)	# of lysers in single pass	
Target (undil.) @OD=2	1000	1.6.E+09	8	125	96	← ① for 1L
Conc 1 (5x concentrate)	200	8.0.E+09	2	100	24	
Conc 1 (5x concentrate)	200	8.0.E+09	4	50	48	← ② for 200mL
Conc 1 (5x concentrate)	200	8.0.E+09	8	25	96	
Conc 2 (20x concentrate)	50	3.2.E+10	0.5	100	6	
Conc 2 (20x concentrate)	50	3.2.E+10	1	50	12	
Conc 2 (20x concentrate)	50	3.2.E+10	2	25	24	← ③ for 40 or 50mL
Conc 2 (20x concentrate)	50	3.2.E+10	4	12.5	48	
Conc 2 (20x concentrate)	50	3.2.E+10	8	6.25	96	

Claremont bio testing of multiplexed lysers to optimize lysis efficiency for different biomass concentrations and volumes (data table). In house optimization testing (photo).

Summary

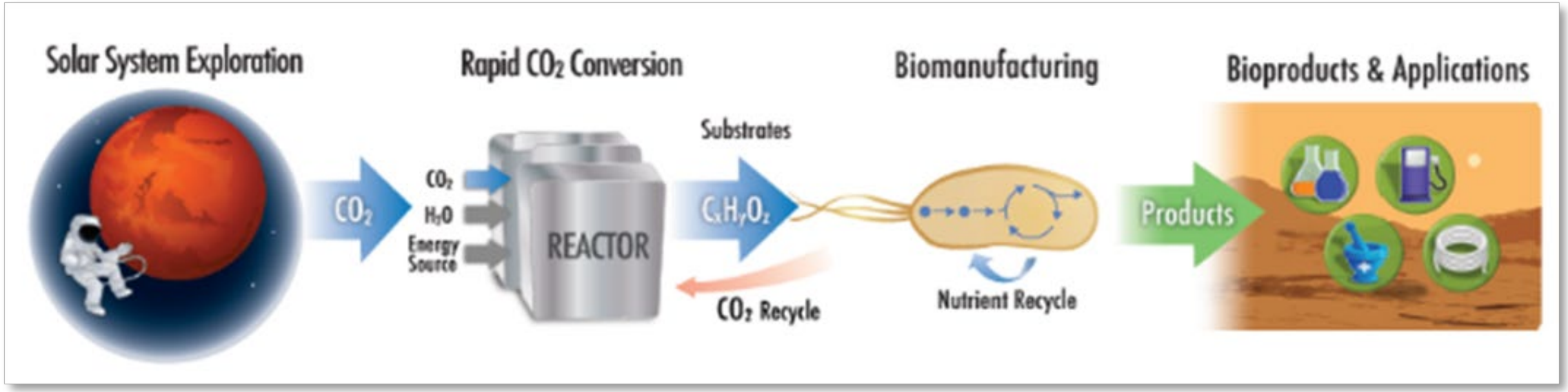
- Platform performed as semi-autonomous system demonstrating growth, harvest, dewatering, lysis, and purification of recombinant proteins from microbial cells.
- Demonstrated gravity-independent high-gas exchange membrane bioreactor capable of supporting multiple organisms (*E.coli*, yeasts).
- Platform demonstrated validity of the concept and identified challenges for future investigation.

High level of purification of recombinant protein



1. Supernatant, 2. Effluent from His-Tag Filter, 3. Wash Effluent, 4. Elution, 5. Post Wash Effluent

A Good Idea – and it's feasible



Space Exploration supported with CO_2-based Biomanufacturing

(Lunar CO_2 sources = humans ~1kg-CM/d, wastes: Mars = atmospheric CO_2 @95%)

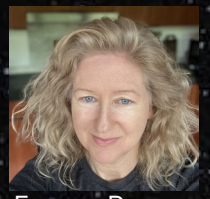
News of CO_2 conversion products on Earth

