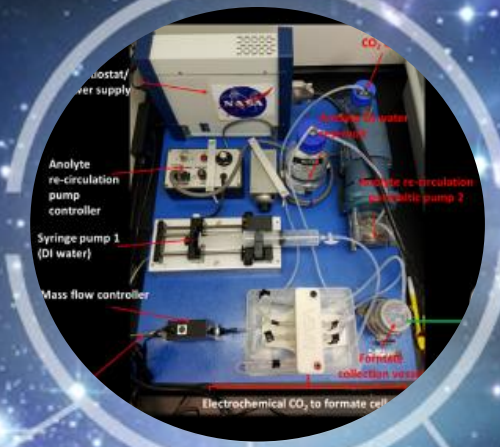
National Aeronautics and Space Administration



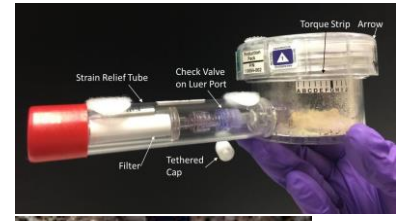
Space Technology Mission Directorate

Game Changing Development Program —Synthetic Biology (SynBio)

Frances Donovan| Synthetic Biology Project Monthly Status Report | 04.2021

Project Overview

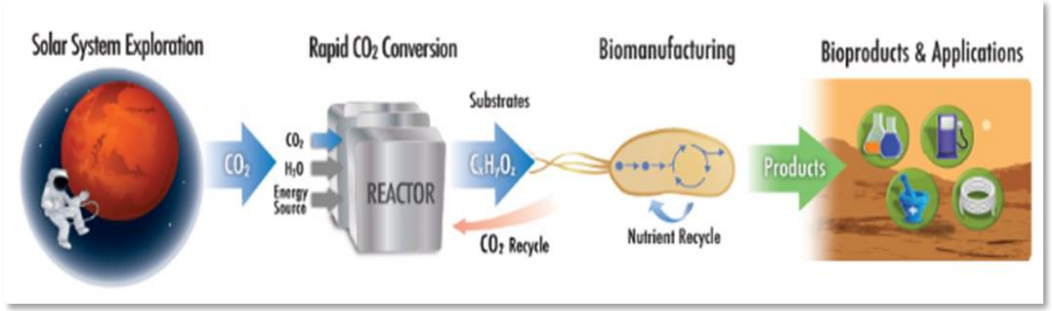
- Technology Product Capability
  - Develop and demonstrate an on-demand nutrient production system for long-duration missions to mitigate demonstrated nutrient degradation in stored foods. Develop an evolvable platform for future surface missions – capable of producing other compounds (e.g., Medicines) requiring minimal resources. Perform an ISS time-course hydration, incubation, freezing and return for analysis over 5 year period.
  - 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.
- Technical Capabilities
  - Reduced gravity compatible bioreactor development
  - Long-duration ambient storage of microorganisms
  - Methods for ensuring quality and safety of biomanufactured products
  - Development of ISRU based growth media
  - Space-relevant biomanufacturing system development
  - Space qualified organisms for biomanufacturing
- Exploration & Science Applicability
  - Cross-cutting: Supports crew health, enhanced logistics, mission sustainability and risk reduction for extended duration habitation missions on the moon (Artemis) and Mars and space-based outposts.



BioNutrients -1 Flight Production Pack for on-orbit testing

Future Implementation Concept

Hybrid CO_2-Based Biomanufacturing Concept



Project Highlights/Accomplishments

Title	Type	Element	Description	New Image for EPO	Highlight Submitted
Yeast expression of a potential therapeutic Follistatin confirmed in BN-2 engineered yeast	test	**BioNutrients**	Production of Follistatin confirmed in BN-2 engineered yeast via FLAG-tag assay. Concentration to be determined with follow on assays. Expression of Follistatin expands the products generated in the BioNutrients element to include both nutrients and potential therapeutics. This result will ultimately be presented via scientific publication.	n/a	No, should wait for BN-2 pre-flight announcements/ scientific publication.

SynBio

Project description:	Build organisms and bioreactor systems to demonstrate the value of emerging synthetic biology approaches to meet mission demands and support in situ resource utilization.	**Current TRL: 3**

Cost:

No cost issues at this time.

Schedule:

BioNutrients-2 production pack development and testing, and CO_2-based manufacturing bioreactor and genetic engineering of organisms delayed due to mandatory telework. Lab access has been regained on a limited basis for BioNutrients activities only (Nov 30), and impacts to the schedule of both tasks are ongoing.

Technical:

No known technical challenges at this point, but schedule delays are impacting our progress.

Programmatic:

The BN-2 ISS Kick-off meeting was completed in February and the draft Payload Integration Agreement (PIA) has been uploaded to the OZ Requirements Baseline and Integration Tool (ORBIT). A CEF to approve the PIA is currently open and review is expected to complete by the end of the March. The BN-1 flight experiment agreement with BioServe to use the SABL incubator for on-orbit runs 4-6 was approved and a kickoff meeting was held March 12. A similar agreement covering use of SABL for BN-2 was approved on March 1.

