



BioNutrients: Microbial on-demand production of short shelf-life micronutrients in space

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Moon to Mars: Human exploration of deep space will require some resources to be produced during missions

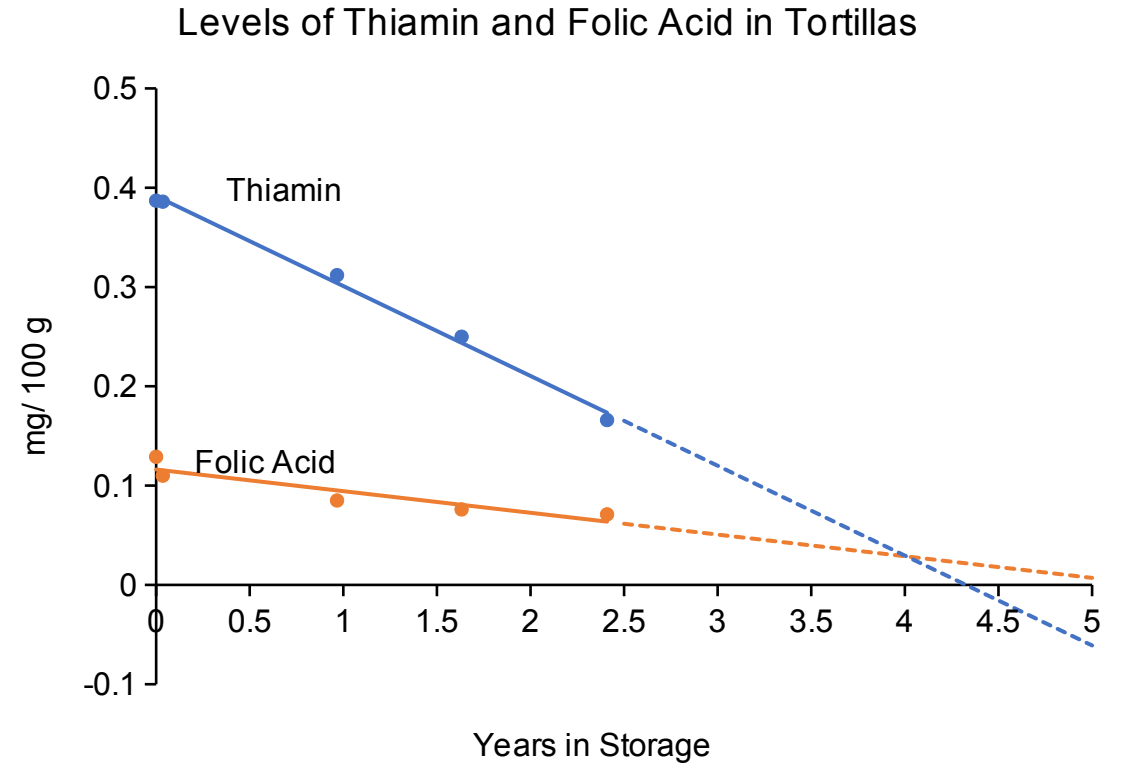


Essential nutrients degrade in long-duration storage

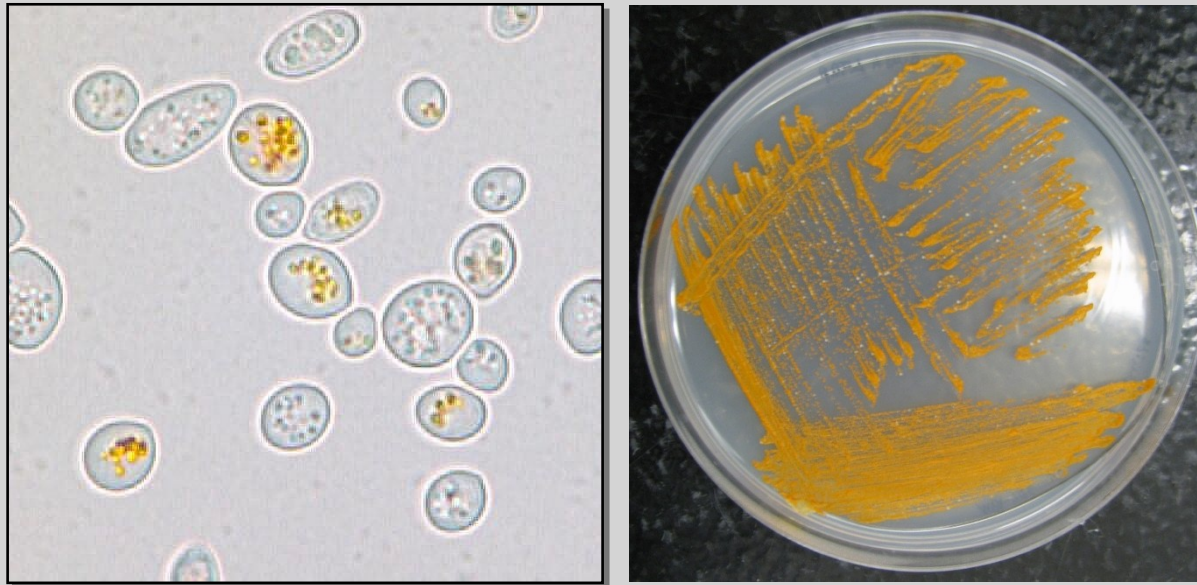
Studies of pre-packaged food in the NASA system have found declines in:

- Folic acid/Folate
- Vitamin A
- Thiamin (Vitamin B₁)
- Riboflavin (Vitamin B₂)
- Vitamin B₆
- Vitamin B₁₂
- Vitamin C
- Vitamin E
- Vitamin K

Need to produce some foods *in situ*



Microbially fermented foods to produce essential nutrients



S. boulardii producing β -carotene

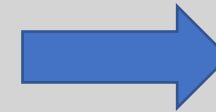
Rationale: Engineer yeast and/or bacteria to produce essential nutrients when grown for a short period of time in edible media.

- Rapid, on-demand production
- Room temperature storage of organisms
- Enriches pre-packaged foods with labile nutrients

Carotenoids selected as initial nutrient:

- Light sensitive and degrades in packaged foods
- Important to protect from macular degeneration
- Antioxidant activity to protect against DNA damage

BioNutrients: Future implementation concept



Premixed dry media and microbes
Ambient temperature storage

Hydrate and dissolve media
Incubate to grow microbes

Deactivate microorganisms
Consume as a supplement



BioNutrients Flight Missions

Mission	Objectives	Launch
BN-1	5-year flight test Engineer yeast strains to produce carotenoids Test media and related packaging Develop Gen-0 bioreactor Test other microorganisms for long-duration storage	NG-11, April 2019
BN-2	<1-year flight test Expand products to yogurt and kefir Engineer medical countermeasure product Develop Gen-1 bioreactor to decrease system mass Develop HACCP food safety plan	Target SPX-25, May 2022
BN-3	Engineer system to produce multiple nutrients Develop Gen-2 bioreactor Demonstrate reliability of HACCP plan	FY24

BioNutrients-1 On-orbit Operations



Clumping Check

Media should be a fine, free-floating powder with no clumps

BioNutrients-1 Effects of Microgravity



Microgravity increases the surface area of the culture media potentially allowing more gas exchange.
Microgravity improves biomass production compared to stationary cultures on the ground.
Active aeration further improves biomass production on the ground.

BioNutrients-1 Stasis Packs

Stasis packs seek to identify species and conditions that will provide 5-year shelf life in ambient temperature storage.

- 9 microbe species investigated
 - Baker's yeast and probiotics
 - Species for yogurt
 - Species that can metabolize diverse carbon sources
- 21 treatments
 - vary storage preparation
 - mutagenize for genetic screens
- 3 media formulations

Compare ground control to ISS storage

Assay for viability at 3- to 6-month intervals over 5 years.

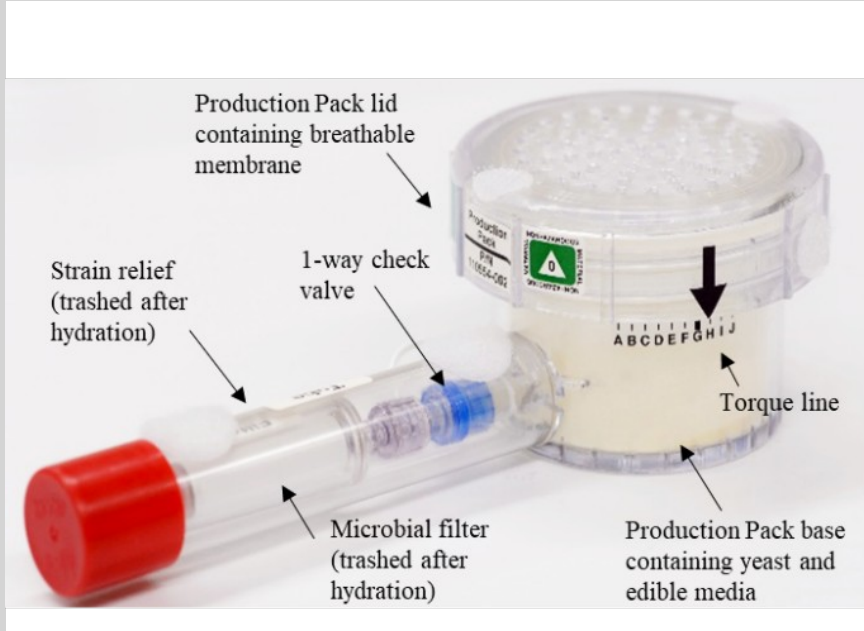
Variation in viability observed by 2 years of storage.

