Synthetic Biology/CUBES Update

NAC Technology, Innovation and Engineering Committee Meeting
October 29, 2019

NASA Kennedy Space Center

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NASA Ames Research Center

Presentation Outline

- NASA Applications of Synthetic Biology
- Internal NASA Projects
- NASA CO_2 Conversion Centennial Challenge
- Space Technology Research Institute (STRI) – Center for the Utilization of Biological Engineering for Space (CUBES)
- Supporting Activities

NASA Applications of Synthetic Biology

Future Missions Need a Different Approach

- Short crew duration
- Frequent resupply of food, water, O_2, medical supplies, replacement parts
- Emergency return to Earth
- No ET planetary protection requirements

- Extended crew durations
- Infrequent or no resupply of food, water, O_2, medical supplies, replacement parts
- No emergency return to Earth
- Possibly strict planetary protection requirements

- NASA needs *In situ* manufacturing, *In situ* resource utilization and life support
- Biological systems offer tremendous potential

Sustaining Future Missions

Capabilities

- ***In situ* Resource Utilization (ISRU)** generates supplies from local resources.
- **In Space Manufacturing (ISM)** provides capability to make needed chemicals, fuels, building materials, pharmaceuticals, etc. on-site and on-demand.
- **Closed-loop life support systems** treat and recover valuable resources via regenerative air, wastewater and solid waste processing systems.
- **Food production** will be required to supply nutritional needs not met by current food provisioning systems. Eventually all food may be produced *in situ*.
- **Space medicine** systems will require the ability to monitor and maintain the health of the crew under very adverse conditions.
- These systems require increased **reliability and self-sustainability,** and decreased mass, power, volume and consumable use.

Potential of Biology – Possible Biological Products:

- Food – plants and microbial products
- Vitamins, nutraceuticals
- Enzymes, flavors, preservatives
- Therapeutics/pharmaceuticals
- Polymers – plastics for parts, habitat construction, radiation protection
- Fuels – hydrocarbons, nitrogen-based
- Primary chemicals for various product synthesis
- Adhesives/biocement - construction
- Specialized function biomolecules:
  - e.g., Carbonic anhydrase for CO_2 management

Potential of Biology in Space

Biological systems are:

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

BioManufacturing Research Challenges

Space-Based Research Challenges:

- Novel, high efficiency, engineered microbes/plants
- Use of unique substrates – *In situ* media generation
- Novel bioreactor designs
- Product harvesting and purification strategies
- Minimization of inputs/wastes
- Reduction and consolidation of separate unit operations
- Enhanced microbial storage and reanimation techniques
- Maintaining pure cultures/controlled consortiums
- Genetic stability in high radiation environments
- Interfacing biology with inanimate components

NASA Alignment

Research alignment with NASA 2015 Technology Roadmaps:

- **TA06 Human Health, Life Support, and Habitation Systems -**. **TA6.3 Human Health and Performance**, **TA6.1 Environmental Control, Life Support Systems and Habitation Systems**, and **TA6.4 Environmental Monitoring, Safety, and Emergency Response**
- **TA07 Human Exploration Destination Systems - TA7.1 In Situ Resource Utilization**, **TA7.2 Sustainability and Supportability**, **TA7.4 Habitat Systems**, and **TA7.6 Cross-Cutting Systems**.

NASA Internal Research

STMD GCD Space Synthetic Biology

STMD Game Changing Division (GCD) is sponsoring the Synthetic Biology Project

- <u>Two major R&TD efforts:</u>

1) **BioNutrients** – On-demand human nutrient production to mitigate demonstrated losses in nutrition in stored food supplies

2) **CO_2-Based Manufacturing** – Developing a platform system to abiotically convert CO_2 to microbial feedstock to enable In Situ Resource Utilization (ISRU) for In Space Manufacturing (ISM) of numerous mission relevant bioproducts

BioNutrients Project Overview

Technology Goal

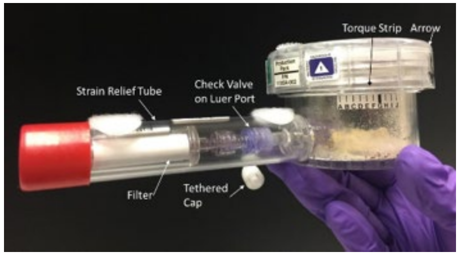
- The BioNutrients technology is developing a platform technology for the on-demand production of nutrients needed for long duration missions to the Moon and Mars to mitigate demonstrated nutrient degradation in stored foods

Technical Capabilities

- Demonstrate 5-year stasis of engineered microbes at room temperature
- Generate target nutrients at consistent amounts during 5-year ISS flight test
- Reliably provide safety assurances of product quality

