

Day 5 - Waste Management

Project: iGEM 2018

Authors: Jessica Laury

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Unlike on Earth, landfills and wasting anything is not a good choice for long term space travel. We will likely be creating a closed system, which means our waste management can't just detoxify and hide away our waste, it needs to turn it into usable tools, materials or fuel.

We will discuss the recycling of water and gas waste in later days. We are going to be looking specifically at stool and urine recycling here.

URINE

The goal is not just to convert waste into safe fertilizer, but also to use it as material for 3D printing of supplies.

Screen Shot 2018-07-25 at 2.48.53 PM.png

Synthetic Biology for Recycling Human Waste into Food, Nutraceuticals, and Materials: Closing the Loop for Long-Term Space Travel

PI: Mark Blenner, Ph.D.
Assistant Professor
Chemical & Biomolecular Engineering
Clemson University

Research Objectives

- Goal: Convert human waste into omega-3 fats and plastic for 3-D printing.
- Innovation: Yeast are a flexible platform for converting algae captured CO₂ and human waste into food, nutraceuticals, and materials.
- SOA: We have engineered several metabolic engineering tools for *Y. lipolytica*.
- Transition from TRL1 (preliminary pathway engineering) to TRL3 (highly productive systems using waste substrates).

In-situ resource utilization of human waste to make food and 3D printable materials

Approach

- Fundamental investigation of algal biomass and urine as a feedstock for yeast.
- Forward and reverse systems engineering for tolerance to feedstock inhibitors.
- Metabolic engineering of omega-3 from lipids.
- Metabolic engineering of PHA from lipids.

Potential Impact

- Recycling of human waste for in-situ resource utilization may reduce the materials needed to sustain long-term space travel.
- A flexible yeast platform may enable the production of other nutrients, therapeutics, and materials during space travel.
- In the process of utilizing waste, water can be recycled as well.
- Waste utilization can positively impact terrestrial processes as well.

<https://www.nasa.gov/feature/synthetic-biology-for-recycling-human-waste-into-nutraceuticals-and-materials-closing-the-loop-for-long-term-space-travel>

Mark Blenner's team at NASA has bioengineered a yeast which uses the nitrogen from urine and the carbon from astronaut's respiration to create plastic. The yeast is *Yarrowia lipolytica* creates both polyester polymers for 3D printing of plastic and omega-3 fatty acids. We have sequenced the genome of this yeast and there is a one-step integration of multiple genes method which is established for the engineering of Blenner's yeast. We aren't using CRISPR-Cas9 with this organism yet, but we are working on it. We have engineered the yeast to work with many carbon sources and nitrogen sources, when we create a pathway that successfully creates are target product in a species we can increase the production rate to an extent by modifying the promoters we use. It is important to note that Blenner's process is strictly aerobic. Additionally, the sugar needed for feeding the yeast would need to be created using cyanobacteria.

Once they are thriving they can be used to create fats needed in astronauts diet, or plastics for building materials (requires more processing).

Another strain of yeast - *Candida tropicalis* has been worked on at New York University with Richard Gross. His team edited the yeast's genome, removing 16 non essential genes, and inserting another, the yeast now makes omega-hydroxy fatty acids. These monomers are then put together into poly(omega-hydroxy fatty acids) that behave like polyethylene (think thin plastics). This production method can be upscaled to work at commercially viable volumes. Together we can make plastic and fats out of waste.

<https://www.deccanchronicle.com/science/science/250817/going-for-a-pee-it-could-make-plastic-for-astronauts.html>

<https://www.sciencedirect.com/science/article/pii/S0163782715300205>

<https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-016-0492-3>

<https://www.hindawi.com/journals/tswj/2014/476207/> **look at 2.4.1 lipid synthesis**

<http://formatex.info/microbiology2/930-944.pdf>

<https://dailytexanonline.com/2018/02/27/ut-chemical-engineers-find-that-yeast-provides-way-to-produce-sustainable-plastics> **Plastics**

<https://www.polymersolutions.com/blog/yeasty-plastics-polymeric-monomers-from-yeast/> **Better plastics**

STOOL

Using the same visual from above as a launch point, but moving to University of Calgary iGEM teams 2017 project

The team engineered E. Coli to turn human stool into bioplastics. Human waste has both glucose and volatile fatty acids which can be used as feedstock for PHB (poly (3-hydroxybutyrate)). The team increased the efficiency of PHB production and engineered the E. coli secretion pathway so the cells would release the PHB they produced. The process begins with waste collection and fermentation *the fermentation increases the concentration of VFAs and is done with naturally occurring enterogenic bacteria. Solids from the waste settles to the base and liquids rise in the fermentation tank. The liquid is separated from the solids by centrifugation (should work on Mars no issue) and sterilized via filtration. The resulting liquid is passed through a bioreactor containing the engineered E. coli, from which the PHB is continuously collected and extracted from the liquid stream. The resulting liquid can be recycled into drinking water while the PHB can be used in a 3D printer. Solid waste can be used as substrate for growing plants.

<https://polymer-additives.specialchem.com/news/industry-news/bioplastic-from-human-waste-space-missions-000212953> **iGEM team**

ACTIVITIES

K-5

Talk about composting - you can compost in class. Talk about the needs of bacteria and how to create a "special soup" that meets the needs of your bacteria. A large pot with felt cut outs of nutrients (or symbols for those nutrients) can be fun, students can take turns adding things to the pot as you discuss the needs of your cells.

Yeast will need - Oxygen (for this set of yeast yes we do) a carbon source, water, nitrogen, potassium, phosphate, magnesium, calcium a pinch of the rest; iron, zinc copper, manganese and molybdenum

Bacteria will need - a carbon source, oxygen, nitrogen, hydrogen, phosphorus, sulfur, potassium, magnesium, calcium and iron

Mix well!

6-8

Draw a theoretical waste purification system. What areas need to be anaerobic/aerobic what bacteria would you need, what resources? How would you know if your system got sick? How could you recover from a system that is "sick". Can you build a model of the proposed system?

What nutrients are in stool and urine? What bacteria could use those nutrients? What components of urine/stool might be dangerous to our bacteria? How could we edit them, or use experimental/directed evolution to make them more resilient to these threats?

9-12

composing....making the igem 2017 system (using a waste analog)

Or turning bacteria into plastics, have it break down a urine/stool analog and then be strained and turned into plastic. Would take time. Much time would be required. We could have them create the system and not turn anything into plastics/edible products but they could sustain the bacteria off of urine/stool replacements.

<https://www.scientificamerican.com/article/turning-bacteria-into-plastic-factories-replacing-fossil-fuels/>

Let them separate and purify the stool analog product. How many kinds of edited bacteria might we need to remove toxins, filter out safe water and then utilize product? How will you co-culture this?

This would be a good chance to talk about the nutrient requirements for our two systems yeast and bacteria. What happens if we don't give them something they need? What if we miss up the balance of the system? If there is too much Urea, not enough carbon. Sometimes it feels like bacteria are so hearty they will grow no matter what we do to them, but especially if we want to keep them operating at peak production we have to work to keep them happy.