Santa Maria et al (2020) found yeast to be stable for 2 years at room temperature storage.

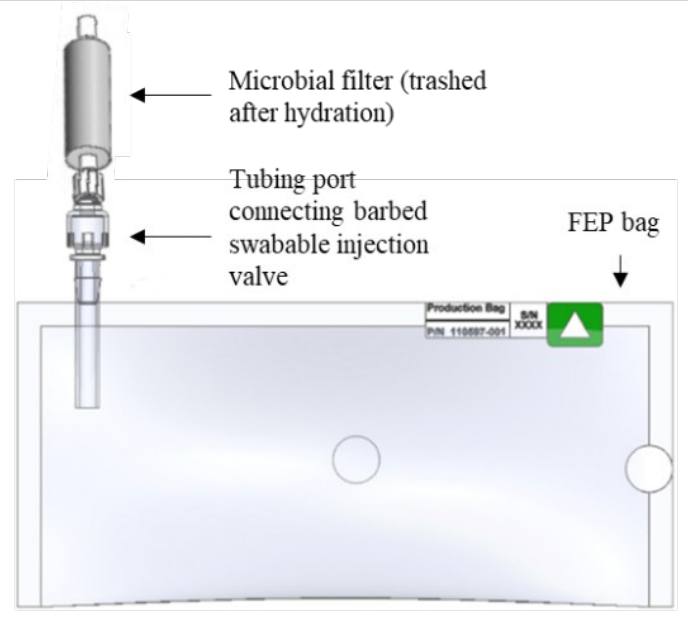
Santa Maria et al (2020) Astrobiology. <https://doi.org/10.1089/ast.2019.2073>



BioNutrients-2 Reduced Mass Hardware



117 g Bioreactor



10 g Bioreactor

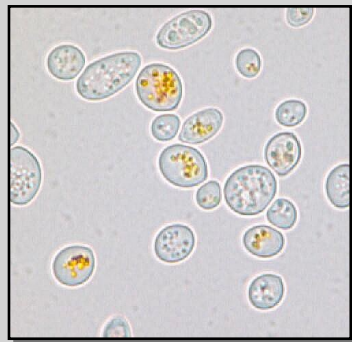
Change to a Fluorinated Ethylene Propylene (FEP) bag bioreactor.

FEP is gas permeable.

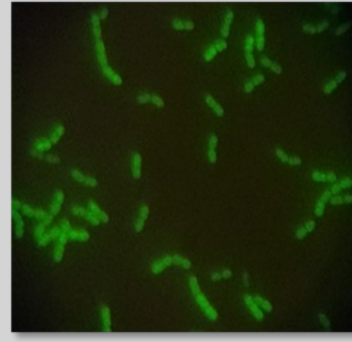


BioNutrients-2 Products

Microbe	GM Product	Purpose	Food Product
<i>Saccharomyces cerevisiae</i>	Zeaxanthin	Prevent macular degeneration	Nutrient broth
<i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>	-carotene	Vitamin A precursor	Nutrient broth
<i>Kluyveromyces lactis</i>	Follistatin	Promote muscle formation to counteract microgravity effects	Nutrient broth
<i>Streptococcus thermophilus</i>	Green Florescent Protein	Demonstrate genetic engineering of species	Yogurt
YO-MIX 151 <i>Streptococcus thermophilus</i> <i>Lactobacillus bulgaricus</i>	None	Increase menu options	Yogurt
C-FIR – proprietary mix	None	Increase menu options	Kefir



S. boulardii
(β -carotene)



S. thermophilus
(GFP)



S. thermophilus and
L. bulgaricus



BioNutrients-2



Bag bioreactors are loaded with sterile media/milk powder and microbe pellets.