Exploration & Science Impact

- BioNutrients system will be able to produce nutritional compounds on-demand
- Technology can be extended to produce medicines and probiotic organisms that improve crew health and safety
- Develops specialized microbes highly suited to long duration storage in space for a variety of biomanufacturing applications



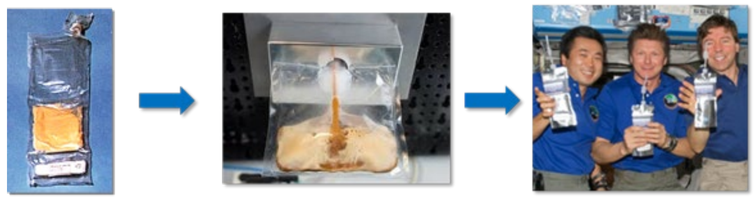
BioNutrients Flight Production Pack for on-orbit testing

Engineering yeast to produce nutrients on-demand

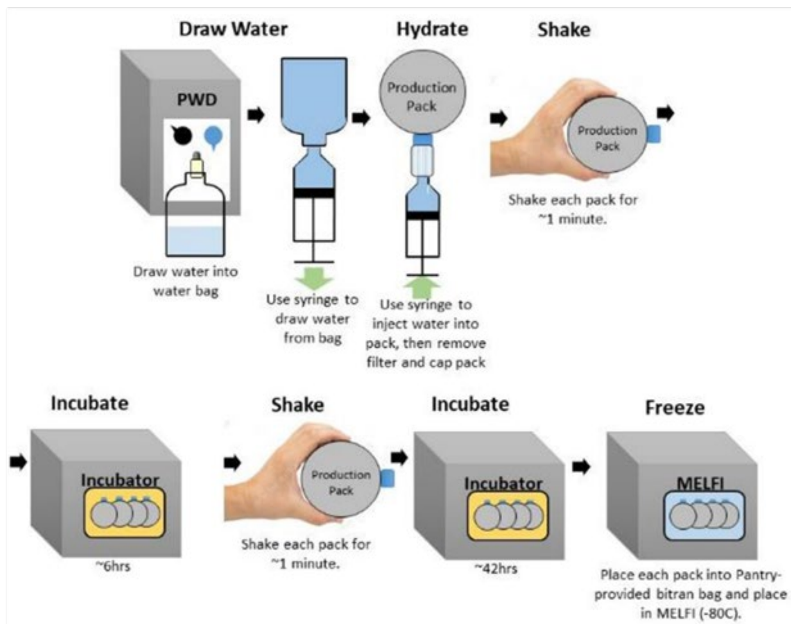
BioNutrients Technical Approach

5-Year ISS Storage and In-Flight Performance Testing

- Developed “edible media”
- Engineered two yeast strains (one is a probiotic strain) to produce the anti-oxidants zeaxanthin and beta-carotene
- Engineered desiccation resistance for enhanced storage
- Developed flight qualified disposable storage/growth pack for in-flight tests
- ISS time-course hydration, incubation, freezing and return for analysis over 5 year period



Overview of On-Orbit Operations



BioNutrients Production Pack (above) and packaged flight article (below)

BioNutrients Technical Approach (2)

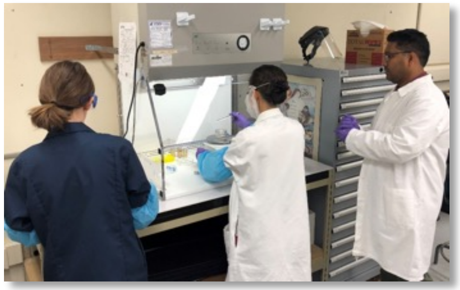
Concurrent 5-Year ISS Cyclic Host Development and Storage Testing

- Stasis testing on a wide variety of relevant microbes, 3 media types
- Studying effects of engineered vs. wild-type, stasis engineering, gene knockouts, encapsulation, and probiotic organism survival
- Survivors characterized, engineered and returned for further storage and flight testing
- Small sample size, low crew effort, high return on science
- No growth in space – storage and return only
- Potential organisms/preparation processes for future commercialization

BioNutrients Stasis Pack contents (above) and packaged flight article (below)

