Microbial Biomanufacturing in Space

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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

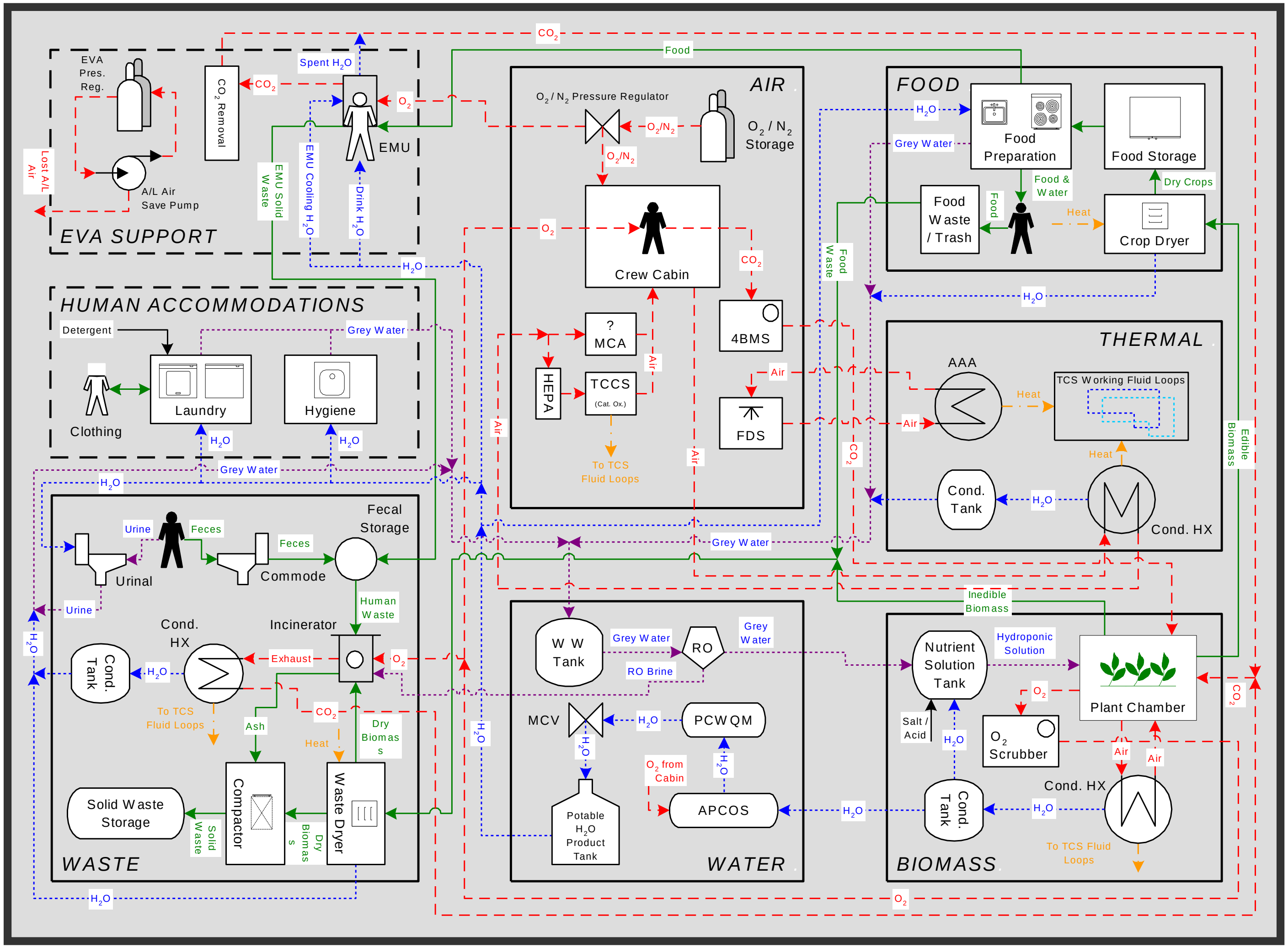
Future Mission Sustainability

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.

Systems Integration

Closing the Loops – "Metabolic Modeling"



From: NASA ALS Reference Missions Document

Bioregenerative Life Support

Food Production
- Produce food, CO_2 to O_2,
- greywater treatment



Wastewater Treatment
- Organics removal, recycling of nutrients


Solid Waste Processing
- Organics stabilization, H_2O/nutrient recovery, plant media, secondary food production


Air Revitalization
- CO_2 to O_2, contaminant biofiltration,, nitrogen conversion


Microbial Issues in Space Exploration

Human-microbe interactions

- Microbiome management, pathogens

Life Support Systems

- Biological waste treatment of wastewater, solid wastes, air contaminants
- Microbial food/nutrient/medicine production
- Microbe/plant associations for plant-based food production systems

Biofouling and Biocontrol

- Fluid lines, surfaces, hardware, technical microbiome

***In Situ* Manufacturing (ISM)**

- Microbial production systems: polymers, adhesives, nutraceuticals, fuels, other chemicals

