U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

Microbial Reactors - Indigenous Feed Stocks to Functional Materials

Katherine Germane, Ph.D.

Team Lead, SBME ARAP Technical Lead

Biotechnology Branch

August 23, 2018

ARMY CHALLENGE

To win in the deep future operational environment, the Army will need to be more adaptive and with less logistic demand.

- Cost/benefit specialty/commodity materials is different than commercial sector
- Synthesis from indigenous resources/waste streams enables:
  - cost reduction
  - minimize materials burden
  - Benefits from waste remediation
- Requires development of biological systems and production pathways
  - robust in Army environments.
  - Process complex inputs
  - High value products/precise hierarchal materials

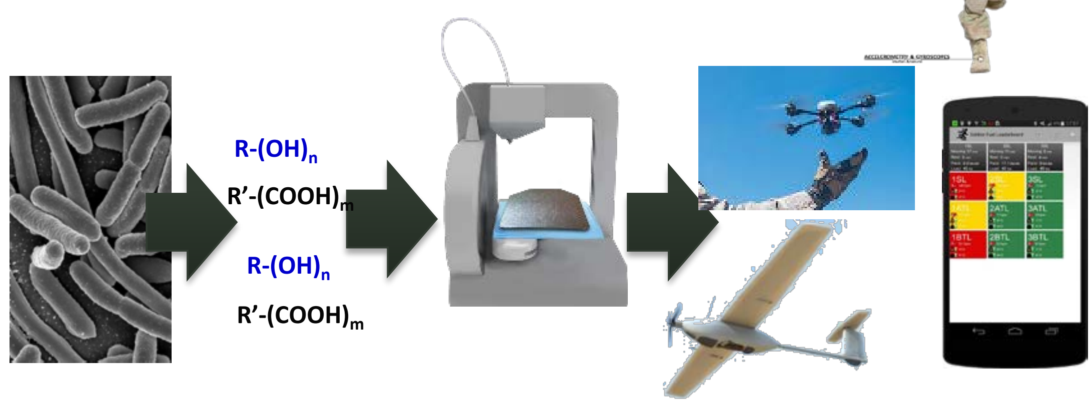
1. **A component of ARL Essential Research Programs: Tactical unit energy independence, Manufacturing at the point of need, Discovery.**
2. **Enables CSA Priority #6 Soldier Lethality: Expeditionary Soldier Power Generation**

TECHNOLOGIES FOR FUTURE DISMOUNTED SOLDIER

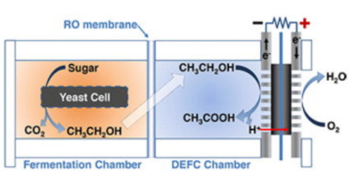
Cumulative, Connective, Converging w/ Outcomes

Soldier System Interfaces & Integration / Sensored Soldier / Expeditionary Power Generation: Self-Sustaining Autonomous System

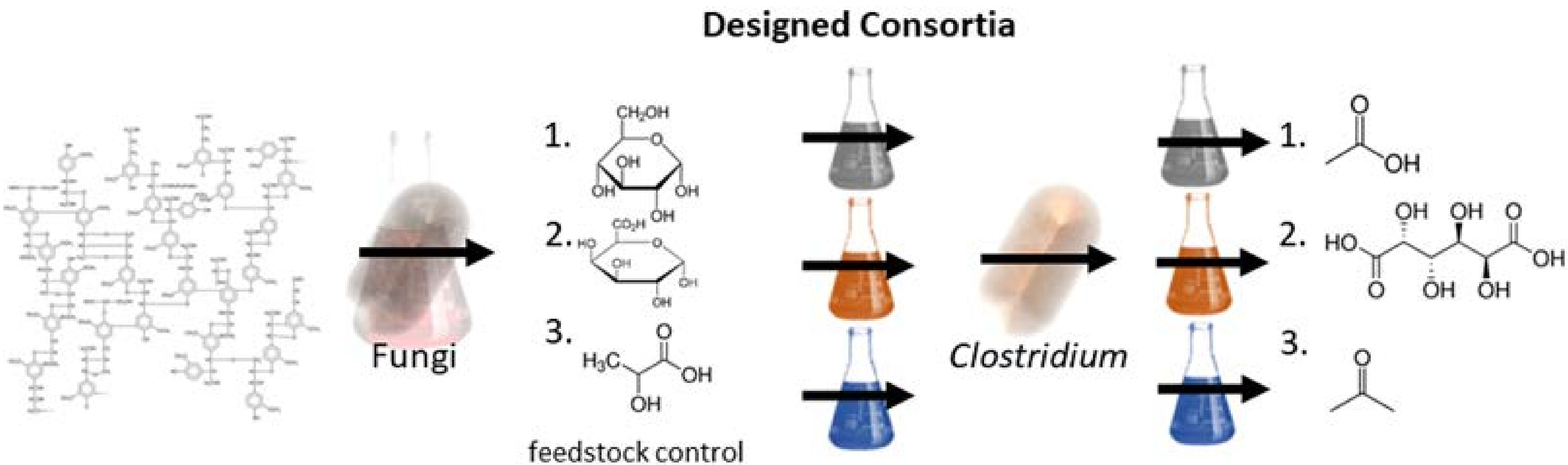
Engineered Organism: Flexible feedstocks to controlled reactant output for advanced polymer systems and sensor reagents