BioNutrients Technical Status

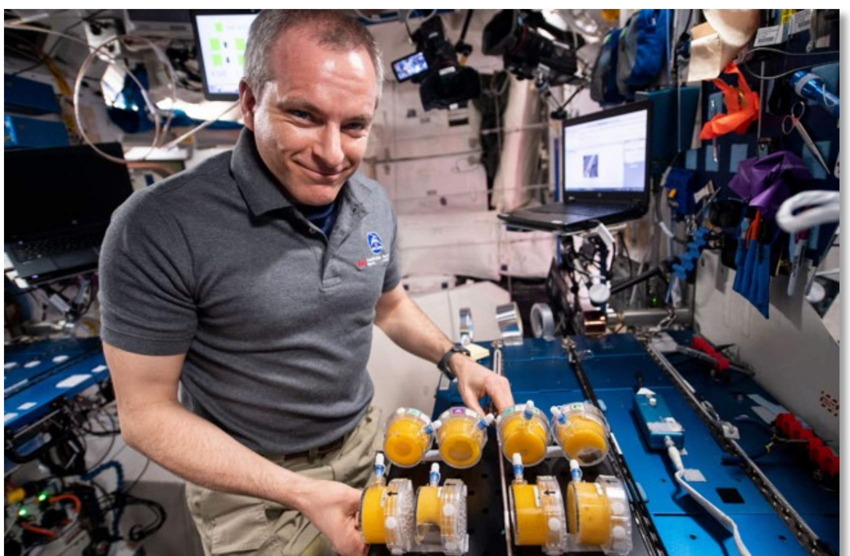
- Production packs were manufactured and ground tested for safety, pressure, and leaks
- Stasis pack was designed and fabricated
- All production and stasis pack test organisms were grown, prepared, verified and loaded into test hardware
- Payload was launched on NG-11 to ISS on 4/17/2019
- Following successful activation of the ISS production packs, concurrent ground controls were successfully activated and grown. The samples have been stored at -80^{o}C mimicking conditions on the MELFI and awaiting return of the ISS packs prior to their analysis.
- Successfully completed growth analysis for all Spx-17 Time 0 and ISS hydrated (Time 1) stasis pack samples.
- Stasis pack "omics" analysis methods and sample preparation (DNA/RNA extraction) continue.



BioNutrients Flight Ops

Crew member David Saint-Jacques hydrating BioNutrients-1 production pack aboard ISS - First BioNutrients crew operations (June 2019).

Production packs in incubator.



BioNutrients production packs removed from incubator after initial growth phase for second agitation.

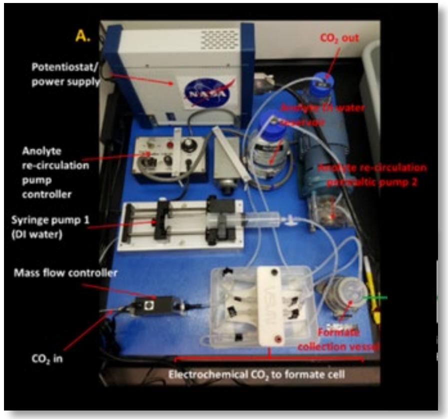
CO_2-Based Manufacturing Overview

Technology Goal

- The CO_2-Based Manufacturing project offers the ability to biomanufacture critical mission consumables and products while minimizing the need for launched resources. The approach is developing systems that can convert locally-sourced CO_2 to organic compounds that can serve as a microbial substrate for microbial biomanufacturing systems.

Technical Capabilities

- Advanced electrochemical CO_2/CO conversion to formic acid and acetate for use as microbial substrates
- Microorganism engineering for production of a CO_2-capture enhancing enzyme (carbonic anhydrase) to enable new life support air revitalization methods
- Low mass/volume bioreactor development for gravity independent operation and improved harvesting/purification



Exploration & Science Impact

- Use of CO_2 for biomanufacturing during surface missions on the Moon and Mars reduces launch mass and increases mission sustainability
- Provides a platform technology to enable a wide range of bio-products

