Harnessing Synthetic Communities and Microbial Recycling of Space Waste Streams for Biomanufacturing Applications

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Introduction

• Long-term spaceflight and habitation of the Moon or Mars require solutions to mitigate the risks and costs for launch and/or resupply.
•In-situ resource utilization (ISRU) via recycling waste into biomanufactured products (food, pharmaceuticals, biofuels, etc.) and bioregenerative life support systems as a particular modality of ISRU offer potential solutions.
• **Research Aims**
1. Formulate an optimal wastewater media on which to grow recombinant microbes for bioproduction in space. Specifically, to create a synthetic wastewater media using synthetic urine (SU) and wastewater processor effluent (AnMBR), combined with a C1/C2 carbon source mix (EtOH/MeOH) and trace minerals/vitamins.
2. Explore stable, metabolically synergistic synthetic microbial communities for large-scale and multi-tiered biomanufacturing. Design and evaluate three synthetic microbial communities grown on an optimal wastewater formulation for stability and metabolic cooperation through pairwise cultures.
3. Measure recombinant enzyme production levels in microbes grown on optimized wastewater media. To this aim: measured recombinant enzyme production using His-Tag ELISA assays, comparing optimized wastewater media with more traditional media.

• **Findings**:
- An optimal wastewater media of (¾ 1xSU - ¼ AnMBR) supports diverse microbial growth and balances key growth metrics.
- Promising stability and synergy observed in *Komagataella phaffii* and *Escherichia coli* co-cultures.
- High recombinant enzyme production sustained in microbes grown on optimized wastewater media.

Materials and Methods

I) Toward an Optimal Wastewater Media

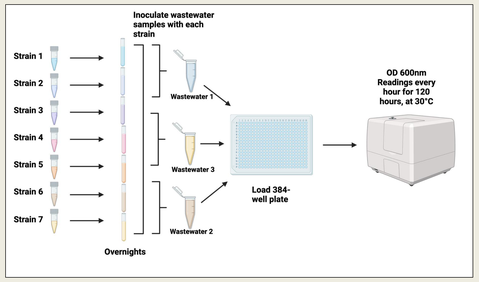

Figure 1: Optimal Wastewater Experimental Setup. Control: 2X YNB without amino acids (pH 5.4). SU formulated according to (Verostco et al., 2004), AnMBR sourced from collaborators at KSC and USF (Bullard et al., 2021; Smith et al., 2022). Carbon sources: 74% EtOH/26% MeOH solution (2% of total volume). Additives: 100X trace mineral, 100x vitamin solution. 384-well plates: 50uL per each well. run at 30°C, OD 600nm reads every hour for 120 hours. Key growth metrics extracted include max OD, doubling time, and lag time.

II) Synthetic Communities on an Optimal Wastewater Media

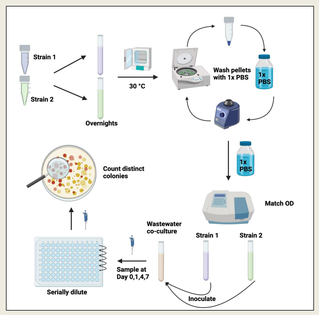

Figure 2: Synthetic Community Experimental Setup. Pairwise competitions involved co-culturing bacteria and yeast on an optimal wastewater media in triplicate over 7 days, with strain ratios assessed at intervals (0, 1, 4, and 7 days) by serial dilution, plating on LB agar, and colony counting, relying on distinct colony morphologies for differentiation.

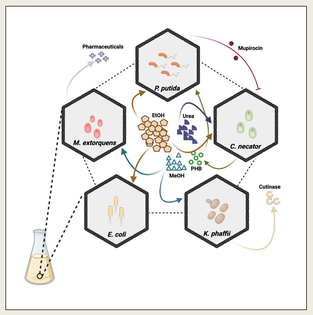

Figure 3: Conceptual Synthetic Community. Depicts conceptual metabolic synergism. Advantages include stability (population consistency, metabolic niches minimized competition, no toxic byproducts), resistance to contamination, and multi-tiered biomanufacturing (distributed metabolic burden, higher production potential).