***In Situ* Resource Utilization (ISRU)**

- Biological extraction/conversion of constituents from planetary regolith/atmosphere

Biosensors

- Human health, process indicators, contamination, radiation

Astrobiology Studies

- Planetary protection, terraforming, ET lifeform detection/control

Microbial Biomanufacturing

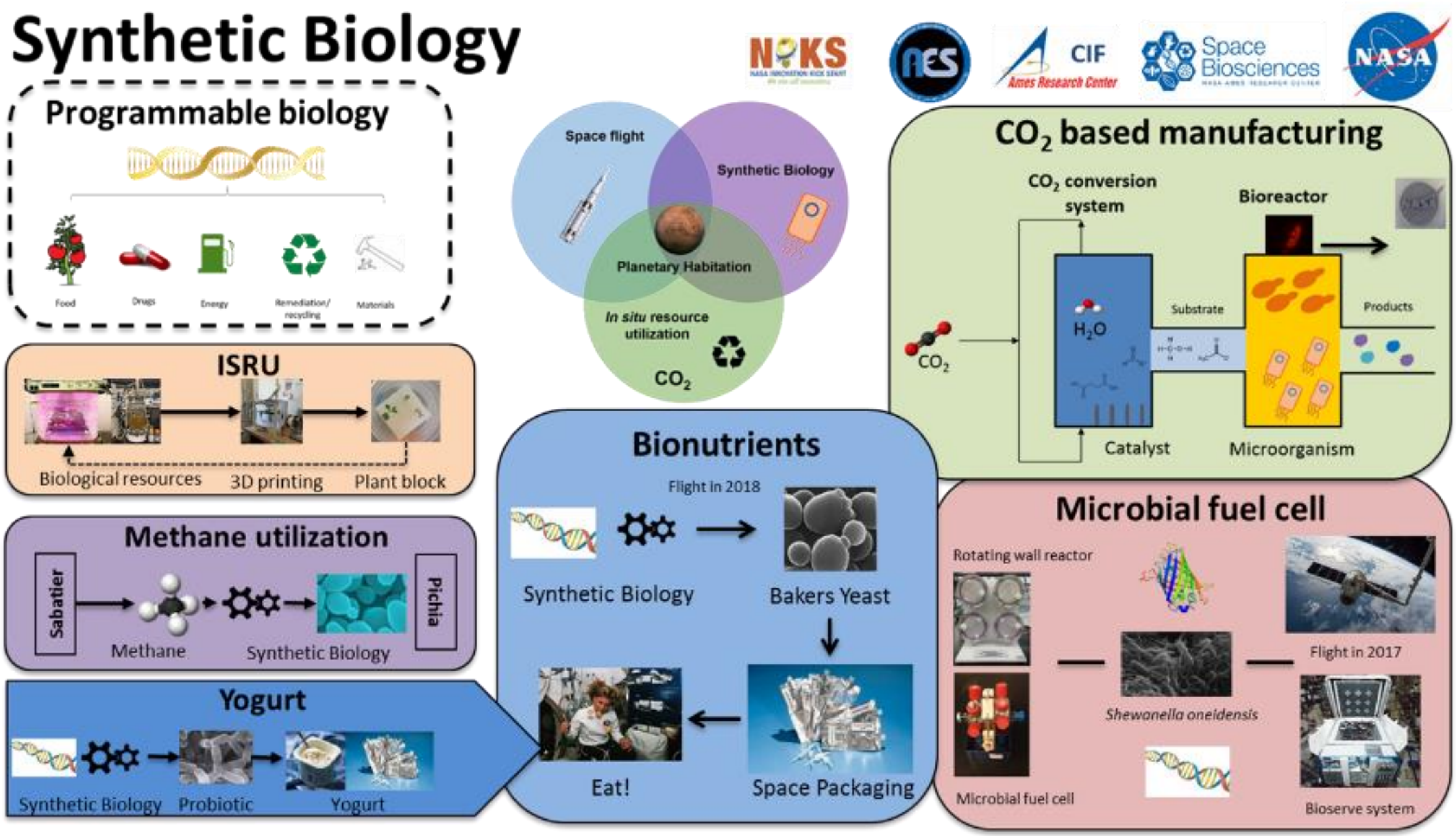
Expanding the role of microbial biotechnology for human missions:

- Microbial production of crucial mission products
- Engineering both the environment and the organism

