National Aeronautics and
Space Administration

The Potential and Challenges of Fermented Food Production in Space

Frances Donovan, Ph.D. | Space Biosciences Division, NASA Ames Research Center | Synthetic Biology Project | Game Changing Development Program, Space Technology Mission Directorate

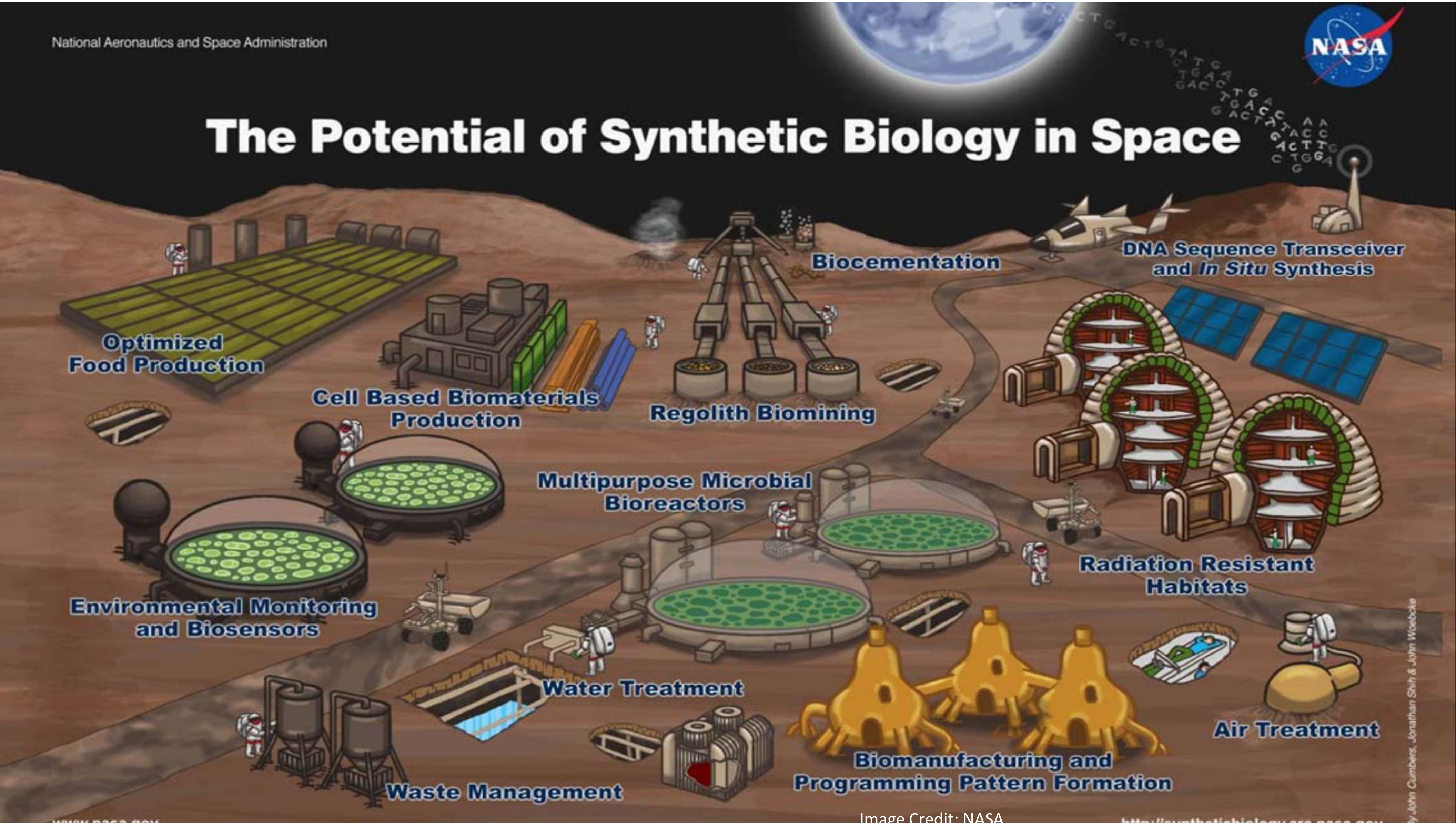


Image Credit: NASA

Nutrition in Space

Nutrients and pharmaceuticals degrade with time in food and supplements.

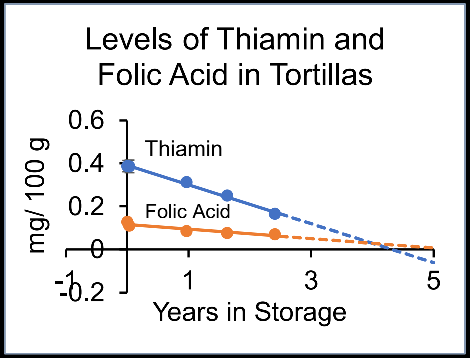
- Enhanced nutritional needs in space travel.
- Radiation and microgravity countermeasures.