- **Savor** – butter and milk ('Butter' made from CO2 could pave the way for food without farming | New Scientist)
- **Air Company** – Vodka, perfume and Jet Fuel
- **C2CNT and academia Licht lab -** carbon nanofibers
- **Carbon Upcycling Technologies** -Nanoparticles for plastics, concrete and coatings
- **Newlight Technologies-** Bioplastics from methane and CO2, **Mango Materials – bio**plastics from methane
- **Breathe -** methanol
- **C4X** -Chemicals, bio-composite foamed plastics

➢ **Upcoming NASA SBIR investments T7.05Climate Enhancing Resource Utilization -- may provide new/better feedstocks for biomanufacturing**

- **Lead Center:** GRC
- **Solicitation Year:** 2023
- **Scope Title:** Sustainable Atmospheric Carbon Dioxide Extraction and Transformation
- **Scope Description:** Component and subsystem technologies are sought to demonstrate sustainable, energy-efficient extraction of carbon dioxide (CO_2) from a defined planetary or habitable atmosphere fully integrated with CO_2 transformation into one or more stable products such as manufacturing feedstock polymers or readily storable, noncryogenic propellants or fuels.

Synthetic Biology Team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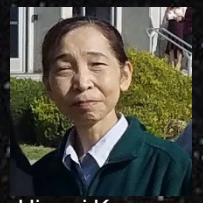
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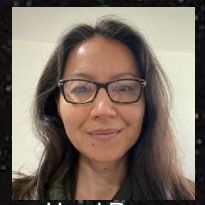
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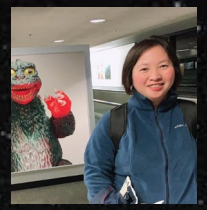
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Candice Tahimic, PhD


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Oscar Roque


Alyssa Villanueva

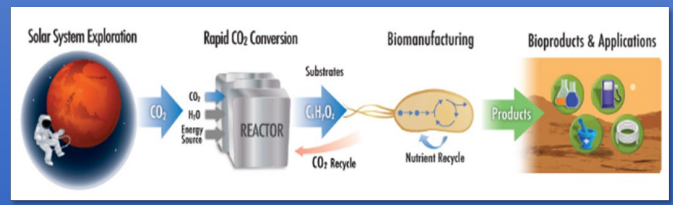

Sean Sharif

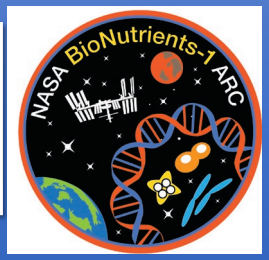

Kevin Sims, Payload Manager


Harry Jones, PhD Systems Engineer

Safety: Daniel Varnum-Lowry
Q/A: Leonard Hee
Logistics at KSC: Satro Narayan

Synthetic Biology GCD project Elements





CO_2 based manufacturing

BioNutrients

Former team members and students: Aditya Hindupur, Amy Gresser, Aphrodite Kostakis, Asif Rahman, Ava Karanjia, Benjamin Alva, Eliza Zaroff, Eric Litwiller, Jason Samson, Jing Li, John Hogan, Jon Galazka, Julie Levri, Katherine Fisher, Leonard Lee, Matthew Kanan, Marilyn Murakami, Mathangi Soundararajan, Michael Dougherty, Paul Milazzo, William Tyukayev

Image Credit: NASA

Thank You

Questions?