Selected Consortia Bioreactor: Conversion of indigenous materials/ wastes for energy generation at expeditionary scale



Technical Challenge



Microbial consortia synergistic production of materials and remediation

Organics, Biosensor Reagents, Probiotics, Enzymes / Catalysts, Adhesives, Polymer Precursors Energetics Precursors, Solvents

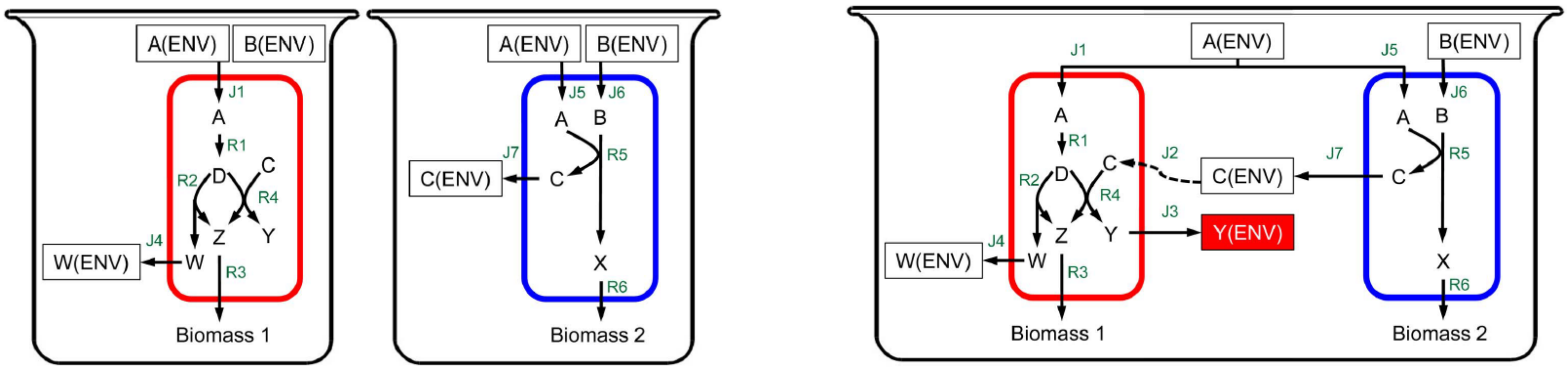
Nano Particles Rare earths reclamation

- **Develop production pathways :**
  - **robust in Army environments**
  - **reconfigurable**

From Monocultures to Consortia

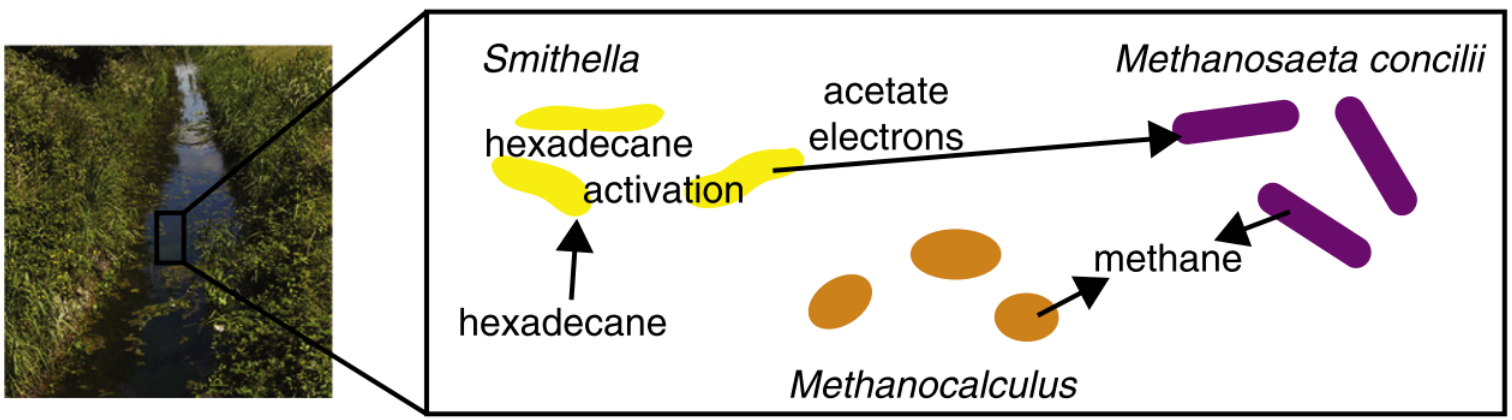
Hypothesis: Microbial consortia will outperform monocultures for waste and indigenous resource conversion to commodity/specialty chemicals

Theory:



Chiu s *et al. PLoS Comput Biol*. 2014.

Nature:



Hays *et al. Current Opin Biotechnol*. 2015.