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

Overview of Our Projects



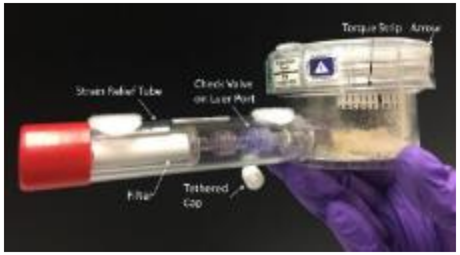
BioNutrients Project Overview

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



BioNutrients Flight Production Pack for on-orbit testing

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

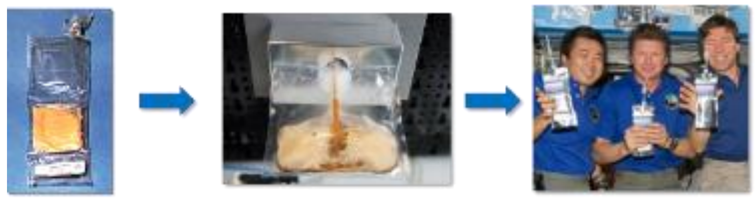
Engineering yeast to produce nutrients on-demand

Funded by STMD Game Changing Division

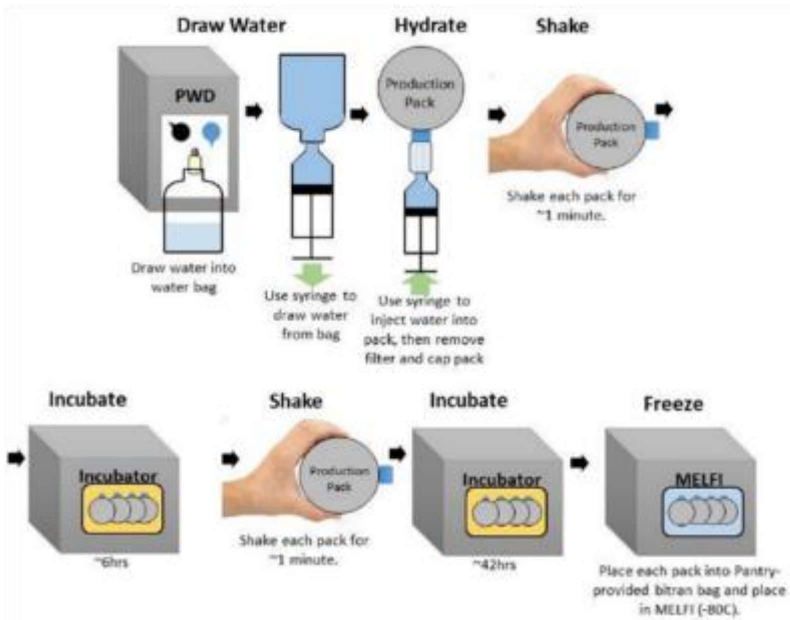
BioNutrients Flight Demo/Test

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



Also developing yogurt-based production systems

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

BioNutrients Production Pack w/ media and yeast

BioNutrients Flight Demo/Test

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 (left) and packaged flight article (right)

BioNutrients flight preparations

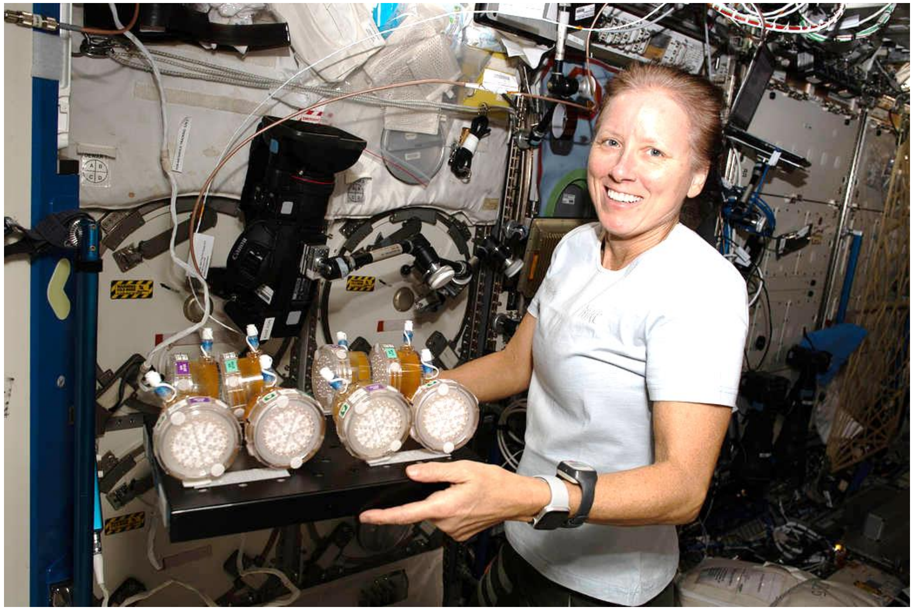
BioNutrients Flight Ops

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

Production packs in SABL incubator.

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

BioNutrients Flight Ops



Crew members Drew Morgan (left) and Shannon Walker (right) conducting operations for BioNutrients-1 production packs aboard ISS – second and third crew flight operations, respectively.

2 more flight tests (BN-2, BN-3) planned

CO_2-Based Biomanufacturing

Objective: Use rapid physico-chemical methods to convert CO_2 to an organic media that is used by heterotrophic microbes to produce mission-relevant products in space.