Food is critical in fighting stress and disease-specific concerns.

- Nutrient deficiency diseases.
- Psychology of food/isolation.

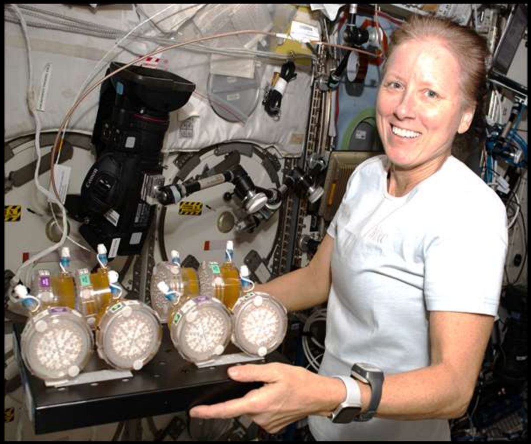
Future, longer duration missions may require production of some foods and supplements *in situ* as mitigation.

STS-127 meal-time. Clockwise from bottom left: ESA astronaut Frank De Winne, astronauts Christopher Cassidy, Mike Barratt and Tim Kopra, CSA astronaut Robert Thirsk, and astronaut Mark Polansky. From left to right at top: JAXA astronaut Koichi Wakata and Russian Federal Space Agency cosmonauts Roman Romanenko and Gennady Padalka.



Adapted from Zwart et al (2009) *Food Science* 74(7):H209-H217. https://doi.org/10.1111/j.1750-3841.2009.01265.x

Astronaut Peggy Whitson working on the Veg-03 experiment.



Fermentation production of nutrients. Astronaut Shannon Walker with the BioNutrients (BN-1) experiment, run 3.

Image credits: NASA

Fermented Foods and Genetic Engineering in Support of Spaceflight

Fermented food production is spaceflight compatible – Small resource requirements, long shelf life, high radiation tolerance.

- ➢ Fermented foods provide beneficial microbes, nutrients, bioactive compounds, flavors and food preservation.
- ➢ Genetic engineering can make fermented food products into a nutrient and therapeutic compound delivery system.

Extensive history of production in non-sterile environments, amazing variety.

- ➢ Fermented foods ~5000 variations worldwide, (Ray, R.C. et al., 2024 https://doi.org/10.1007/978-3-031-72000-0_1)
- ➢ Earliest evidence for the deliberate application of fermentation circa 7000 BC, (McGovern 2004 https://doi.org/10.1073/pnas.040792110).(Tamang 2020 https://doi.org/10.1111/1541-4337.12520)

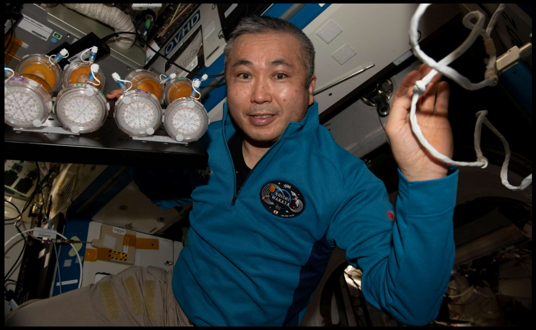
What Could Fermentation in Space Look Like?

- Yogurt
- Kefir
- Cottage cheese
- Essential Fatty Acids
- Vinegar
- Miso
- Yeast based production
- Kombucha

Feasibility

- ➢ Feedstocks required and shelf-life of that feedstock – any ISRU?
- ➢ Biproducts or waste generation
- ➢ Gas/ethanol production
- ➢ Is it ready to consume? Post processing requirements
- ➢ Can it be pasteurized or otherwise made safe?
- ➢ Can the product be removed from the live culture?

https://www.sciencenews.org/article/ferment-miso-orbit-space-food-taste-iss. Coblentz et al10.1016/j.isci.2025.112189



BioNutrients 2 production of carotenoids and follistatin above. Bionutrients 1, Koichi Wakata (Run 5, January 2023) right.

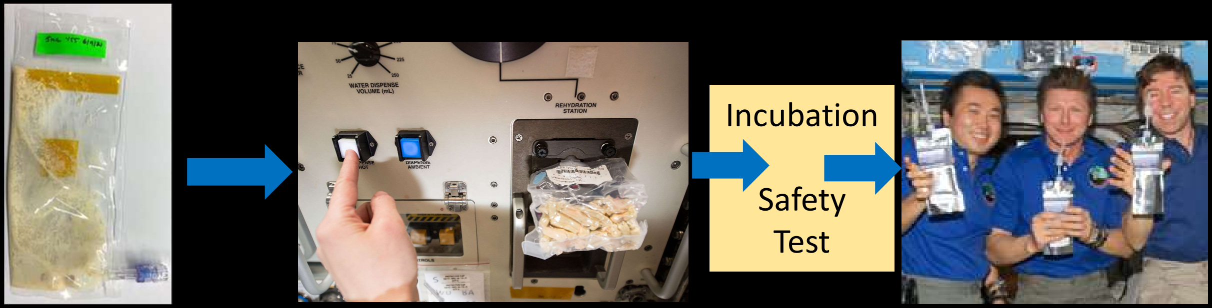
❖ Fermentation of waste products to generate fuels, feedstocks, fertilizers, generate enzymes or compound for further purification

