National Aeronautics and Space Administration

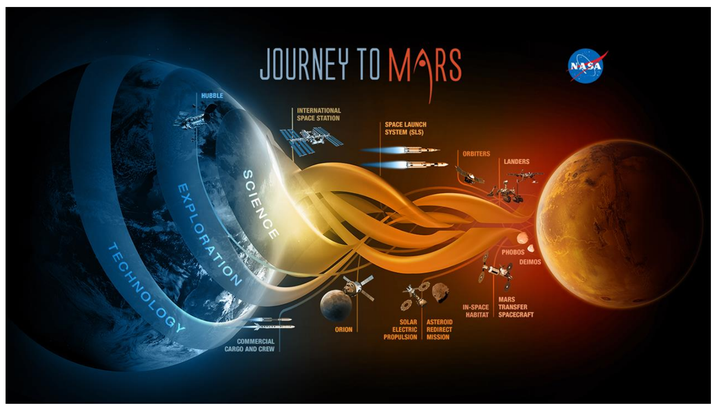
Enhanced Polyhydroxybutyrate Production For Long-Term Spaceflight Applications

Ryan J. Putman[1], Asif Rahman[2], Charles D. Miller[1], Masood Z. Hadi[2]

[1]Department of Biological Engineering, Utah State University, Logan, UT
[2]Synthetic Biology Program, Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA

Introduction

Long duration spaceflight missions present many new challenges and payload limitations. Maintaining and utilizing finite resources during long-term spaceflight will require cyclical biological systems to both reduce and recycle materials. Biologically made plastics can be a valuable biomaterial to use during long-term space missions (e.g. Mars, lunar outpost). The aim of this project is to optimize the production and recovery of bioplastic from a genetically modified *Escherichia coli* (*E. coli*) bacterium. Improving total bioplastic production and purification procedures will enable less mass to be launched as payload aboard manned space missions, therefore reducing costs and leaving room for additional cargo pertinent to mission success. Using this biomaterial as a building block, the digital schematics for human habitats, replacement parts, or new tools can be sent directly to astronauts to be 3D printed on-demand. For this project, *E. coli* was cultured in 5L batch fermentations with focus on optimizing parameters that affect bioplastic production such as fermentation time, dissolved oxygen content, as well as the quality and quantity of nutrients in the aqueous growth media. Lastly, by defining the minimal media components necessary for adequate production of bioplastic, a good approximation of the effectiveness of growing *E. coli* using *in situ* resource utilization on Mars or the Moon can be determined.



The advantages of microbial bioplastic production will help to accelerate NASA's mission of transitioning from "Earth reliant" to "Earth independent" (figure from nasa.gov).

Experimental process

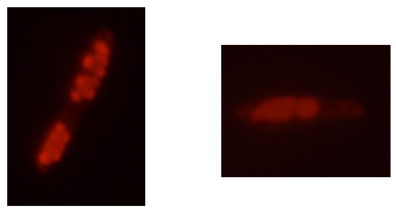
Fermentations were carried out at constant temperature (37° C), pH maintained at 7.0, dissolved oxygen cascade set to 40%, and were under antifoam control. All runs were batch fermentations carried out for 24-48 total hours.

Biomass growth *Centrifugation* *Drying* *Extraction* *Application*

Cell pellets were lyophilized and small aliquots were weighed for extraction. Chloroform was used to separate bioplastic from the biomass, which was subsequently analyzed using gas chromatography-mass spectrometry (GC-MS) to determine the % PHB per dry cell mass. The remaining dried biomass can be processed in a larger reaction. Resulting bioplastic is then extruded into filaments that can be fed into a 3D printer to create useful tools or products (see test tube holder, far right above).

Analysis

Cells were sampled at different time points throughout the fermentation process and stained with Nile red to visually confirm the accumulation of polyhydroxybutyrates within the cell. 96-well microtiter plate fluorescence studies will be used for rapid quantification of intracellular PHB production. Selected pictures below show individual *E. coli* cells with distinct bioplastic granules (1000x magnification).

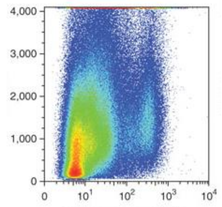


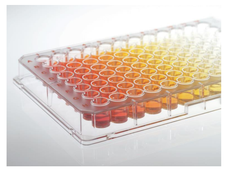
Nile red is a lipophilic stain which can be used to both qualitatively and quantitatively determine bioplastic accumulation.

Future work

Samples from all eight 5L fermentation runs are currently being analyzed for % PHB content. Once complete, the 100+ grams of dry cell mass will be processed. Other goals include:

- Total sugar analysis to determine if system is carbon limited
- 3D print biologically made bioplastic
- Further optimize fermentation parameters and nutrient additions for greater bioplastic production
- Use fluorescence-activated cell sorting to select for higher PHB producers





www.nasa.gov