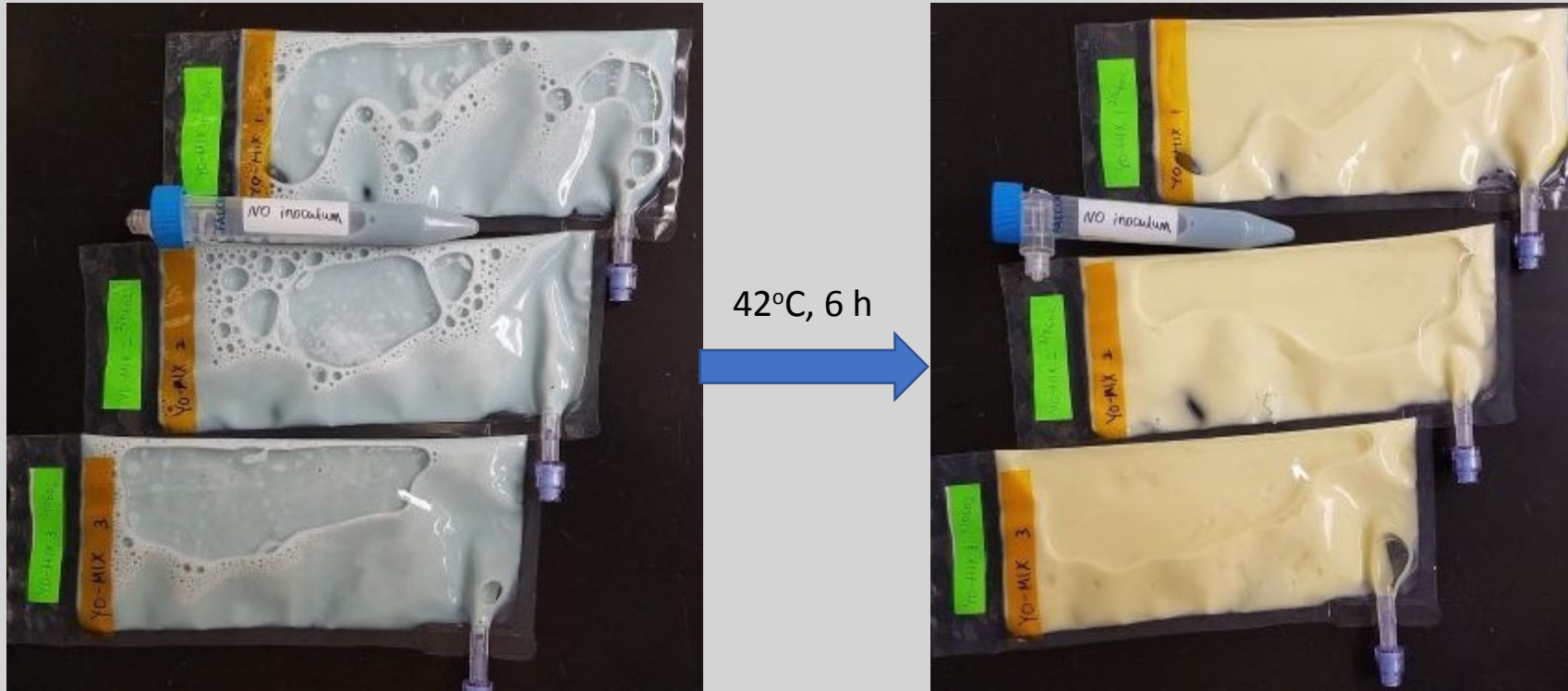
Luer lock port is used to attach a sterile water filter for hydrating the culture.

Carotenoids are produced similarly to BioNutrients-1 with a 48 hour incubation at 30°C.



BioNutrients-2 Products

Yogurt and kefir are produced by lactic acid fermentation.
pH should be <5 when the product is ready.
Will be monitored with bromocresol purple on-orbit





Summary

Fermented foods provide an additional strategy to produce essential nutrients for extended shelf life

- Microbes can be engineered to produce multiple nutrients or countermeasures
- Media and microbes need to be shelf stable for 5 years
- Growth cycles are short, on the order of a few days instead of weeks to months

Development needed for full implementation

- More comprehensive microbe engineering
- Continue to improve flight hardware
- Engineering food safety process controls in space: cooking or other methods

Space Synthetic Biology



BioNutrients Authors

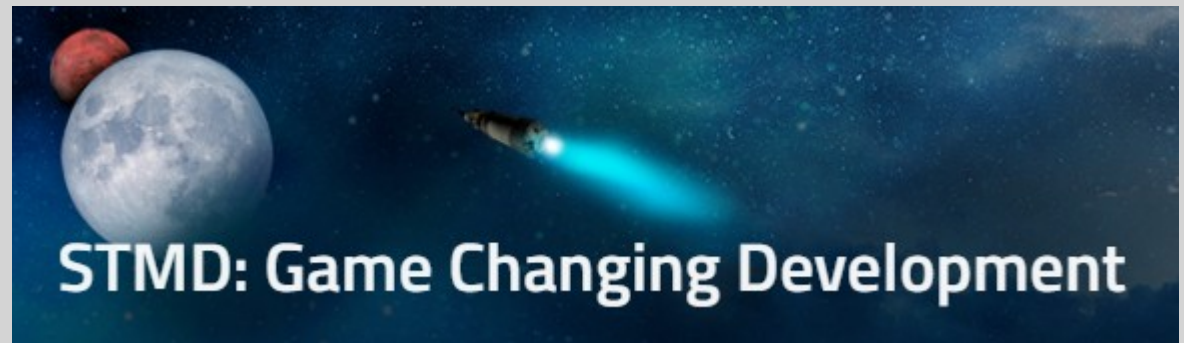
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STMD: Game Changing Development