BioNutrients – native and engineered nutrient supply

Aim: Develop and demonstrate an on-demand nutrient production system for long duration missions to support nutrient degradation in stored foods.

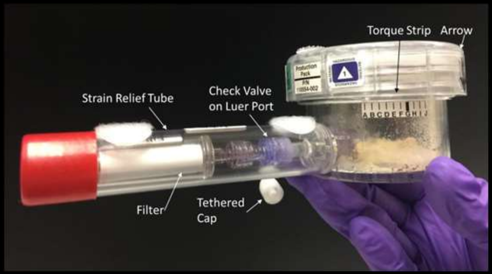
- Flight-tested, evolvable microbial production platform capable of producing many compounds (e.g., nutrients, medicines).
- Space-adapted microbial hosts for future generic use and genetic engineering.
- On-orbit safety and operational systems for future implementation.

Mission Implementation Concept



Incubation

Safety Test



BioNutrients-1 Flight Production Pack Development for 5 year on-orbit shelf-life testing

Engineering microbes to produce nutrients and medicines on-demand

Developing microbes adapted for use in space

BioNutrients-2/Gen-1 System: FEP bioreactor, new products, including yogurt, kefir and a protein therapeutic.

ISS

BN-2 yeast ISS production packs (*S. boulardii* producing beta-carotene, *S. cerevisiae* producing zeaxanthin, and *K. lactis* producing follistatin).

Run 1 of yogurt and kefir on ISS. Left: kefir (blue color at initial hydration turns yellow as culture grows and pH lowers), Upper right: yogurt.

Ground Control

BN-2 yeast ground controls (*S. boulardii* producing beta-carotene, *S. cerevisiae* producing zeaxanthin, and *K. lactis* producing follistatin).

BN-2 ground controls yogurt top, kefir bottom.

Nicole Mann and Josh Casada hydrating and shaking the yeast and yogurt packs respectively. Featured in Space Station Science Highlights: Week of January 2, 2023 | NASA ISS Research on Twitter: "BioNutrients-2 tests an on-demand system to produce key nutrients from yogurt, kefir, and a yeast-based beverage. The study could help maintain the crew's health while reducing launch mass and volume requirements. https://t.co/fuxW39uTWd https://t.co/bzFZVYMIxS" / Twitter

BioNutrients production after 5 years, 10 months

iss072e616432 – ISS Commander Suni Williams holding a SABL Short Tray with eight BioNutrients-1 Production Packs attached. Taken during BioNutrients-1 Run 7 Ops on February 11, 2025.

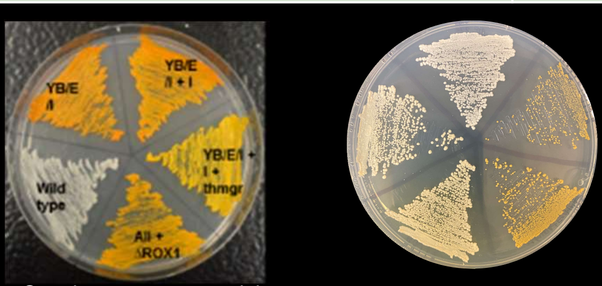
Crew comments remain positive; multiple mentions of beer or baking bread odor that is deemed neutral or positive.

BN-1 Run-4 production packs in SABL incubator on ISS, (2/2022). Astronaut Tom Marshburn agitating the cultures at the 6-hour timepoint.