Concept Example:

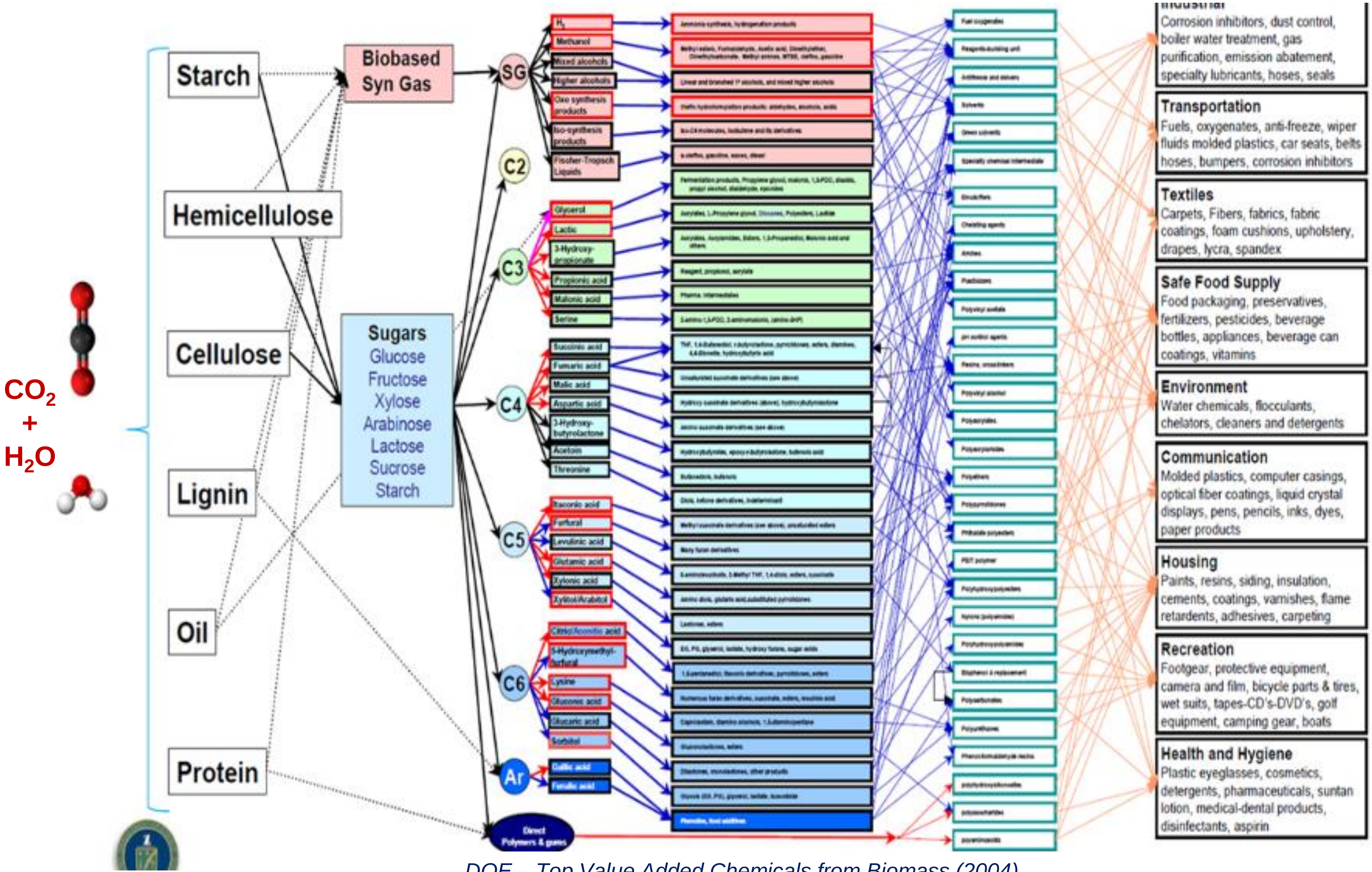
$CO_2 + H_2$

↓

P/C Conversion System → Microbial Medium → Growth System → Product ↑