Terrestrial Chemical Manufacturing

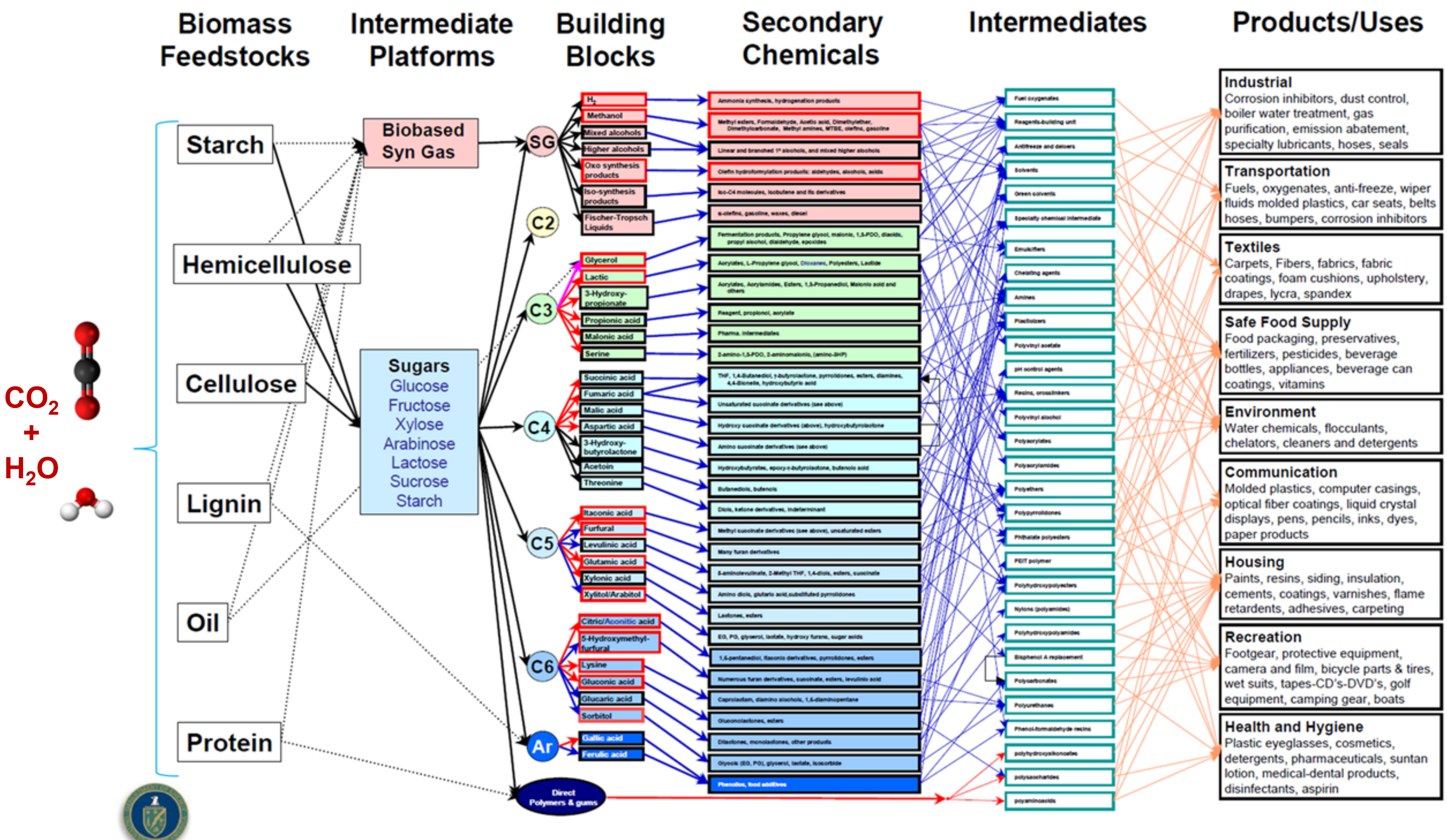
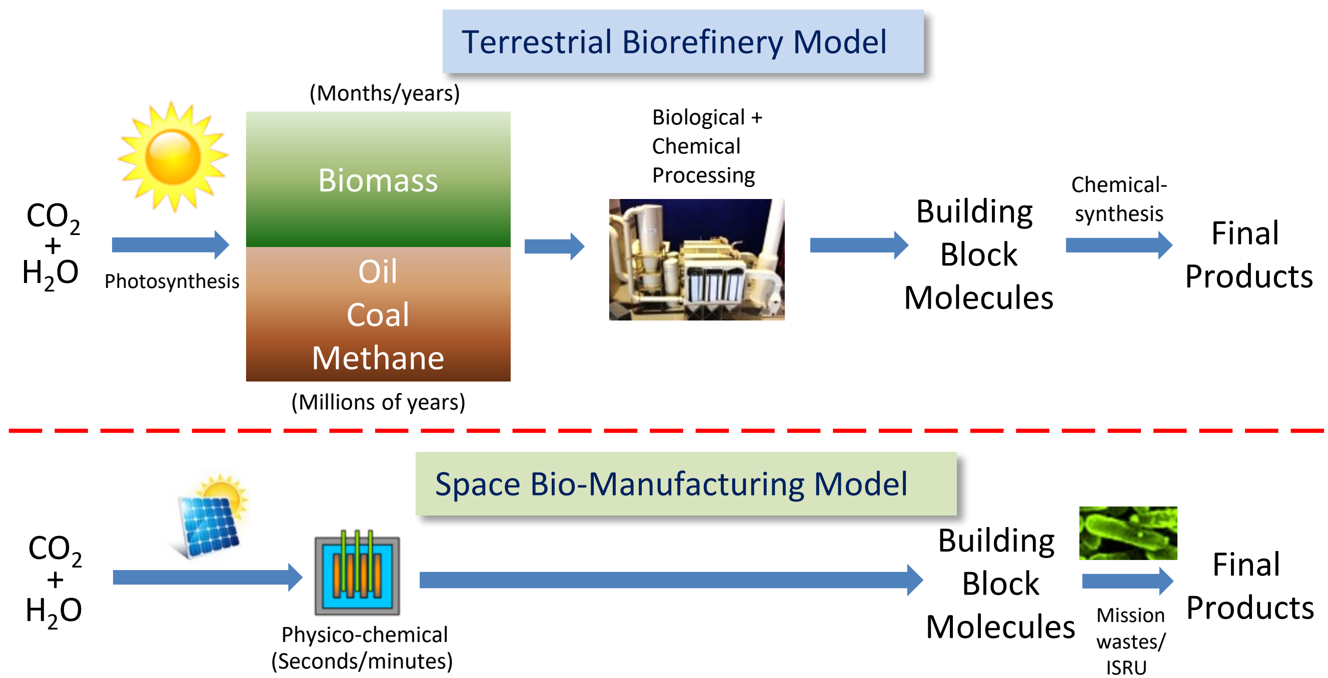