Probiotics and Fermenting Organisms Flown on ISS

BioNutrients Strains

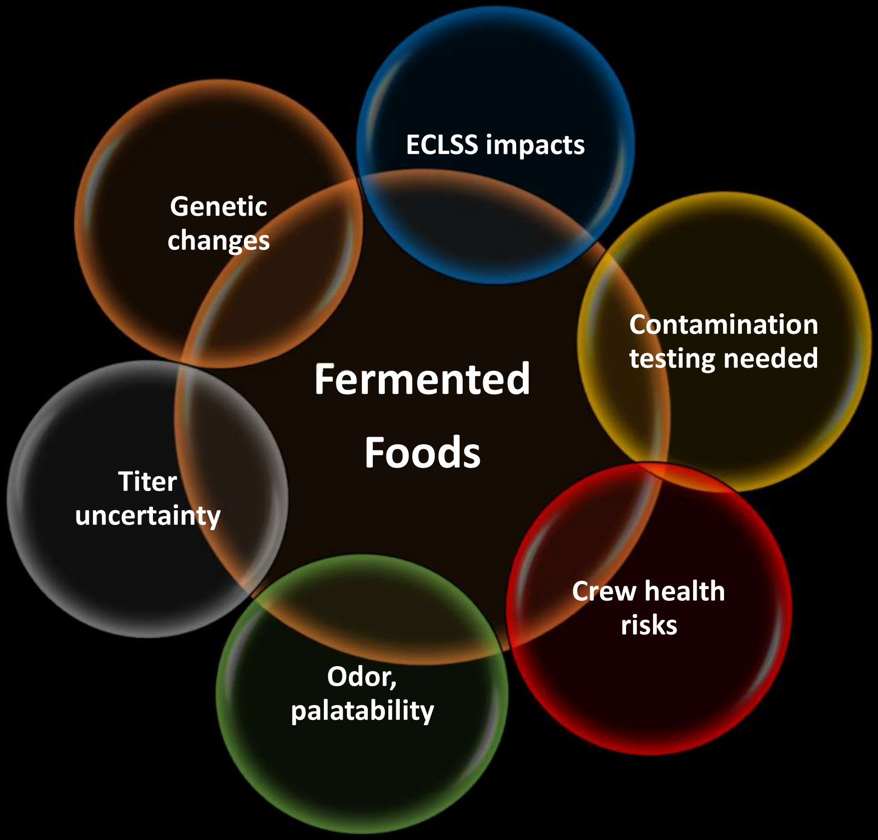
Flight Strain	**Product**
Saccharomyces cerevisiae Y55 (Carotenoid strain)	Beta-carotene, zeaxanthin
Saccharomyces boulardii (Carotenoid strain)	Beta-carotene
Saccharomyces cerevisiae Y55 (Multi-nutrient co-culture)	Beta-carotene, zeaxanthin, riboflavin, and folate
Kluyveromyces lactis	Follistatin
Streptococcus thermophilus/ Lactobacillus bulgaricus	Yogurt
Mixed organism culture	Kefir



Saccharomyces cerevisiae
Engineered to produce β-carotene and zeaxanthin (left) and riboflavin and folate overproducer (right) (planned BN-3 mission)

Organism	**Experiment**
Bacillus subtilis	Various, never as a probiotic
Escherichia coli	Various, never as a probiotic
Lactobacillus acidophilus ATCC 4356	Growth on ISS
Lactobacillus casei strain Shirota	Long term viability (1+ month on ISS with JAXA)
Miso (Mixed community)	30 days on ISS
Saccharomyces cerevisiae	Various, never as a probiotic
Yogurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus)*	Swinburne Youth Space Innovation Challenge/Rhodium Probiotics (Active) Arla Foods (inactive) ISS Baseline Food List (inactive)
proprietary probiotic in dehydrated whole milk	Arla Foods - commercial mission

Challenges with Fermented Food Products



Fermented Foods

ECLSS impacts

Genetic changes

Contamination testing needed

Titer uncertainty

Crew health risks

Odor, palatability

Photo credits NASA

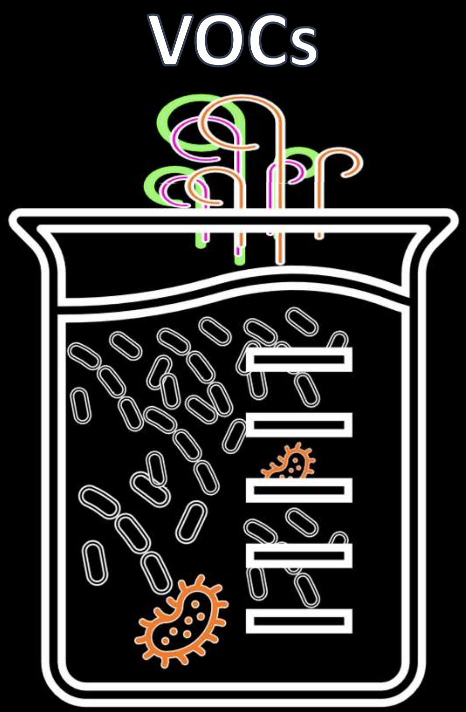
Food Safety and Contamination Detection Challenges

NASA requires zero salmonella and between 10 and 100 cfu/ml or gram depending on the pathogen/food spoilage organism and product.