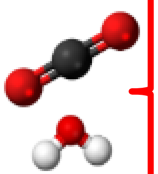
5/20/2017

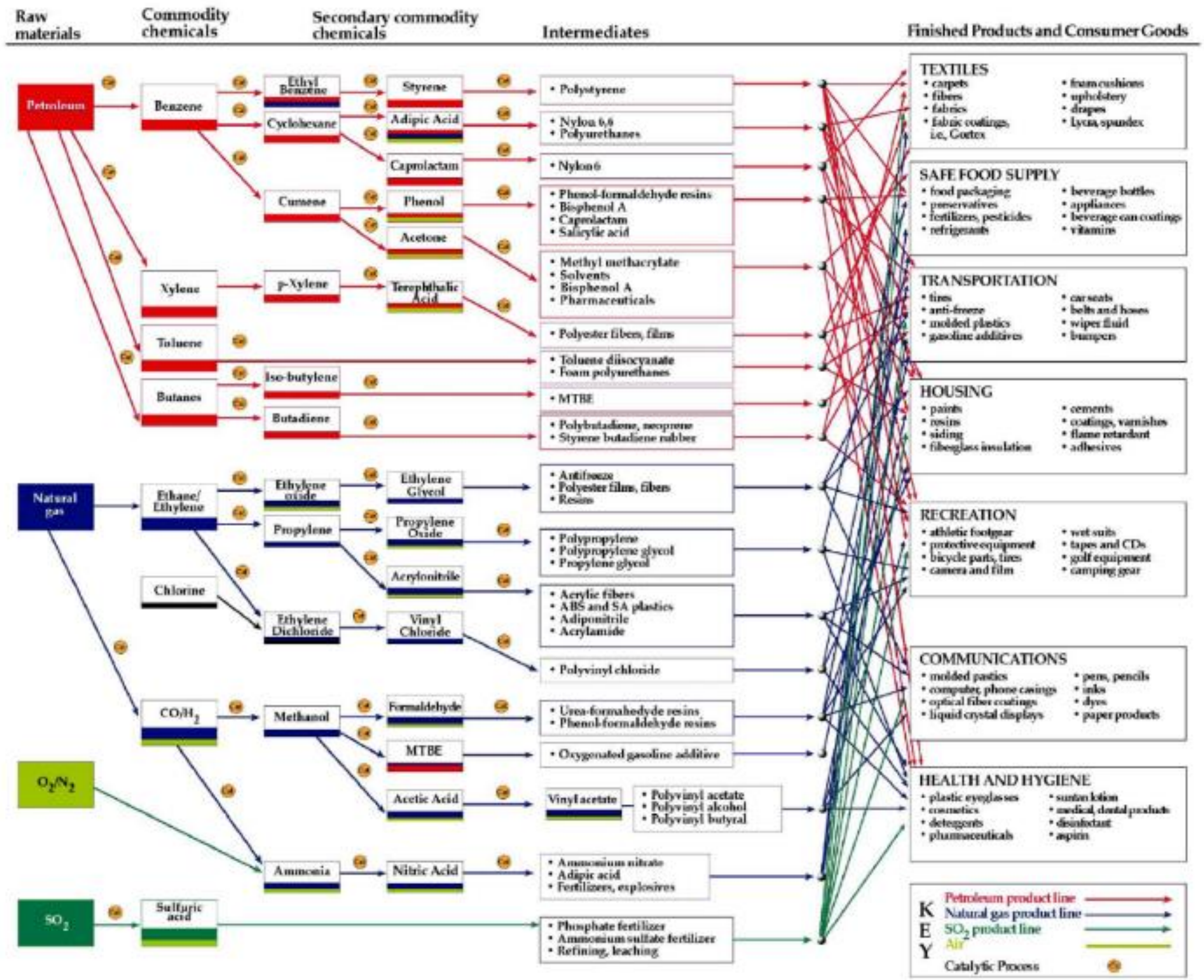
Biomass-Based Manufacturing



DOE – Top Value Added Chemicals from Biomass (2004)