Image Credit: NASA

Space Feasible Process Flow

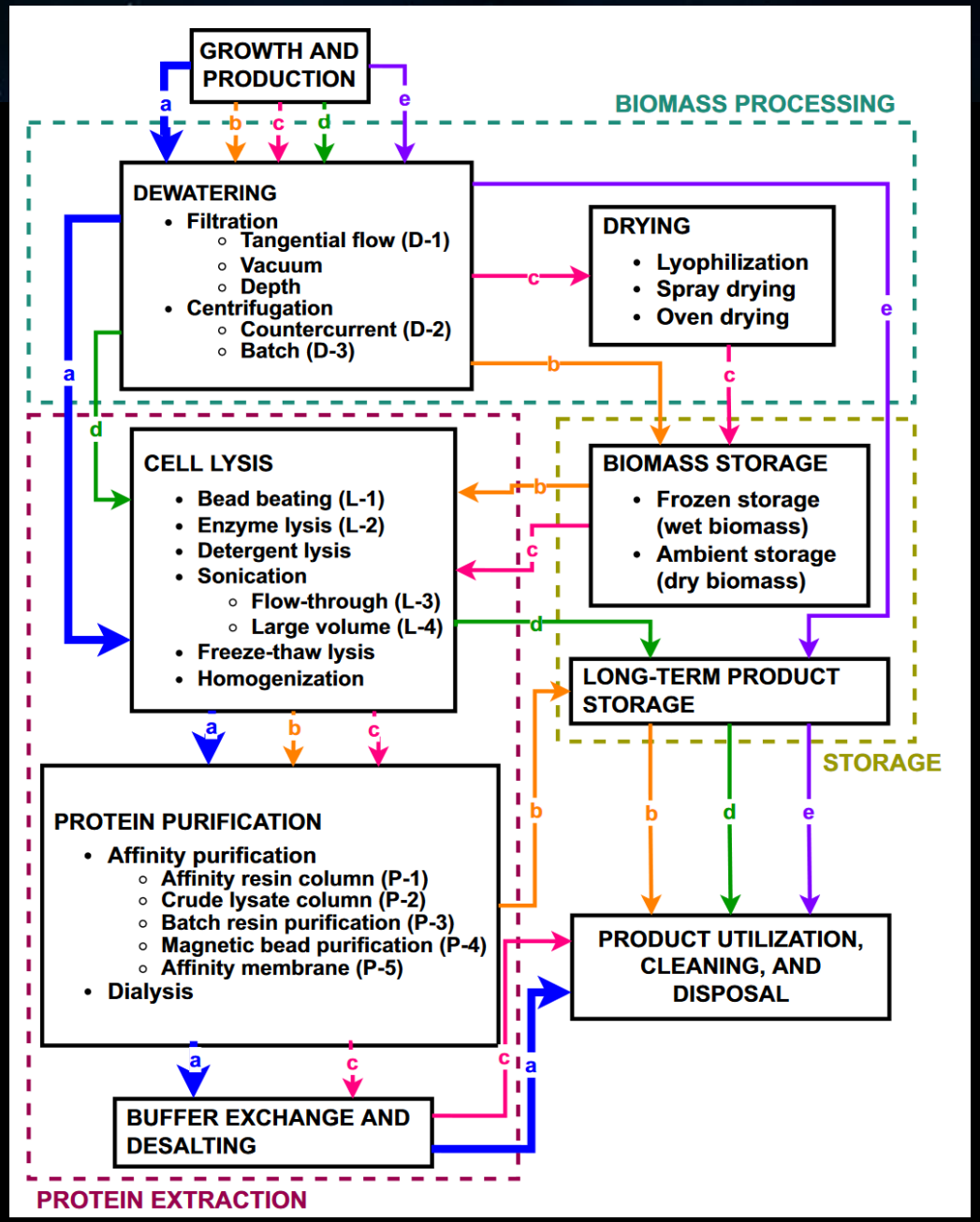
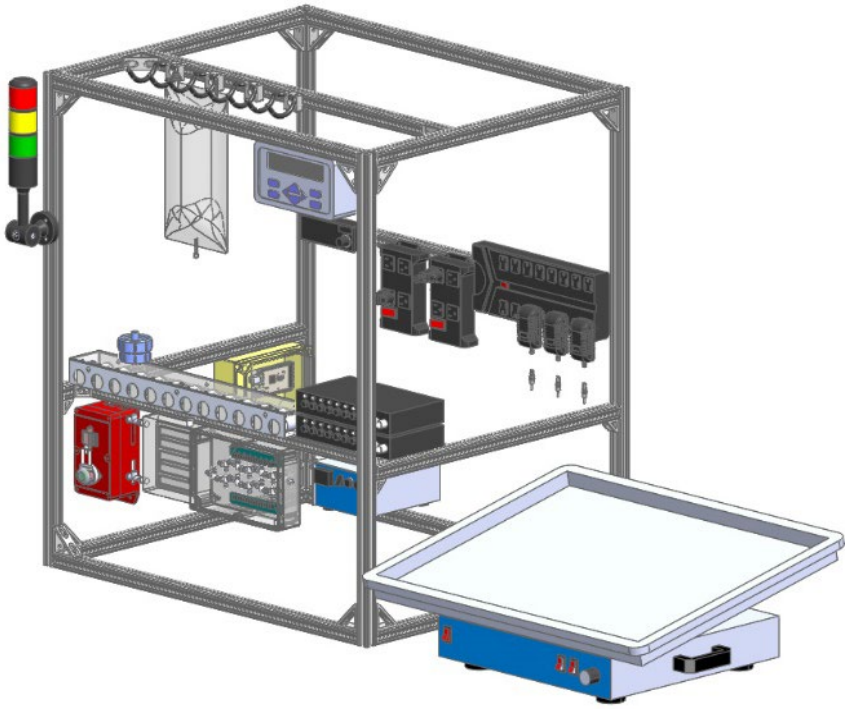