Bottom-up Assembly of Consortia

Food waste

Indigenous resources (e.g. grass)

Commodity / Specialty Chemicals

- Leverage advancements in human microbiome research in new context.
- Plethora of genome-scale metabolic models
- Transitions to Natick Soldier Research and Development Center
- Partnership with John La Scala (WMRD)

- Leverage rumen microbiome that degrade lignocellulose
- Leverage past ARL research on *Clostridium acetobutylicum*
- Untapped potential, particularly lignocellulolytic fungi – collaboration with Michelle O'Malley at UCSB

Non-homogenous food waste → Tunable outputs

Key questions:
Hundreds of species, so how do we choose members for consortia?
Do combinations of microbes outperform monocultures?

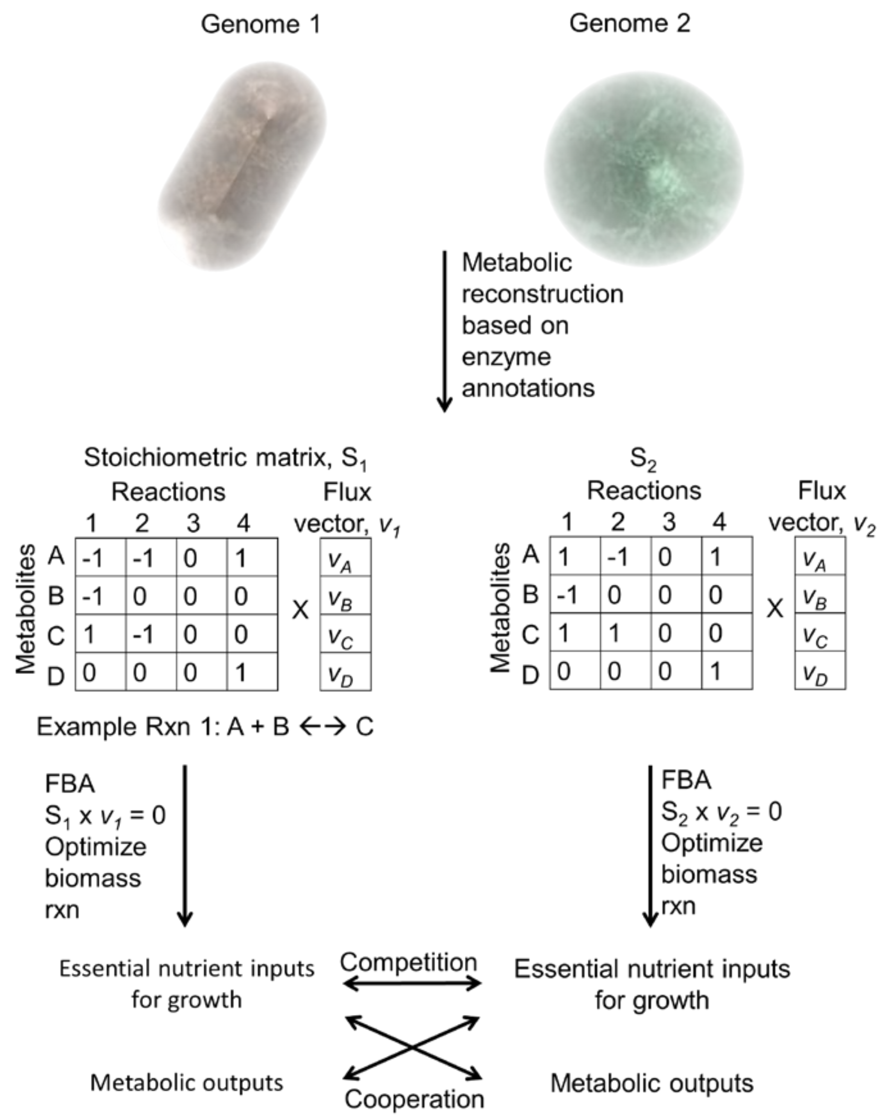
Approach: Flux balance analysis (FBA) of genome-scale metabolic models

Genome-scale Metabolic Modeling

- **Modeling predicts potential of microbes to cooperate/compete**
- **Plethora of available models**:
  - Assembly of gut organism
    - reconstruction and analysis (AGORA)
  - **773 microbes**
  - Includes 205 genera and 605 species
  - Simulate steady-state growth on Western diet

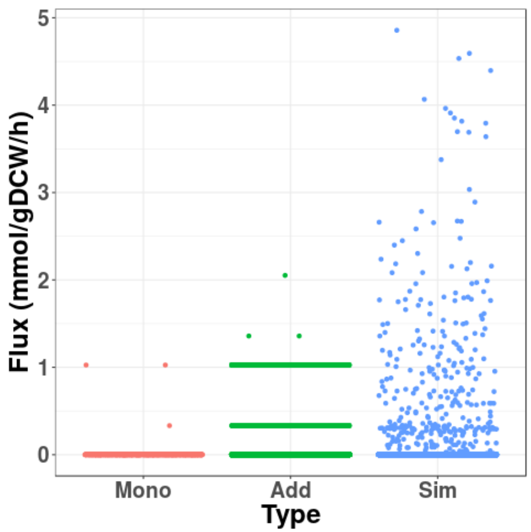
Magnusdottir *et al. Nat Biotechnol*. 2016.