Figure 3 – Analogous Model of a Biobased Product Flow-chart for Biomass Feedstocks

DOE – Top Value Added Chemicals from Biomass (2004)

Biomanufacturing Approaches

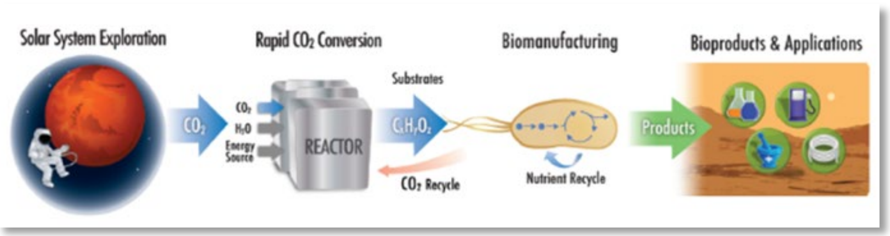
- Long duration missions/settlements require extensive loop closure, *in situ* resource utilization and *in situ* manufacturing
- Terrestrial "biorefineries" use low-efficiency biomass processes - not viable in space



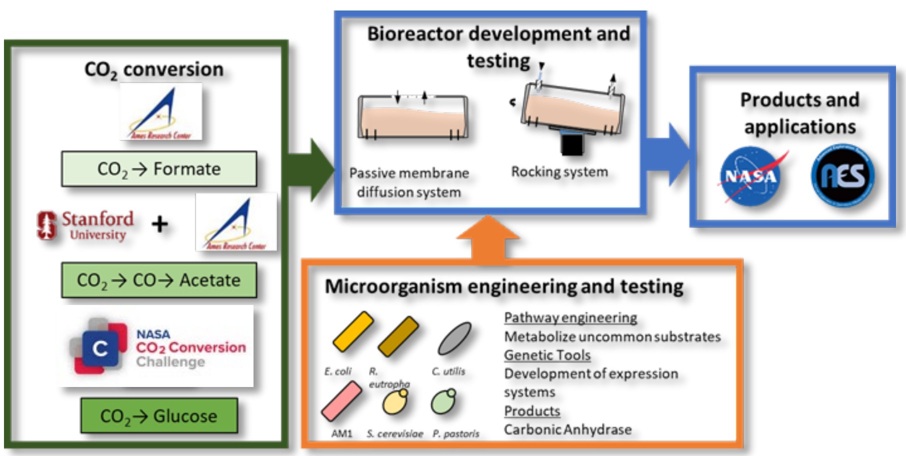
CO_2 - Based Manufacturing Approach

- Develop advanced electrochemical conversion systems for conversion of CO_2 to formic acid (Ames) and CO to acetate (Stanford Univ.) to serve as microbial substrate. Leverage technologies from NASA CO_2 Conversion Centennial Challenge for sugar production.
- Engineer microbial strain(s) for production of mission products. Down-selected product to a carbonic anhydrase enzyme adapted for use in a liquid sorbent CO_2 removal system for air revitalization.
- Develop space-relevant, enclosed membrane bioreactor system for product formation and harvesting.
- Develop compatible product harvesting and purification techniques
- Develop and test space-compatible (not flight ready) integrated prototype and develop future R&D needs for future maturation and applications.

Hybrid CO_2-Based Biomanufacturing Concept



Project Efforts



CO_2-Based Manufacturing Technical Status

CO_2/CO Conversion

- Stanford collaborator (Matthew Kanan) demonstrated record single pass efficiency for acetate formation from carbon monoxide. Published high-impact paper in Joule. Ames making formic acid from CO_2 using SOA reactor.

Organism and Product Engineering

- Engineered *E. coli* host strain to produce a carbonic anhydrase enzyme (used for CO_2 removal) using acetate and formate as the carbon sources.
- Led to additional AES Life Support funding to enable novel CO_2 removal systems.