Image Credit: NASA 29

Platform Volume vs Middeck Locker

- Requirement 1.3:The System shall be implemented in a flight-like configuration that would operate in a volume similar to an ISS middeck locker and under moon and Mars gravity.
- Subsystem volume within the platform is **approximately 770 in ^{3}**. This does not include heated rocking table, light tower, frame or any electrical harness or flexible tubing. **Internal Volume of Middeck Locker is 3512 inch ^{3}**



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Revision S

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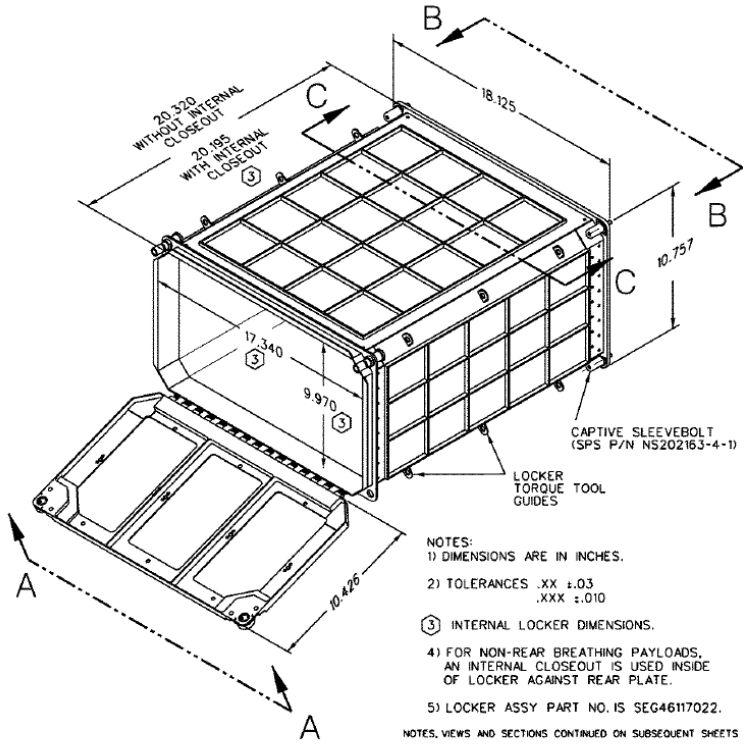

FIGURE F.3.1.2.4-1 ISS LOCKER (PAGE 1 OF 2)