III) Production of Recombinant Cutinase on an Optimal Wastewater Media

A His-tag ELISA, using a modified Cayman kit protocol, was performed to detect cutinase secretion from *Komagataella phaffii* cultured in optimized wastewater media compared to more conventional media. The assay required no sample lysis, as cutinase was secreted directly into the supernatant, and involved serial dilutions, antibody binding, and colorimetric detection, with absorbance measured at 405–420 nm to quantify cutinase levels.

Results

I) Toward an Optimal Wastewater Media

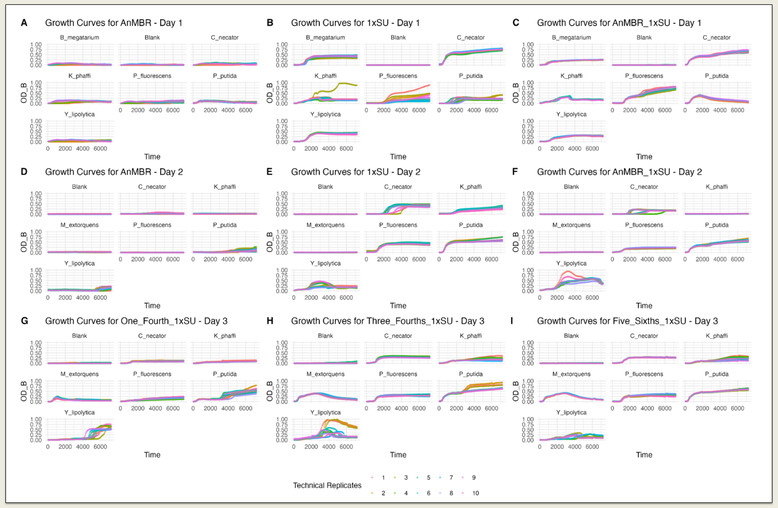

Figure 4: Growth Curves of Several Microbial Strains on Various Wastewater Media
Each subplot depicts the average optical density (OD) readings at 600nm of ten technical replicates (represented by the multi-colored lines) each of six distinct microbial strains growing in varying wastewater formulations and one blank, within a standard 384-well microplate. Microplates were incubated for a period of 120 hours, generating the observed microbial growth curves.

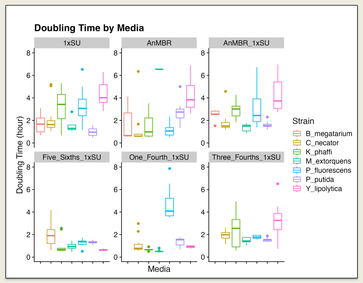

Figure 5: Average Doubling Time by Media
Each subplot depicts the average doubling time, in hours, across ten technical replicates of either six or seven distinct microbial strains in varying wastewater formulations. Doubling time refers to the time it takes for a microbial population to double in size or value.

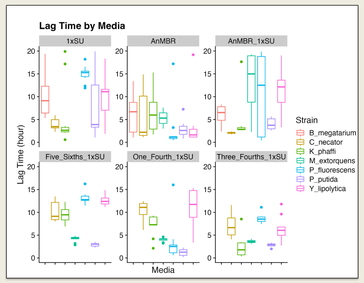

Figure 6: Average Lag Time by Media
Each subplot depicts the average lag time, in hours, across ten technical replicates of either six or seven distinct microbial strains (distinguished by color) in varying wastewater formulations. Lag time, or lag phase, refers to the period of time in which a microbe adjusts to a new environment before entering an exponential growth phase.

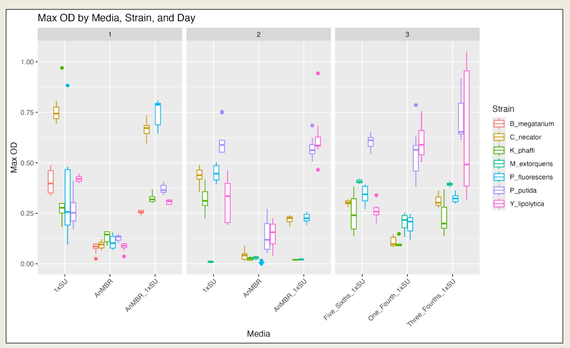

Figure 7: Max OD of all Strains on Various Wastewater Media
A comprehensive plot of the average maximum optical density (OD) measurement across ten technical replicates of seven microbial strains in varying wastewater formulations. Maximum OD, here, refers to the highest or final OD reading over the entire 120-hour microplate incubation period as a measure of growth potential.