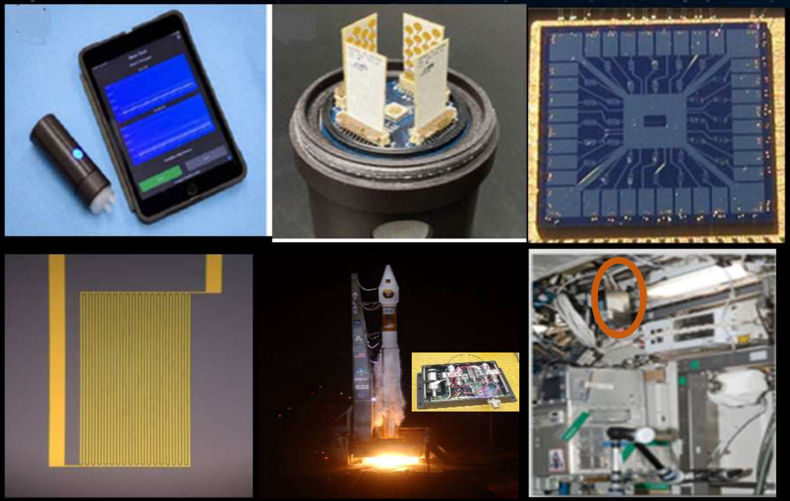
  - Current food system is thermostabilized or gamma irradiated to ensure sterility.
  - Fermented foods cannot be sterile by definition.

- **Identification by VOC analysis:** Electronic nose devices sample the VOC from the entire product (less sample bias) without destruction of the product. Can measure CO2 and ethanol production, may be able to detect changes in culture composition.

- Standard methods are prone to sampling limitations and limits of detection, not developed for use in microgravity.
  - Other limitations include shelf life, chemical requirements, gravity sensitive processes, require computational power/time, and destruction of the sample.



ARC Electronic Nose / Chemical Detection “E-Nose”



TECHNICAL / SCIENTIFIC CHALLENGE ADDRESSED

An intelligent, compact, highly sensitive, low-powered, fast responding analytical tool replicating the human olfactory system capable of identifying and classifying complex mixtures of chemicals and odors.

SUMMARY

NASA Ames spaceflight-proven robust E-Nose technology with its current TRL of 5-6 has been demonstrated for many NASA, DHS, HHS, DoD, and other commercial applications. Integration of sampling, sensing and computing in a one platform allows in-situ, real time, automated measurement compared to other SOA detection systems.

KEY ATTRIBUTES / COMPETENCY

- Individually addressable multi-channel sensing
- Integrated temperature, pressure, and humidity sensing
- Detection limit range: ppm to ppb
- Response time in seconds at room temperature
- Reproducible from sensor to sensor
- Room temperature operation
- Low power: µW to mW/sensor
- Easy integration
- Sensor chip size is 1x1cm^2 with 12-256 channels

HERITAGE

- Tested aboard a US Navy Satellite and International Space Station
- Multiple peer-reviewed journal publications and US patents
- This E-Nose technology is licensed by many startups for their applications in health diagnosis (diabetes, flu, cardiovascular disease, various types of cancers, etc.), and environmental monitoring.

APPLICATIONS BEYOND

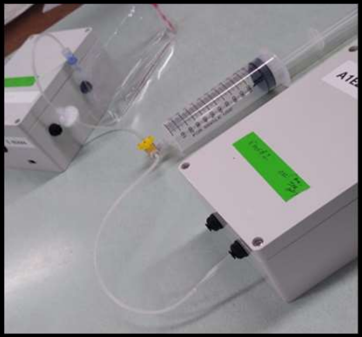
E-nose can be utilized for fire detection, crew health and safety, fuel leak detection, food safety and air quality monitoring.

E-Nose Food Spoilage/Pathogen Detection in BioNutrients System

Spoilage and pathogen detection via E-Nose: successful discrimination between samples demonstrated.

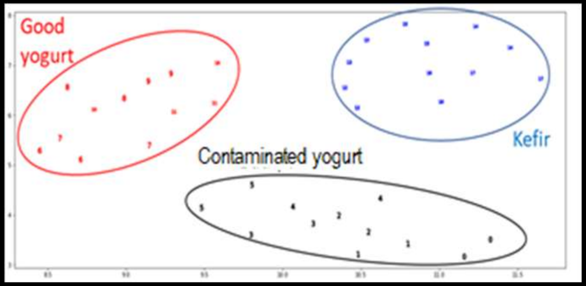
- Three categories of milk products (good yogurt, *Kluyveromyces marxianus* contaminated yogurt, and kefir) were tested.
  Linear support-vector machine (SVM) cross-validation showed 100% identification accuracy.
- A total of 27 samples from four different categories (100% good, 1%, 5%, and 10% contaminated yogurt) were tested to learn E-Nose detection limits in identifying *K. marxianus* contaminated samples.
  - Linear SVM model showed 96% identification accuracy.
  - 37 CFU/ml (1.3 x 10^3 CFU/35 ml of bag) of the intentional contaminant was successfully detected in the 1% contaminated yogurt.