- **Simulated monocultures and all co-cultures**
  - 298,378 co-cultures
  - Computation time = 7 days
  - Would be a major experimental challenge
  - 96 well plate format – 3,109 plates
  - With automation ~ 50 plates/day
  - 62 days to setup
  - Metabolite analysis: 10 min/sample
  - ~ 5.5 years total

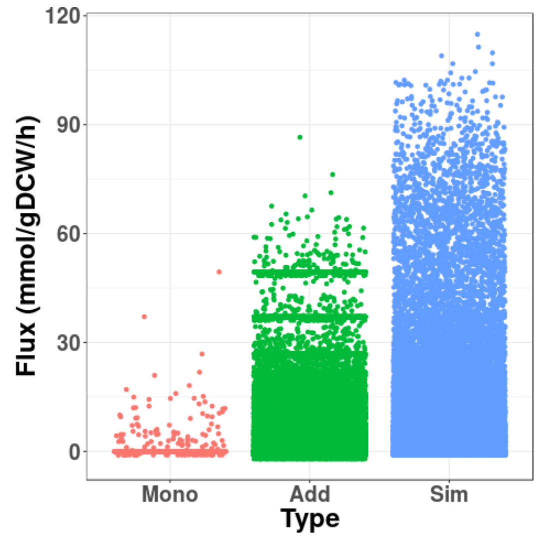


Co-cultures Outperform Monocultures

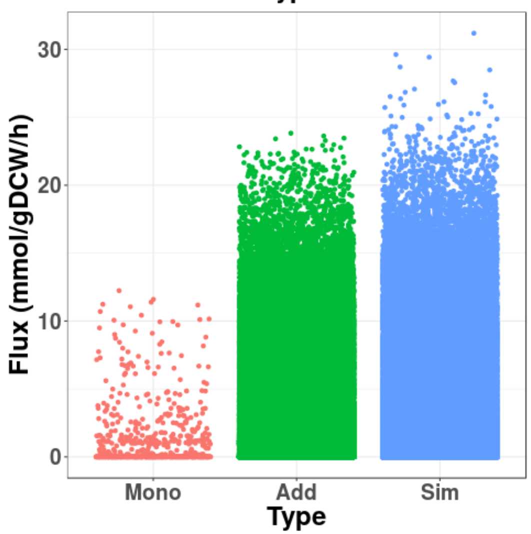
Butanol



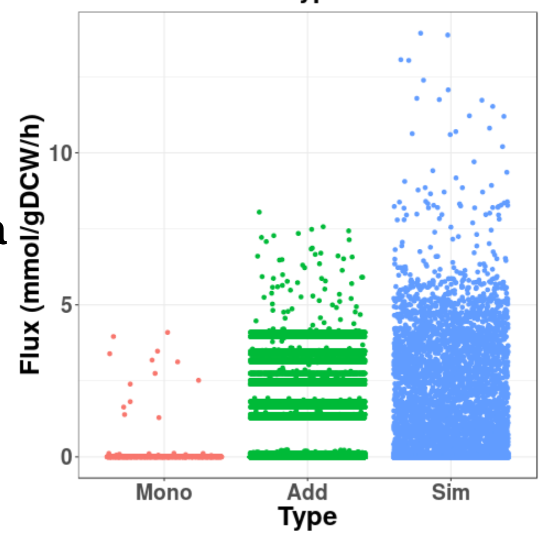
H_2



Propionate



Urea



Mono: all monoculture fluxes

Add: All pairwise monoculture fluxes added together

Sim: All pairwise simulated co-culture fluxes

Models: co-cultures capable of greater than additive production

Microbe production of Useful Chemicals

Products based on single microbe model simulations:

Organics	Alcohols	Nitrogen	Fatty acids	Gases
Acetate	Ethanol	Ammonium	Tetradecanoate	H_2
Formate	Butanol	Amino acids	Octadecanoate	N_2
Succinate	Methanol	Indole	Hexadecanoate	CO_2
Propionate	Propylene glycol	Urea		CH_4
Butyrate				
Lactate				
Glycolate				
Pyruvate				

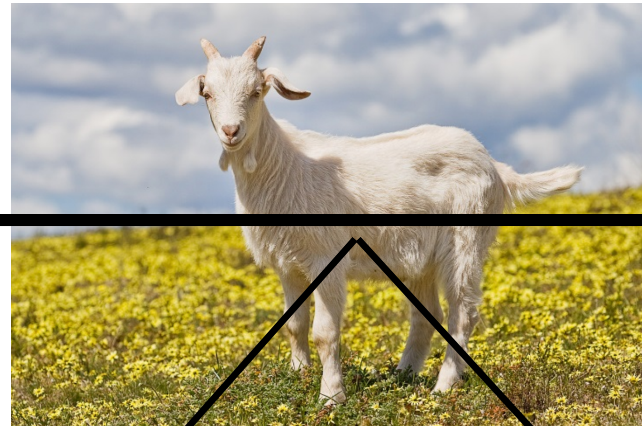
Emergent products from co-culture simulations:

Citrate	2,3-butanediol	Vitamin B_1	Laurate	N_2O
Diacetyl		Vitamin B_2		
Oxaloacetate		Vitamin B_9		

Di-carboxylic acids and alcohols have potential for additive manufacturing (John La Scala - WMRD)

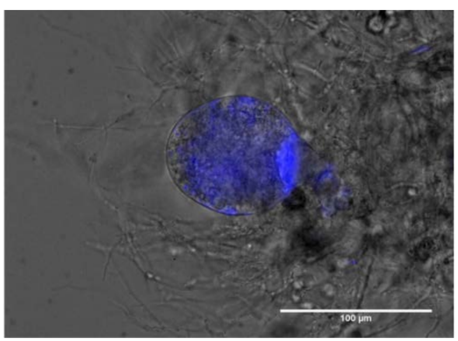
RUMEN MICROBIOME AS GUIDE

Non-homogenous lignocellulose feedstock



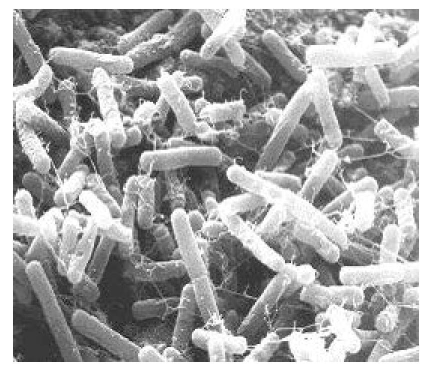
Tunable outputs

Anaerobic, lignocellulolytic fungi



Haitjema *et al*. *Biotechnol Bioeng.* 2014.