II) Synthetic Communities on an Optimal Wastewater Media

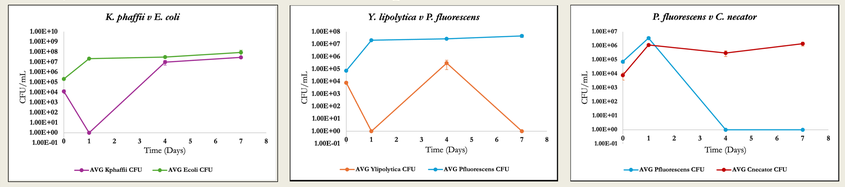

Figure 8: Microbial Communities on an Optimized Wastewater Media
Each log-scale subplot depicts the change in the number of colony-forming units (CFUs) of all strains within the context of pairwise competitions unbuffered in an optimized wastewater media over a 7-day time period. Each point represents the average CFU/mL count of all biological replicates (n=3) for that competitor within the co-culture at that given time point (0, 1, 4, 7 days). Error bars represent standard deviation. Across all competitions, values that appear as zero CFU/mL indicate that strain was not detectable at the plated dilutions, not that strain is no longer present in the co-culture entirely. Refeeding with EtOH/MeOH mixture occurs on Day 4, post-plating.

III) Production of Recombinant Cutinase on an Optimal Wastewater Media

Sample	[Recombinant Cutinase] (ug/mL)	R^2
BMGY 1:100	43.8	0.997
BMGY 1:1000	52	
2x YNB 1:10 (2% EtOH/MeOH)	54.87	0.998
2x YNB 1:100 (2% EtOH/MeOH)	57	
¼ AnMBR - ¾ 1xSU 1:10 (2% EtOH/MeOH)	35.26	
¼ AnMBR - ¾ 1xSU 1:100 (2% EtOH/MeOH)	37	

Figure 9: Summary of His-Tag Recombinant Cutinase Assays
A strain of *K. phaffii*, optimized for recombinant cutinase production was grown on three distinct media (BMGY, 2x YNB, ¼ AnMBR - ¾ 1xSU 1:10). Cultures were spun down and supernatants containing free-floating recombinant cutinase were subjected to a modified Cayman His-Tag ELISA. Dilution-corrected concentrations of ug of recombinant cutinase per mL of media are presented. Data in Figure 8 indicates that *K. phaffii* cultured on an optimal wastewater media (¼ AnMBR - ¾ 1xSU) not only produces recombinant cutinase but does so at levels comparable to those of a generic yeast minimal media (2x YNB) and a specialized yeast media (BMYG).

Summary

I) Optimal Wastewater Media
•Synthetic urine (1xSU) supports better microbial growth than AnMBR alone.
•A mixed wastewater medium (¾ 1xSU - ¼ AnMBR) provides optimal growth, with shorter lag/doubling times and higher optical densities.
•Microbial growth varies across media, suggesting the potential for tailored formulations for specific strains.
II) Synthetic Communities on an Optimal Wastewater Media
•*P. fluorescens* outcompetes *Y. lipolytica*, while *C. necator* outcompetes *P. fluorescens* (likely due to predatory behavior).
•A stable co-culture of *K. phaffii* and *E. coli* was established on the optimal wastewater medium, offering the potential for biomanufacturing without engineered metabolic interdependence.
III) Production of Recombinant Cutinase on an Optimal Wastewater Media
•His-Tag ELISA confirmed recombinant cutinase production in *K. phaffii* cultures
•Supports the potential for using waste streams (1xSU and AnMBR) for bioproduction in space environments.

Attributions

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NASA Space Biology Program: https://science.nasa.gov/biological-physical/programs/space-biology
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Smith, A., Bullard, T., Seatta, D., Hoque, B., Devito, C., Haarmann, K., Bair, R., Yeh, D., Long, P., Fehrenbach, M., Fischer, J., & Roberson, L. (2022). Management of fecal waste utilizing a hybrid organic processor assembly unit designed for resource recovery. Paper presented at the 51st International Conference on Environmental Systems, St. Paul, MN. https://ttu-ir.tdl.org/server/api/core/bitstreams/5bef30bb-a672-4b4b-afcf-5bf3399db0b6/content
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