E-Nose ground unit integrated design including pump

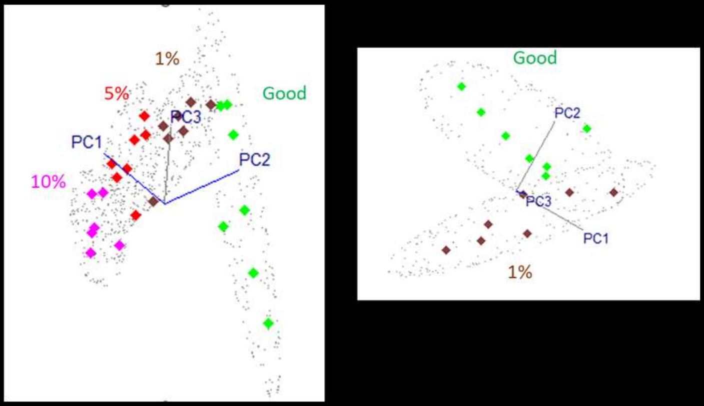


E-Nose Interface design for use with BioNutrients Bag designs

A.



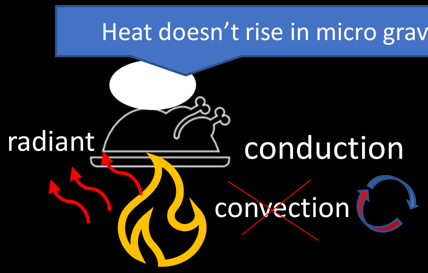
B.



(A) Uniformed Manifold Approximation and Projection (UMAP) plot showing discrimination of three different groups: Good yogurt (red), *K. marxianus* contaminated yogurt (black), and Kefir (blue). (B) Principal Component (PC) analysis 3D scatterplots showing the first three PCs discriminate good yogurt (green) relative to contamined groups: 1% (brown), 5% (red), and 10% (purple).

Potential Methods to Reduce Microbial Load

- **ISS food warming system – potential for low temperature "cooking", pasteurization.**
  - Temperature range is ~145-190°F, top temperature inner chamber ranges 175-190°F (85°C)
  - Heat is transferred by conduction, from the outside in.



Heat doesn't rise in micro gravity

radiant

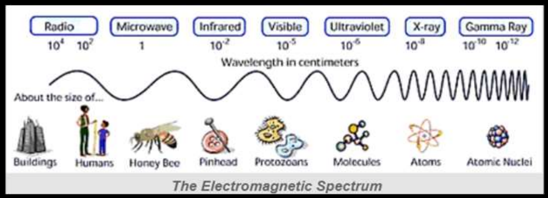
conduction

~~convection~~

- **Zero G oven**
  - Heat via conduction from hot wires to air filled chamber.
  - Not currently on orbit.

Cookie baking test result from Zero G oven, image courtesy of NASA

- **Microwave oven** -Heats via electromagnetic radiation. Wave energy vibrates molecules.
  - Frequencies are very close to Radio Frequencies that interfere with ISS communications.
- **Infra-red technologies** –Heat the surface of objects/food via infrared non- ionizing electromagnetic radiation.
  - May generate radio frequencies that could interfere with ISS systems, less likely than microwaves.
  - In absence of convective forces might result in crispy exterior but raw interior of items.



- **Ohmic** (pulsed electric field)
  - High-intensity pulsed electric field (PEF) processing involves the application of pulses of high voltages (typically 20–80 kV cm −1) of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, sub-ambient, or slightly above ambient temperatures for less than 1 s to foods.
  - Pulses does not overtly heat the product but are thought to rupture to microbial cell walls (electroporation that is unrecoverable).
- **Ethanol production and acid pH as food safety**
  - Although sometimes pasteurized, there are foods and beverages that create enough alcohol to inhibit alternate organism colonization and result in only limited culture viability (beer, wine) or are further purified via distillation (hard alcohols, vinegars).
  - Yogurt cultures lower the pH <4.5 which prohibits the growth of many competing organisms.

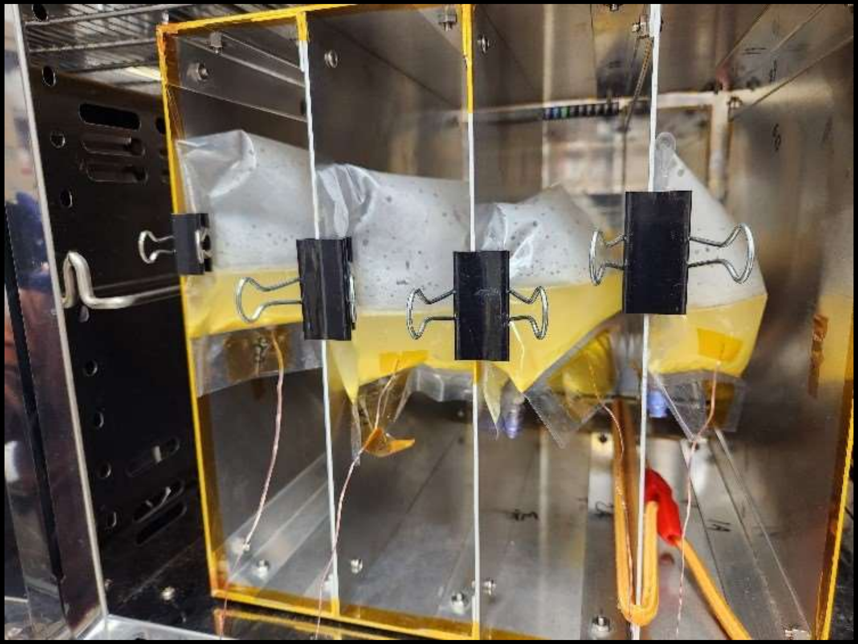
Pasteurization Test Planned for Galley Rack Food Warmer