Reactor Development

- Designed, fabricated and successfully tested a gas-permeable bioreactor bag prototype with membrane protection layer.
- Demonstrated production/separation of carbonic anhydrase
- Integrated prototype (FY 21) - "space-compatible"

Automated colony picker for directed evolution studies

NASA CO_2 Conversion Centennial Challenge

NASA
CO_2 Conversion
Challenge

NASA CO_2 Conversion Centennial Challenge

GOAL: Non-biological conversion of CO_2 to sugars to feed microbial biomanufacturing operations

PHASE 1: CONCEPT

Goal: Provide a preliminary design schematic and description of the physicochemical conversion system the competitor(s) could construct to demonstrate the production of selected carbon-based molecular compounds.

Duration: 8 Months

Participation: Submissions from 20 teams were evaluated for a Prize

Awards: $250,000 Prize Purse
Five teams were awarded $50,000 each

COMPLETE

PHASE 2: DEMONSTRATION

Goal: Demonstrate a physicochemical system that is able to produce one or more of the targeted compounds.

Competitors will: Build a system; submit video evidence of their successful process; host the Challenge judges for an on-site evaluation and submit a sample for analysis.

Duration: 12 months

Awards: $750,000 Prize Purse

- 1st Place - $400,000
- 2nd Place - $250,000
- 3rd Place - $100,000

PHASE 1 AWARDS

5 Winning Teams each received $50,000

Dioxide Materials	Boca Raton, Florida
Lotus Separations	Princeton, New Jersey
Peidong Yang Group	Berkeley, California
RenewCO_2 LLC	Jersey City, New Jersey
The Air Company	Brooklyn, New York

Criteria for Judging

- **Technology overview-** Description of Physiochemical Overview and its Chemistry
- **Assumptions**- Operations/Tactics Critical to Overcoming Implementation Challenges
- **Design Schematic-** Can operate continuously for 7 hours, produce product sufficient for analysis
- **Physical Properties-** Physical characteristics of system
- **Data Analysis-** Supporting calculations/preliminary laboratory analysis data
- **Project Plan-** Milestones for building the technology

NASA CO_2 Conversion Challenge

Challenge Target Compounds

Product Constituent	Weight Factor
D-Glucose	100
Other 6-carbon Sugars (hexoses)	80
5-carbon sugars (pentoses)	50
4-carbon sugars (tetroses)	10
3-carbon sugars (trioses)	5
Glycerol	5

Space Technology Research Institute:

Center for the Utilization of Biological Engineering in Space

CUBES

CUBES

The Center for the Utilization of Biological Engineering for Space

CUBES

Lead Institution:

University of California - Berkeley

- Dr. Adam Akin, PI

Collaborating Institutions:

Stanford University

University of California – Davis

Utah State University

University of Florida

Physical Sciences Inc.

5 years - up to $3M/year budget

Vision Statement

The Center for the Utilization of Biological Engineering in Space (CUBES) is leveraging partnerships between NASA, other federal agencies, industry, and academia to:

- Support biomanufacturing for deep space exploration;
- Create an integrated, multi-function, multi-organism biomanufacturing system for a Mars mission; and
- Demonstrate continuous and semiautonomous biomanufacture of materials, pharmaceuticals, and food in Mars-like conditions.

https://cubes.space

- ✓ 2.5 Years Complete
- ✓ 4 Divisions
- ✓ 5 Universities
- ✓ 15 Professors; 2 Research Scientists
- ✓ 12 Postdocs
- ✓ 21 Graduate Students

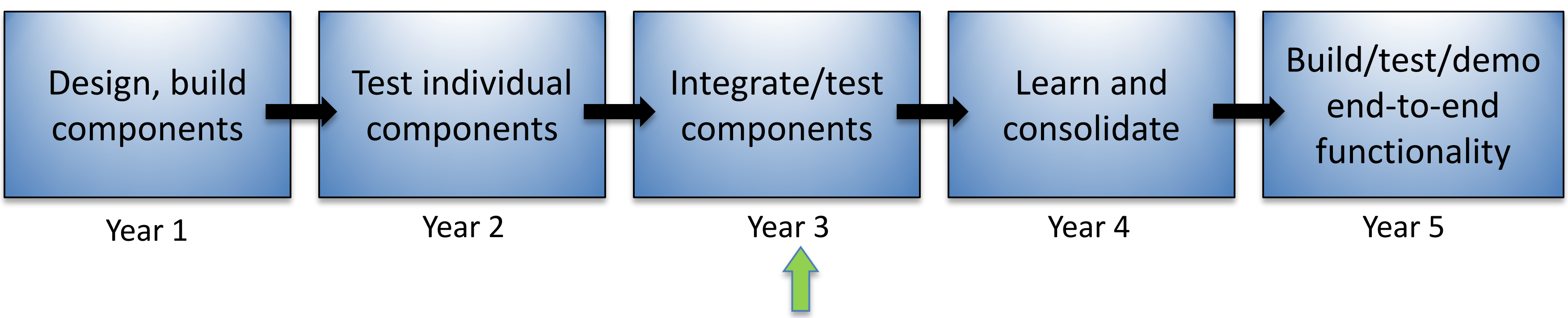
Berkeley UNIVERSITY OF CALIFORNIA — Stanford University — UtahState University — UCDAVIS — UF UNIVERSITY of FLORIDA