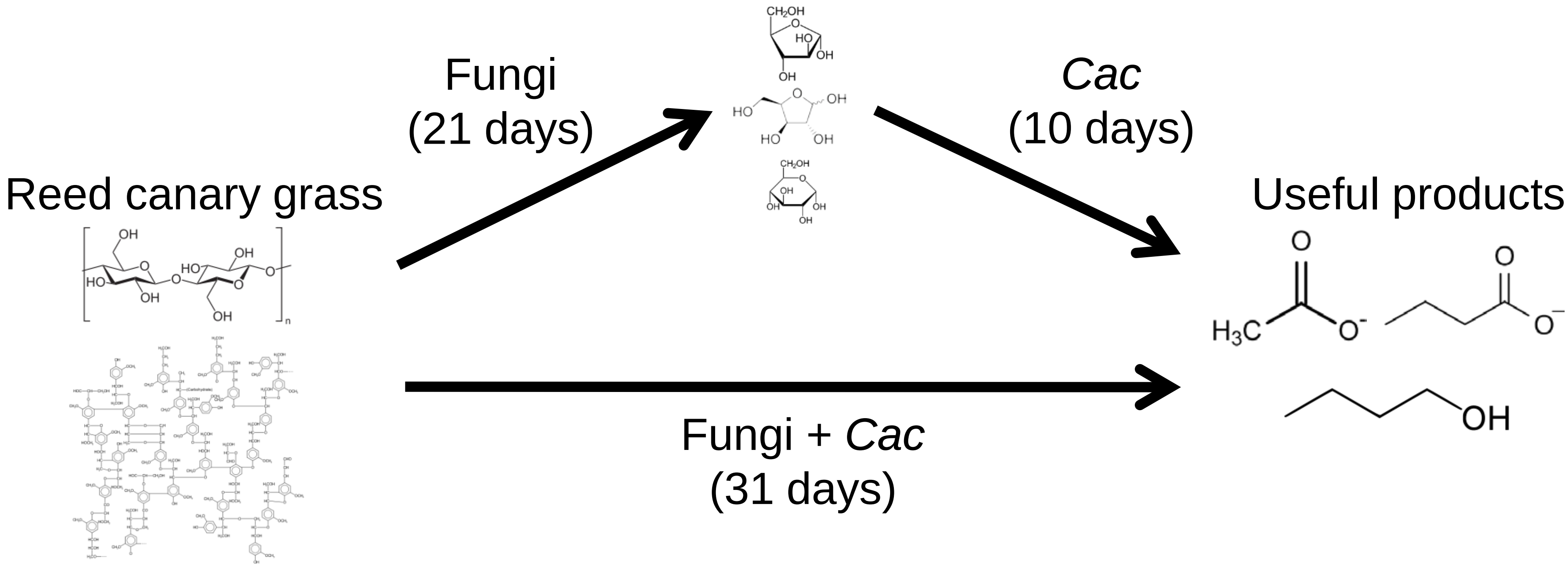
Clostridium acetobutylicum



Functional Ecological Approach

Ecological strategies: Division of labor, temporal organization

2 Stage

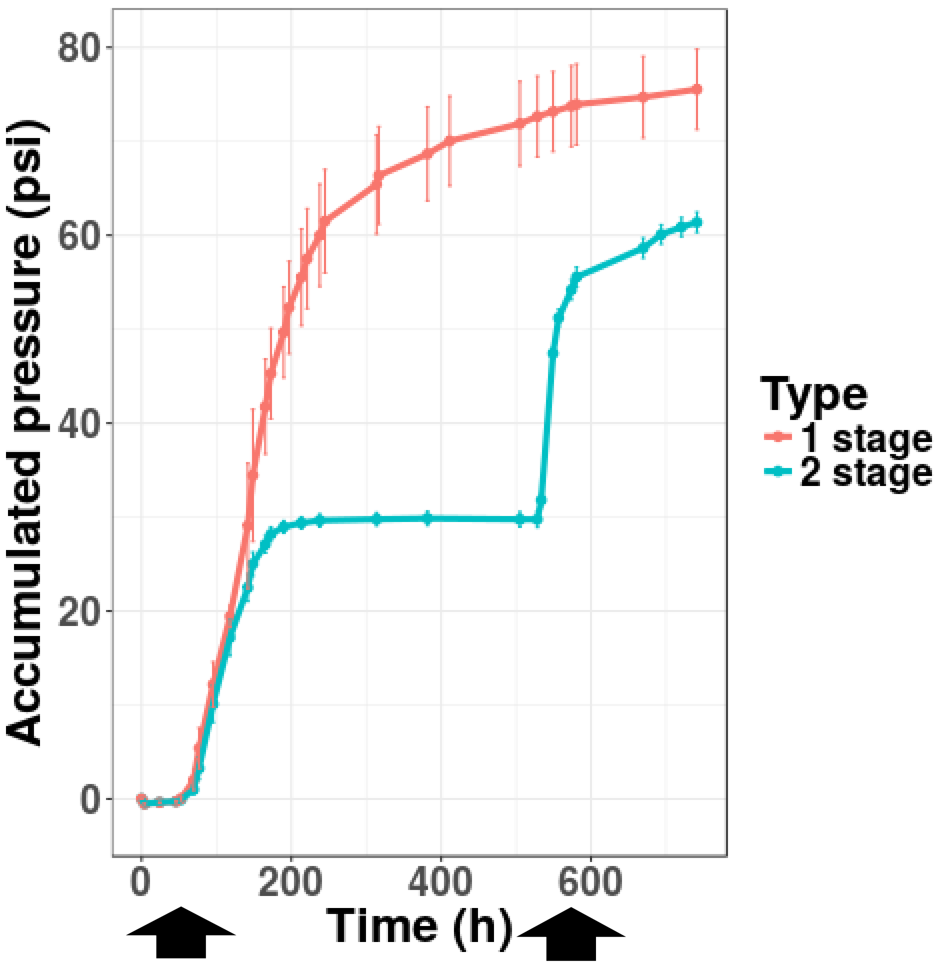


1 Stage

Fungi: *Neocallimastix californiae* *Cac: Clostridium acetobut*

Fungi/*Cac* Co-cultures Result In Synergistic Growth

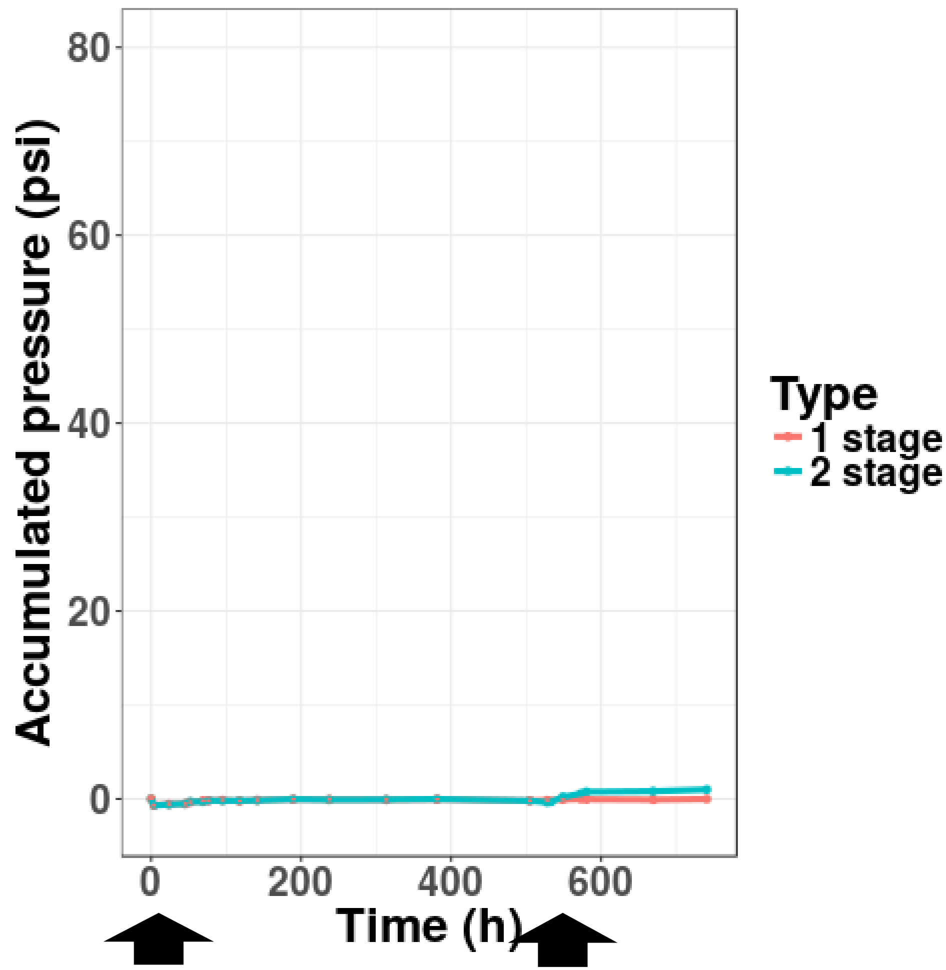
N. californiae + Cac



1 stage*: Cac* + fungi
2 stage: fungi

2 stage*: Cac*

Cac alone

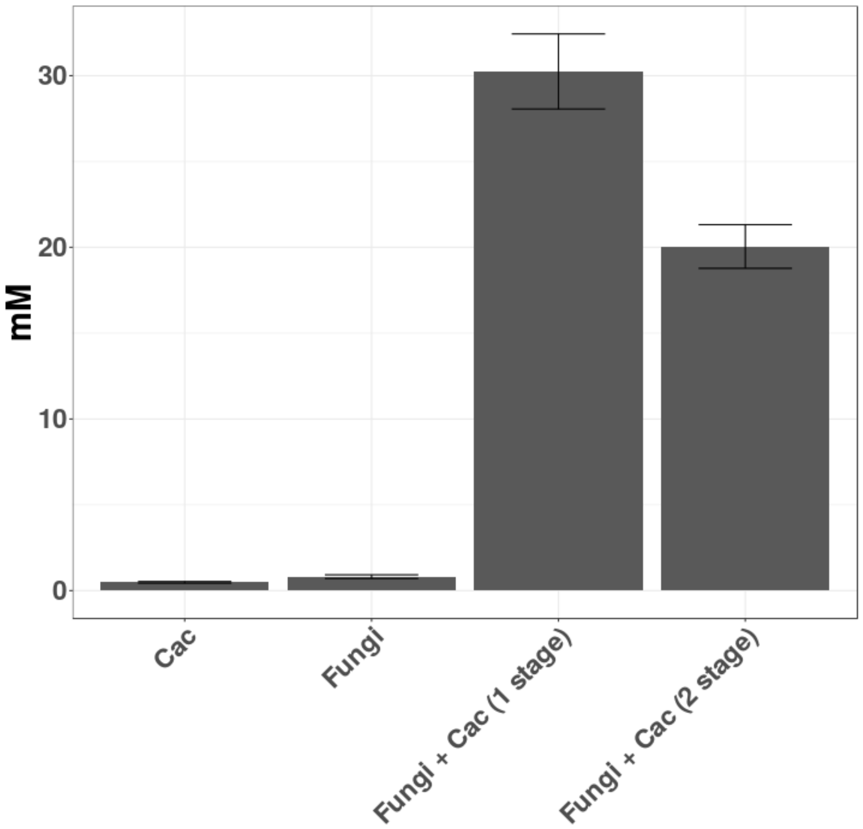


1 stage*: Cac* + media
2 stage: media