Pasteurization in mock ISS Foodwarmer

- 4 sample bags prepared per experiment
    - 99.99% inactivation at 1 hour
    - 100% inactivation at 1.5 hours

- Galley Rack Food Warmer ground unit testing now complete, granting higher fidelity results prior to launch.

- Bag integrity testing (pressure testing, handling post pasteurization) is critical to assess risks of implementing on the ISS.

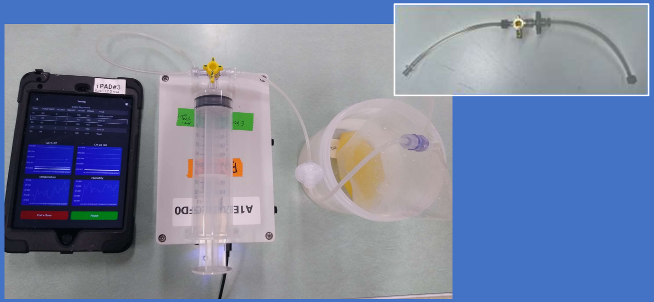


Mock ISS Food warmer – sample bags shown post-pasteurization

(Upcoming) BN-3: developing processes for safe consumption

Improved Products and Designs

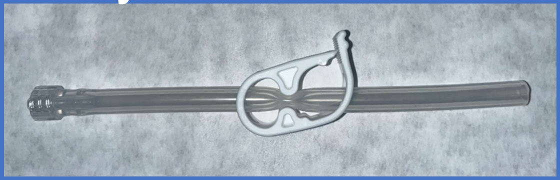
E-Nose Compatible Design



Products

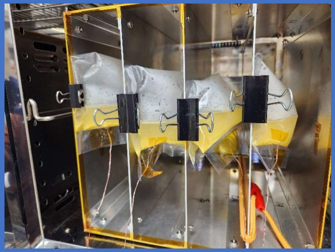
- Yeast: carotenoids
- Yeast: carotenoids, riboflavin, folate (multi-nutrient)
- Yogurt – commercial
- Kefir – commercial
- Serial production – yogurt

Crew access – attachable straw, same pinch closure as current food system.



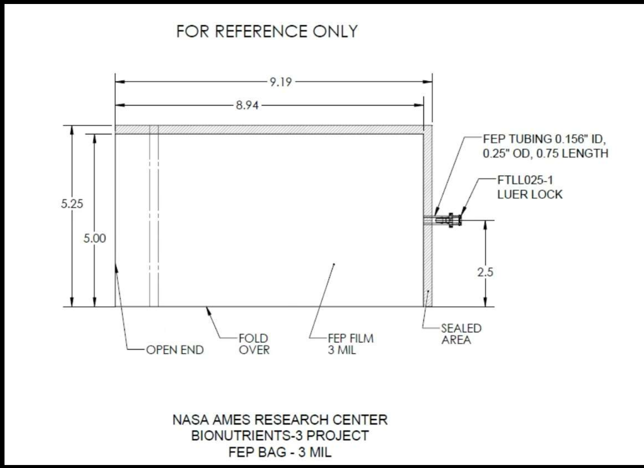
Pasteurization Capable

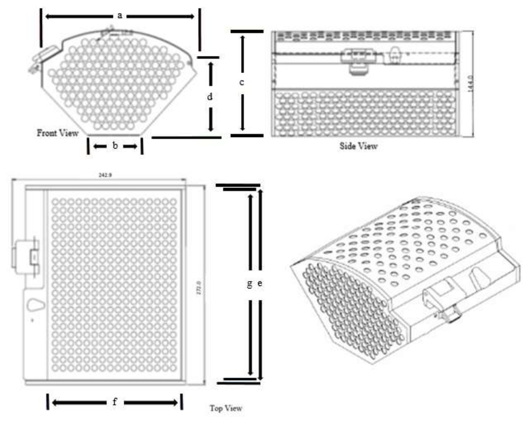

ISS GRFW


ARC test unit

Bioreactor Bag Design Drivers (for BN-3 and for ISS)