CUBES Approach

Four Integrated Research Divisions

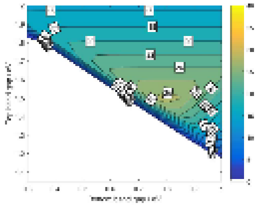
- **Systems Design and Integration (SDID)**: Optimally allocate and utilize Mars resources, tightly integrate and automate internal processes, and to satisfactorily achieve performance per mission specifications (U Florida/Menezes)
- **Microbial Media and Feedstocks (MMFD):** Harness *in situ* resources, decontaminate and enrich regolith, and transform human/mission wastes to media and feedstocks for utilization by downstream processes (Utah State U/Seefeldt)
- **Biopolymer Bio-Manufacturing (BBMD):** Produce biomaterials, biopolymers, and chemicals from media and feedstocks, recycle products at end-of-life, and use generated biopolymers in 3D-printing (Stanford U/Criddle)
- **Food and Pharmaceutical Synthesis (FPSD):** Engineer plants and microbes to produce food and medicine for use by astronauts. (UC-Davis/McDonald)

Design-Build-Test-Learn continuously for Year 5 Demonstration



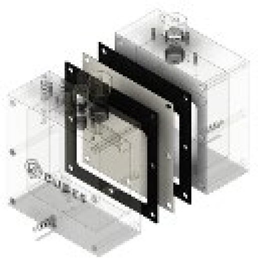
Systems Design and Integration Division Accomplishments

Design of a biomanufacturing-driven Reference Mission Architecture
- Developed Martian climate and environment models
- Agriculture scheduling and crop models
- Calculated solar cell production capacity for Mars surface missions


Modeling for Mars agriculture

Dynamic bioreactor modeling and surrogate model-based uncertainty quantification
- Developing modeling for optimizing photobioreactor design for the hybrid acetate production reactors – true first principle design

Space biomanufacturing optimized decision making
- Developed a space biomanufacturing optimization model to reduce equivalent system mass (ESM)


Reactor modeling/design

ESM reduction via waste recycling and bioreactor commonality
- New effort to examine biological degradation systems to recover valuable molecules for reuse in biomanufacturing system.

MMFD Division Status

Microbial Media and Feedstock Division Accomplishments

Electrochemical CO_2 fixation by microbe/silicon nanowire electrode hybrid systems

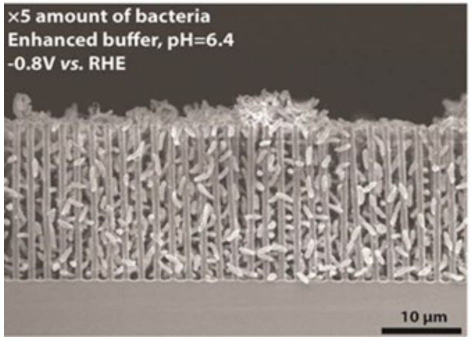
- Significantly improved strain tolerance and acetate yields

Selective evolution of nitrogen fixing organism under Mars nitrogen conditions (2%)

- Successfully adapted target organism to grow and generate organic nitrogen sources under the low N_2 conditions found in the Martian atmosphere

Use of bacterial biomass for plant growth

- Successful demonstration of using biomass from nitrogen fixation systems to serve as a nitrogen source for crop production



Hybrid electrochemical CO_2 conversion to acetate

Advanced nitrogen fixation for nutrient supply

BBMD Division Status

Biopolymer Bio-Manufacturing Division Accomplishments

Methanotrophic growth and production of bioplastics (PHAs)

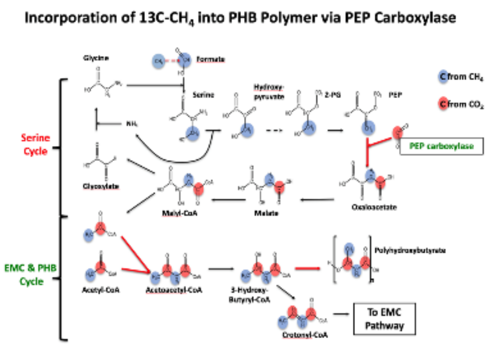
- Developed systems that can metabolize methane and other related compounds to produce bioplastics – optimizing bioreactor design and conditions

Metabolic engineering for increased product quality and quantity

- Developing advanced engineering techniques for precise engineering of host organisms for specific products