Petroleum-Based Manufacturing

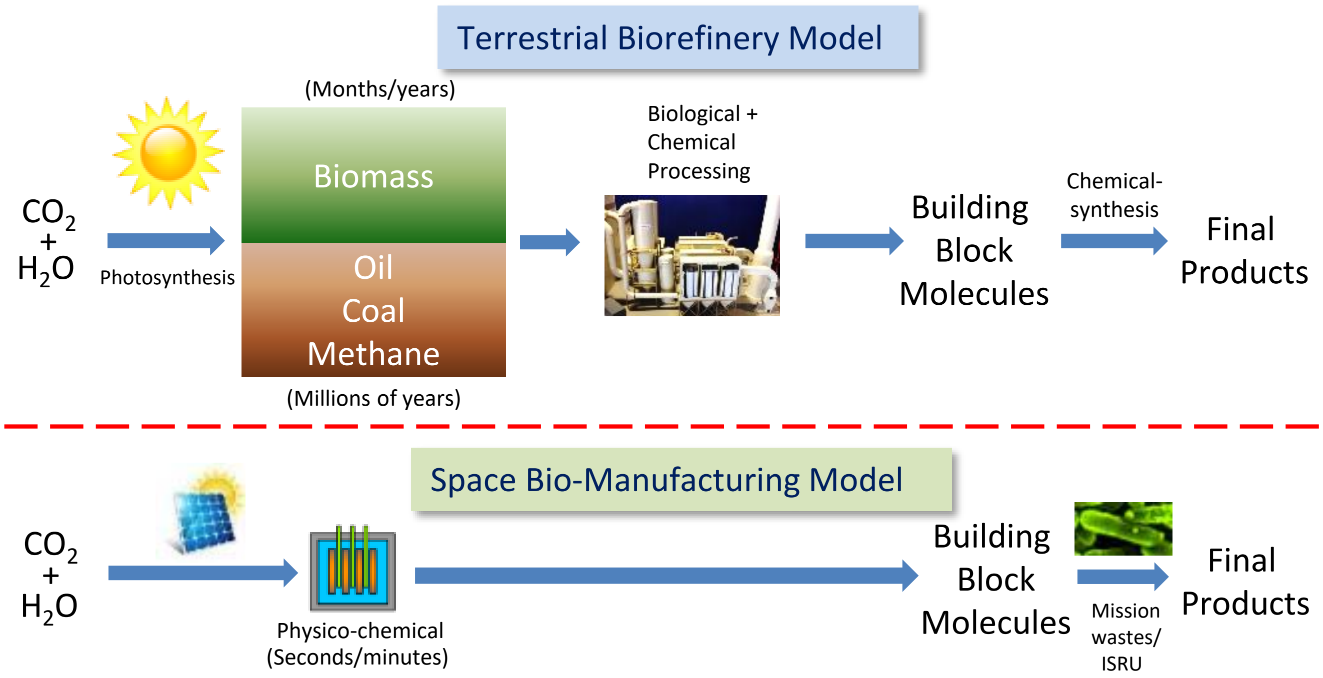




From: Top Value Added Chemicals from Biomass Vol.1. US DOE 2004

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 Project

CO_2 conversion

CO_2 → Formic Acid

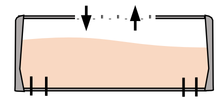
Stanford University + Ames Research Center

CO_2 → CO → Acetate

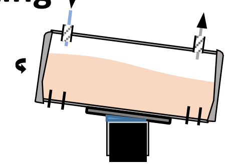
NASA Centennial Challenges — CO_2 Conversion Challenge

CO_2 → Glucose

Bioreactor development and testing



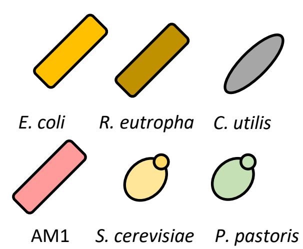
Passive membrane diffusion system



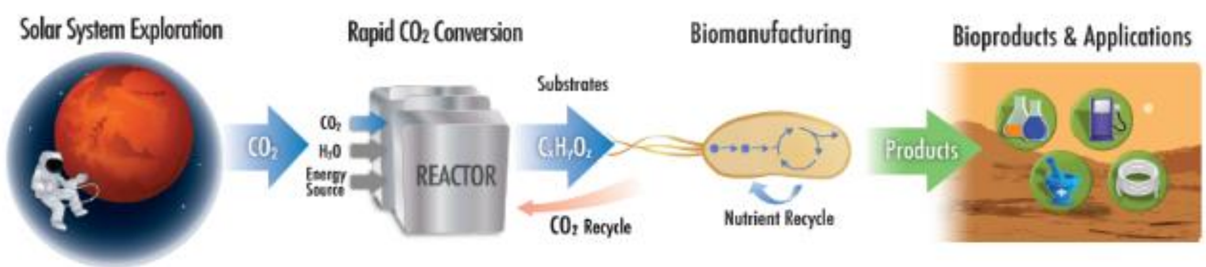
Rocking system

Products and applications

Microorganism engineering and testing



Pathway engineering
Metabolize uncommon substrates
Genetic Tools
Development of expression systems
Products
Carbonic Anhydrase



Funded by STMD Game Changing Division

NASA CO_2 Conversion Centennial Challenge

NASA CO_2 Conversion Challenge

GOAL: Abiotic production of sugars from CO_2 to support to feed heterotrophic 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

NASA CO_2 Conversion Centennial Challenge

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

Exoelectrogens in Space

Goal: Develop exoelectrogen-based systems for life support in extended duration missions

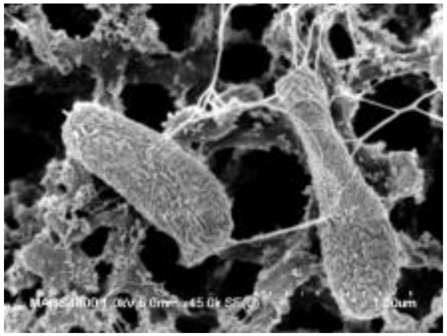
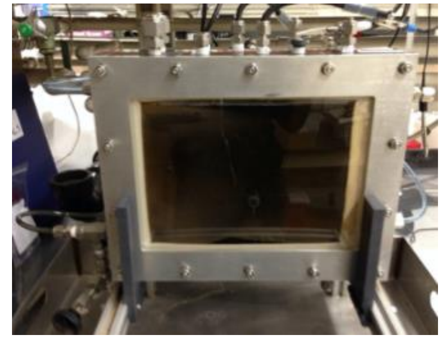


Photo: Natalie Ball



Investigated bio-electrogenesis and bio-electrosynthesis using extracellular electron transport - Developed bioelectrochemical system for treating urine with concomitant reduction of CO_2 to CH_4

Funded by NASA STMD Game Changing Division

Shewanella oneidensis MR-1 - Flight Experiment (Micro-12)

- Measure *S. oneidensis* MR-1 **extracellular electron transport** (EET) rates and determine the EET mechanism(s) impacted by microgravity using biochemical and genetic approaches.
- Characterize and quantify *S. oneidensis* MR-1 **biofilm development** under microgravity conditions using confocal laser scanning microscopy (CLSM).
- **Identify other key molecular and genetic components** for biofilm formation and extracellular electron transport that are affected by microgravity by using genome-wide gene expression and mutant fitness profiling.