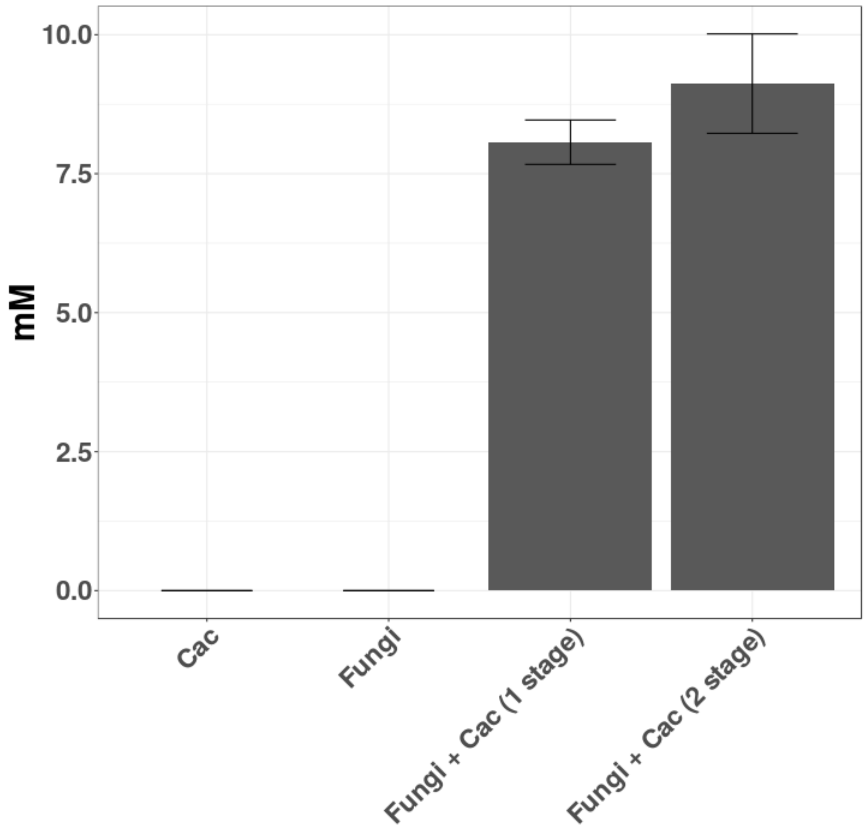
2 stage*: Cac*

Cac Metabolic Outputs

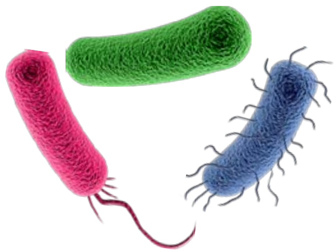
Butyrate



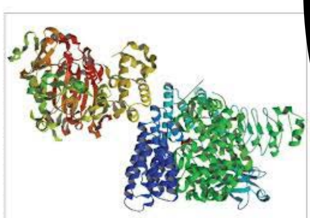
Butanol

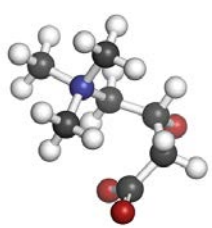


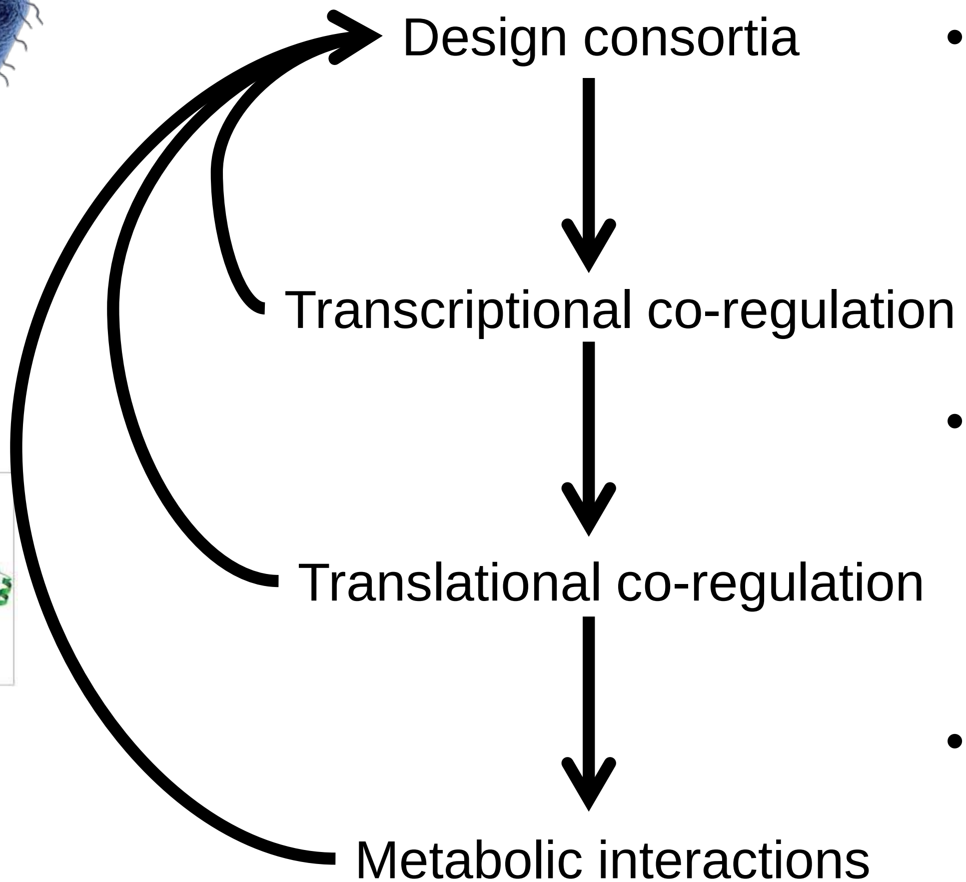
Path Forward



© Buzzle.com







- **Understand synergistic interactions that result in robust, productive consortia**
- **Develop intervention strategies to control consortia assembly and outputs.**
- **Convert food waste and indigenous resources to useful chemicals.**

Discussion & Conclusions

- Co-culturing as alternative to genetic manipulation for robust microbes recalcitrant to genetic changes
- Consortia design allows for:
  - synergistic growth
  - targeting of new output
- Engineer functional and stable interactions between consortia and abiotic components
- Work with NRL, AFRL, MIT to transition functional chemical targets for further analysis and scale up.

Team- Dr. Matthew Perisin, Dr. Theresah Zu, Dr. Sanchao Liu, Dr. Rebecca Renberg, Dr. Nathan Schwalm, Dr. Christian Sund (DARPA detail), Elliot Gerlach, Marcus Benyamin