Demonstrating extrusion and printing with bioplastics

- Developed increased base-plate adherence method to increase print accuracy for bioplastics



Methane utilization for bioplastic production

FPSD Division Status

Food and Pharmaceutical Synthesis Division Accomplishments

Optimizing plant production

- Demonstrated substantial increases in growth rates in lettuce with far-red wavelength addition
- Engineering rice to increase photosynthesis efficiency
- Developing microbiome management methods for increasing plant health/growth
- Developing optical fiber system for enhanced plant lighting

Plant-based production of biopharmaceuticals

- Engineered lettuce to produce a bone-regenerating therapeutic (PTH-Fc fusion protein) for crew bone health.
- Validating drug activity using cell-based assays
- Demonstrated Viral Immunosorbent Nanoparticles (VINs) for protein purification in plants to reduce needed purification resources


Grand Rapids Great Lakes Bibb Iceberg

Pharmaceutical production in cyanobacteria

- Novel engineering of *Spirulina* for production of acetaminophen – potential breakthrough as a scalable photosynthetic drug production platform

CUBES Forward Activities

CUBES is On Schedule (Grant Mid-Point)

To Date:

- Conducted modeling for development of individual systems, and overall system planning (with technology down-selection)
- Very strong progress in diverse individual system development efforts

Present:

- Currently in process of pair-wise integration of 6 separate technology couplings
  - Modeling specific integration strategies between divisions
  - Physical integration of system component inputs/outputs

Year 3:

- Review results of preliminary integrations, down-select successful approaches, and model/conduct further integration scenarios of increasing complexity

Year 4:

- Refine integration approaches and individual technology performance – begin design and fabrication of final integrated demonstration system(s)

Year 5:

- Conduct testing and refinement of demonstration units, perform use-case analyses for space applications

Supporting Activities

STMD Early Career Faculty Program

- Dr. Mark Blenner – McQueen-Quattlebaum Associate Professor in the department of chemical and biomolecular engineering at Clemson University
- Completed 3 year ECF research project on “Synthetic Biology for Recycling Human Waste into Nutraceuticals and Materials: Closing the Loop for Long-Term Space Travel”
- Recipient of the Presidential Early Career Award for Scientists and Engineers, also known as PECASE in 2019.
- Currently on six month sabbatical at NASA Ames Research Center working in the Synthetic Biology Group on improved product harvesting in biomanufacturing systems.

http://www.clemson.edu/cecas/departments/chbe/documents/news/blenner-nasa-award-sep2015.pdf

Dr. Mark Blenner at NASA Ames Research Center

CLEMSON UNIVERSITY

Supporting Activities

Related STTR Efforts:

- **Mango Materials, Oakland, CA**
  - "A Novel Membrane-Based Bioreactor Design to Enable a Closed-Loop System on Earth and Beyond" – currently in Phase IIE
  - Designing a novel, membrane-based bioreactor to enable bacterial production of biopolymer in microgravity environments on moist membranes that are sandwiched between layers of the gaseous feedstocks methane and oxygen

- **Sustainable Bioproducts, Bozeman, MT**
  - "A Robust Biofilm-Biomat Reactor for Conversion of Mission-Relevant Feedstocks to Products" – Finished Phase I
  - Developing an encapsulated biofilm-biomat reactor that will efficiently convert mission relevant feedstocks to usable products under zero gravity conditions
  - Recently received $33M funding infusion for terrestrial applications

Supporting Activities

Notable Conferences:

- **SynBioBeta** October 2019 – Jitendra Joshi (NASA HQ) and Adam Arkin (UC Berkeley CUBES PI) supported a panel discussion on Synthetic Biology for Space
- **2nd Annual Space Travel: Adaptive Research and Technologies from Biological and Chemical Engineering (STAR Tech)** – November 18-19/2019 Boston, MA
  - Al Sacco and John Hogan – Conference Chairs
- **Veriditas Workshop** - Shannon Nangle Organizer - Harvard Medical School November 20, Boston MA - Viriditas is a focused, discussion-based workshop geared towards using biological approaches to support sustainable settlements on Mars.

Supporting Activities

NASA Food System Road Mapping Effort

- New effort in the Human Research Program (HRP) to expand the Food System Roadmap to provide a pathway to safe food supply for future long duration missions.
- Prepositioning of stored foods on the Moon and supplies for Mars missions require shelf-life of up to five years, and requires food diversity and palatability.
- Current food system requires improvement to meet these challenges.
- Synthetic Biology team is partnering with HRP to provide input on novels methods for potential food and nutrition production during missions to meet requirements.

Biomanufacturing Mission Product Identification

- Working with various exploration and medical personnel to identify required products that either terrestrial or in-space biomanufacturing processes can provide.

Questions?