Group Activation Pack (GAP)

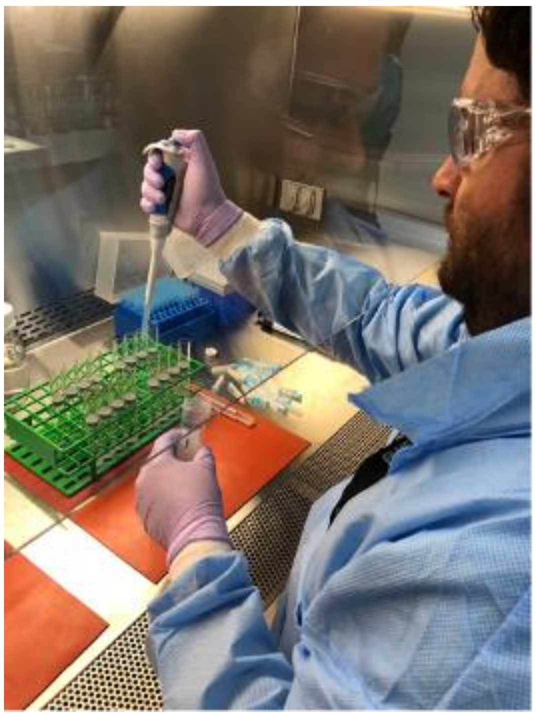
Funded by NASA Space Biology Program

Exoelectrogens in Space

Space Station Processing Facility – Kennedy Space Center

Exoelectrogens in Space

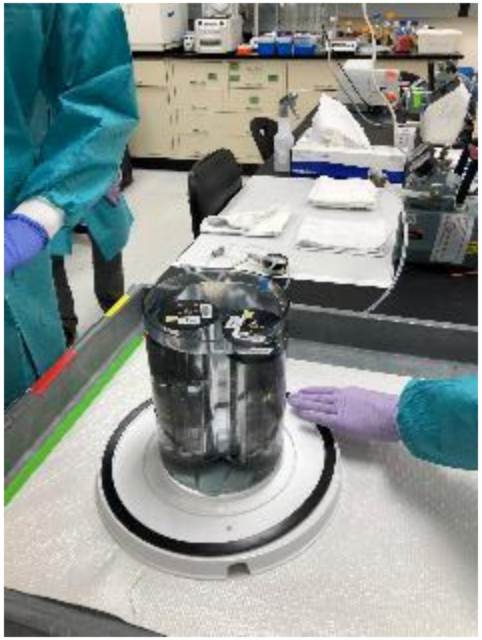
Space Station Processing Facility – Kennedy Space Center

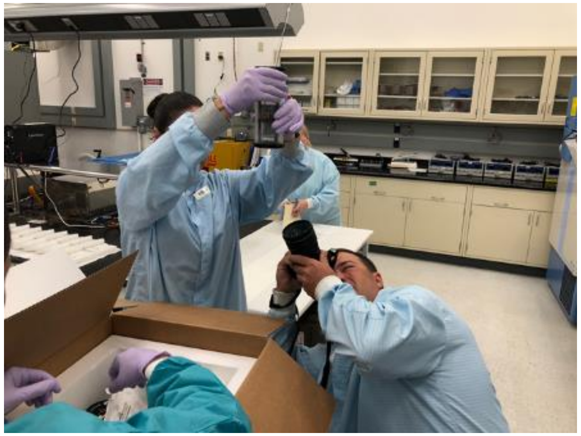


Funded by NASA Space Biology Program

Exoelectrogens in Space

Space Station Processing Facility – Kennedy Space Center





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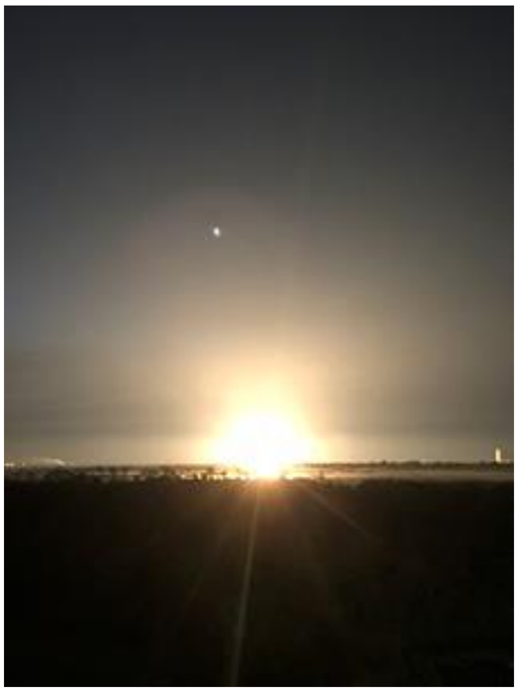
Exoelectrogens in Space