- Product volume
  - The various pathogen detection protocols and quantification of nutrients/production yield dictates a larger sample be produced per bag.
- Product yield
  - higher oxygen availability correlates with production in many aerobically growing yeast strains. PDMS has significantly higher gas permeability than FEP.
- Head space volume/gas expansion volume
  - Yeast cultures create CO2 and ethanol as they grow, and this causes the bag to expand.
  - E-nose testing requires creation of a head space and pumping in air to mix and sample from the gas content of the bag.
- Ethanol production limits
  - Production of more than 10mg/day would require a category 1 Volatile Organic Compound Usage Agreements (VUA).
- Multifunctional port
  - Product access for crew, for E-Nose air sampling requires a port that allows open passage (the needle-less septa port must be removable).
- Fit into SABL, MELFI, POLAR
  - To grow and return.





MELFI dimensions

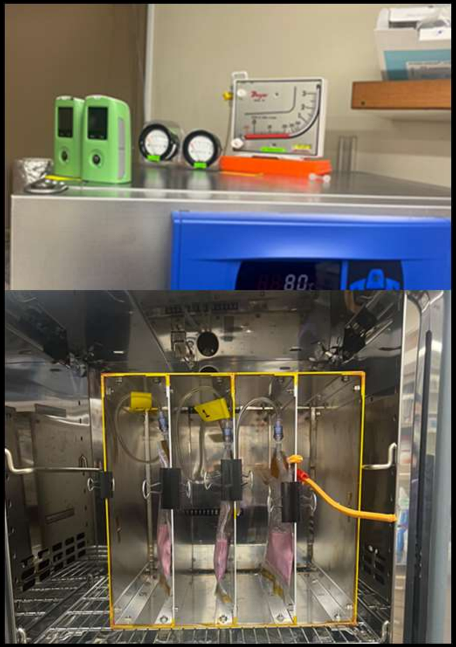
FEP bioreactor Design testing

Objective: to determine the maximum pressure achieved in the FEP bags during the incubation and pasteurization phases of planned flight experiment. (Images below show Kefir experiment.)

Procedure: Autoclave/dry, load with media and organisms, heat-seal, kit, hydrate, incubate, pasteurize, pressure test to 2.5X MDP.

Pre- and post- hydration, hand centrifugation, folding

Incubation



In food warmer mockup for pasteurization.

No loss of bag integrity observed following experiment and maximum design pressure tests, static load test and freeze/thaw testing.

Future Bioreactor Design

- 3D Printing Bioreactors and Membranes
- Recyclable/Reusable materials
- Bio-produced plastics

https://grabcad.com/challenges/3d-printable-bioreactor-for-deep-space-food-production/entries
NASA Astronaut Barry (Butch) Wilmore holds a ratchet wrench created in 2014 with the 3D printer aboard the International Space Station using a design file transmitted from the ground.
International Space Station’s 3-D Printer - NASA

NASA Gen-1 Reusable Recycla...
0 16

3D Printable Bioreactor for ...
0 16

BioPack-X | 3D Printable Bio...

DONUT (Dynamic Oxygen and

Fermentation Considerations

- \+ On-demand, ready in 8-48 hrs
- \+ Shelf-life of organisms
- \+ Shelf-life of media
- \+ Potential for production of wide array of nutrients and therapeutics
- \+ Small volume and mass
- \+ Low resource requirements
- \+ Broad applicability (waste stream remediation, valorization, recyclable)

- \- Bi-product impacts on ECLSS
- \- Genetic changes
- \- Titer uncertainty
- \- Crew health risks
- \- Contamination testing requires development and resources

Potential of Synthetic Biology in Space

Biological systems are:

- Scalable
- Programmable
- Precise (pure isomers)/selective
- The only route of production in some cases (protein therapeutics)
- Low T^o and pressure
- Regenerable

Synthetic Biology Team


Frances Donovan, PhD Project Manager, PI


Natalie Ball


Hiromi Kagawa, Ph.D.


Sandra Vu

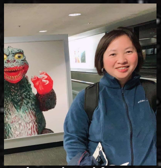

Sadie Downing

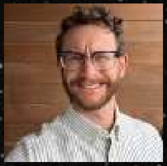

Matthew Paddock


Ami Hannon Ph.D.


Hami Ray, Ph.D., dPM


A. Mark Settles, Ph.D.


Jessica Kong


Philip Sweet, PhD


Candice Tahimic, Ph.D.


Lisa Anderson


Oscar Roque


Alyssa Villanueva

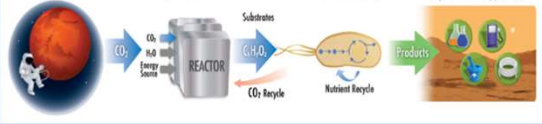

Sean Sharif


Kevin Sims, Payload Manager


Harry Jones, Ph.D. 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