SynBio Additional Project Information

BioNutrients

- BN-1 results: Production Pack run 3 ISS on-orbit operations and ground control were completed January 25-27. Biomass comparisons of the first two runs now complete (Fig 1). Production packs flown to ISS are viable and outperform ground controls. This is likely due to settling of microorganisms under gravity conditions. Stasis pack viability assessment is ongoing.
- BN-1 production pack safety analysis reveals no contamination as far as testing can determine (Fig 2). KPP exceeded for time points up to 1.75 years.
- BN-2 Gen 1 bioreactor bag test with yeast, kefir and yogurt samples is underway. Preliminary pressure testing of FEP bag indicates full tolerance above expected pressure values. Safety review on track for 4/28. Shelf-life study of yogurt and kefir strains continues, status shown in Fig 3. Results indicate acceptable viability up to 3 months. Study will continue to assess a minimum of 6 months.
- BN-2 new product Follistatin confirmed as expressed in yeast (Fig 4). Follistatin is a potential therapeutic agent supporting muscle growth and may be indicated as a countermeasure to spaceflight induced sarcopenia.

Fig 1 BN-1 T1- and T3- ISS PP flight & ground control Biomass results. Both *Y55* and *S. Boulardi* yeast strains grew with flight packs (F) outperforming controls (G)

CO_2-Based Manufacturing System Development

- Commercial support for test and fabrication as well as organism bio-engineering is being pursued.
  - We are working with a vendor to obtain a product-secreting capability in our chassis organism which will expand our capabilities and simplify product purification for secretion products.
  - We have identified specialized filters for product recovery and have obtained quotes.
  - Discussion is underway with a commercial vendor for proprietary cell lysis technologies to be tested for our system.
- Evaluation of commercial products and activities in the CO_2 based manufacturing space has led to ESM analysis of various COTS options.
- System requirements are being refined with systems engineering support, requirements document is being drafted.

Fig 2 BN-1 run 1 and 3 have no detectable levels of Aerobic, Coliform, Staphylococcus, or Salmonella contamination. Yeast counts are expected viable cells from inoculum.

Project Management/General

- BN-2 System Requirements Review (SRR) internal project milestone is pending review by ARC chief engineer's office and review team. Science and Demonstration Requirements Document and Concept of Operations to be baselined post review.
- CO2 conversion Centennial Challenge status: Phase 2 judging is commencing for three teams
- Summer internships: two students have been selected for summer internships

Fig 3 BN-2 commercial yogurt and kefir strain shelf life experiments show acceptable viability out to 3 months. Study is ongoing.

Fig 4 BN-2 yeast expression of Follistatin confirmed.

Project Plan - Milestone Status

Milestone Title (Mirror Project Plan)	Baseline Date	Planned Date	Variance Explanation
Status of milestones due since last report			
C3-BN Generation 1 Flight Pack Design Review – will now combine with TAPR, targeting mid-June	01/19/21	TBD, targeting mid June	Delay due to delays in lab access, delays in procurement, CR has been submitted.
Status of milestones due in the next 60 days			
C4-CO2 Space Compatible System Design Review	04/15/21	TBD, targeting Dec 2021	Delay due to lack of lab access, target date is for 6 months after expected return to lab. CR will be submitted in combination with PPBE per direction at PM forum.

Project Risks, Issues/Concerns

Project Risks

Risk ID	Affinity	Likelihood/ Consequence (if applicable)	Description/Status	Trend	Proposed Mitigations
Risk 1	S	5/2	If delays in lab access result in incomplete testing and data acquisition, the Design Review for BioNutrients-2 will not occur on Jan 19th as planned.	realized	Delay the Design Review until the required testing can be completed. CR has been submitted.

Issues/Concerns

Issue Title	Description/Status	Proposed Mitigations
COVID-19 schedule impacts	The progression of several tasks on the project has been delayed due to lack of access to laboratory facilities. Upcoming controlled milestone, a design review for the BioNutrients-2 next generation bioreactor will not occur per plan and will likely have >30 day slip.	Delay the Design Review until the required testing can be completed. For other tasks the level of optimization and development will be impacted if schedule relief is unavailable.

Education/Public Outreach

EPO Involvement

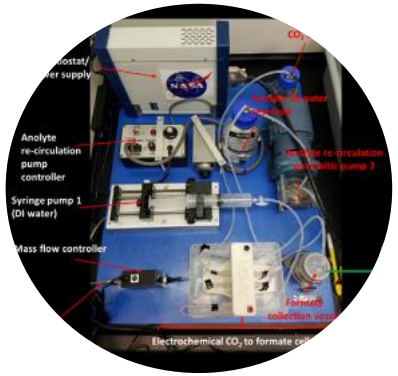
- Paper submitted to 50th International Conference on Environmental Systems - ICES 202, July 12-14, team will attend virtually.

EPO Calendar Outlook (High Priorities):

30 Day Look-Ahead	
60 Day Look-Ahead	

Space Synthetic Biology

View of BioNutrients-1 production packs during ISS crew operations. Packs were removed from incubator for mid-course agitation.



CO_2-Based Manufacturing project prototype that demonstrated the production of formic acid from CO_2 and water, while also producing oxygen.

Ground testing of BioNutrients-1 production packs in SABL flight incubator (ground unit) to verify temperature, fit and no leakage.

View of open BioNutrients-1 production pack during loading. Dried edible media (powder) and yeast pellets (red) are visible.

State-of-the-Art CO_2 electrochemical system that converts CO_2 and water to formic acid without electrolytes. The formic acid serves as an media for microbial production systems.

Loading BioNutrients-1 production packs with media and organisms under sterile conditions. The pack lid and sterile water filter are attached during this assembly.