Micro-12 Launch on CRS-15 – Kennedy Space Center



Funded by NASA Space Biology Program

Exoelectrogens in Space

Astronaut performing a GAP (Group Activation Pack) operation on the ISS.
https://www.nasa.gov/mission_pages/station/research/news/micro_6.html

Funded by NASA Space Biology Program

The Center for the Utilization of Biological Engineering for Space

CUBES

Lead Institution:
University of California – Berkeley; Dr. Adam Akin, PI

Vision:
- 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.

5 years - up to $3M/year budget

https://cubes.space

Berkeley University of California | Stanford University | Utah State University | UC Davis | UF University of Florida

Bio-Manufacturing for Deep Space Exploration – CUBES Activities

Use of Local Resources to Support Biomanufacturing

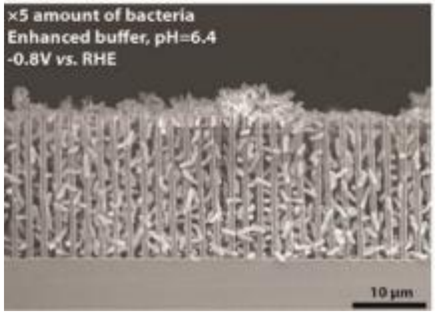
- Conversion of carbon dioxide, water, and other needed local resources to support rapid plant and microbial growth systems
- Develop nanowire/bacteria hybrids for solar-driven CO_2 fixation to organic substrates for microbial growth
- Develop novel hybrid N_2 fixation methods for nitrogen capture/supply

Biomanufacturing of Mission Products

- Optimize media/microbial engineering to produce bioplastics
- Advanced additive manufacturing techniques for mission products
- Increase yield, volume efficiency, and photosynthetic efficiency
- Pharmaceutical synthesis in plants and cyanobacteria
- Microbiome engineering for enhanced plant growth/performance

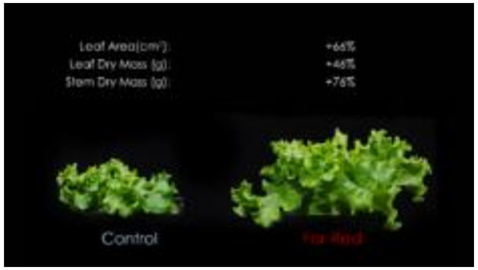
Systems Analysis, Integration and Demonstration

- Determine resource availability to guide technology development efforts
- Develop performance requirements, architecture and process models
- Integrate components for a scaled biomanufacturing demonstrationand assist in fabrication/testing



Nanowire/Bacteria Hybrid Reactor for acetate feedstock production

In-Space Bioplastic synthesis and product manufacturing



Far-red wavelength benefits

Academic 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 project on "Synthetic Biology for Recycling Human Waste into Nutraceuticals and Materials: Closing the Loop for Long-Term Space Travel"
- 2019 Recipient of the Presidential Early Career Award for Scientists and Engineers.
- Currently on six month sabbatical at NASA ARC - improved product harvesting systems.

Dr. Fuzhong Zhang – Francis Ahnmann Career Development Associate Professor at Washington University in St. Louis

- Completed 3 year ECF project on "Engineering Cyanobacteria for the Production of Lightweight Materials"
- Developed new methods for spider silk proteins for high strength materials

Dr. Mark Blenner at NASA Ames Research Center

Team

PI: Dr. John A. Hogan (NASA)
Co-PI: Dr. Mark Settles (NASA)
PM: Dr. Francis Donovan (NASA)

BioNutrients:
Dr. Aditya Hindupur (KBR)
Dr. Hiromi Kagawa (SETI)
Natalie Ball (KBR)
Aphrodite Kostakis (KBR)

BioNutrients Payload Development Team:
Julie Levri – BN-1 PM - NASA
Dr. Amy Gresser – BN-2 PM (KBR)
Kevin Sims - (Wyle Labs)
Sean Shariff - (Logyx)
Alyssa Villanueva – KBR)
Alan Noblitt – JSC Safety

CO_2-Based Manufacturing for Mission Extensibility:
Dr. Jonathan Galazka (NASA)
Dr. Michael Dougherty (Wyle Labs)
Mattie Soundararajan (U. of New Mexico)
Matthew Paddock (KBR)
Dr. Matthew Kanan (Stanford U.)
Jason Samson – (NASA)


Thank you!
Questions?