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Spatiotemporal metabolic modeling of multispecies bacterial biofilms

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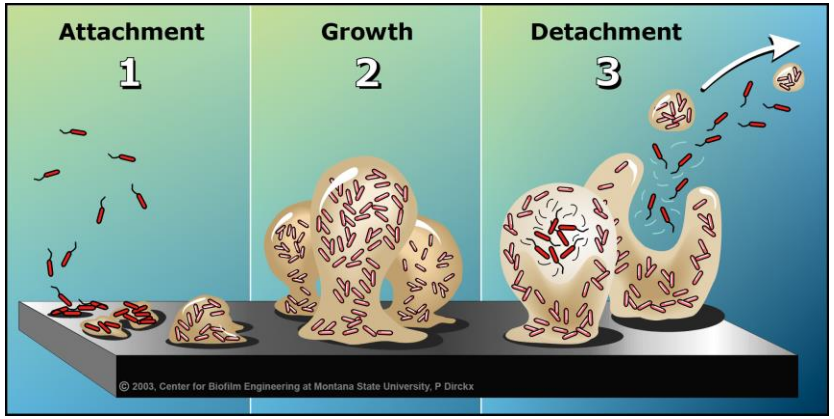
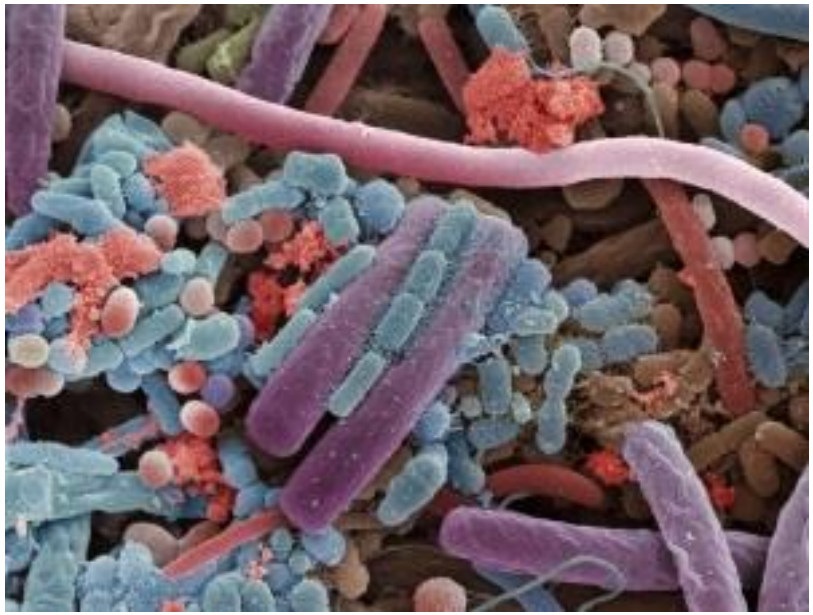
1. Background



Spatiotemporal Behavior of Microbial Systems

- Most microbes evolved and exist in environments with both temporal and spatial variations
- Spatial heterogeneities allow the development of unique metabolic niches critical to system function

Human Gut Microbiome

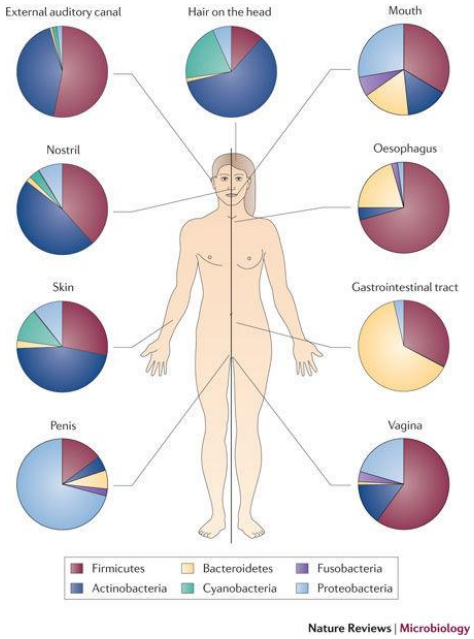


Biomass Degrading Communities

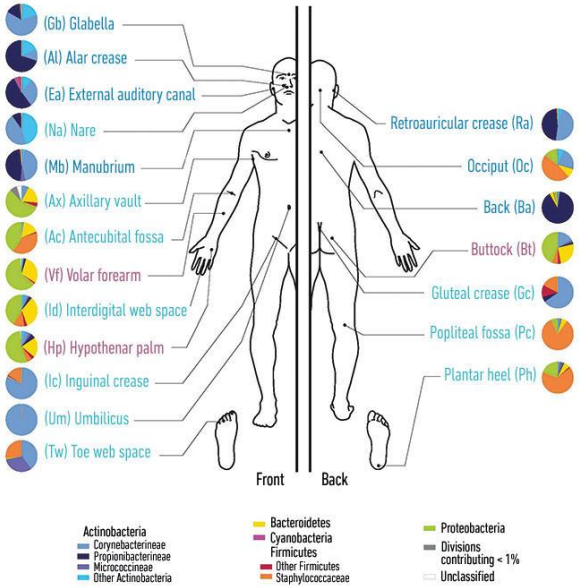
Images from www.autismspeaks.org, www.bbc.com

Multispecies Communities Important in Human Health

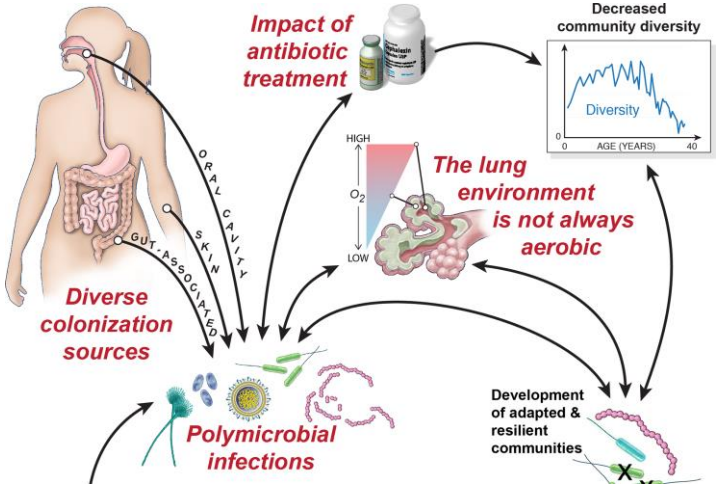
Commensal Communities



Chronic Wounds

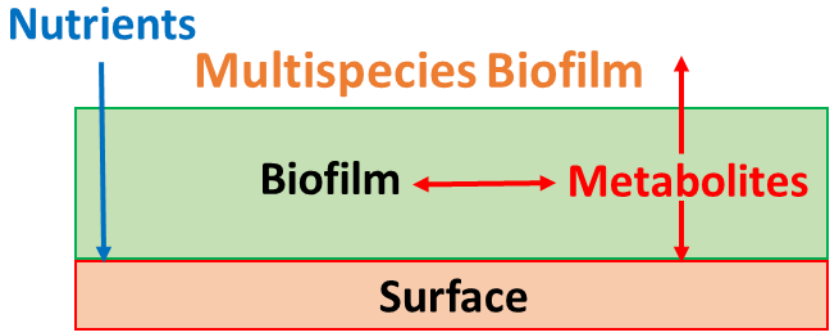


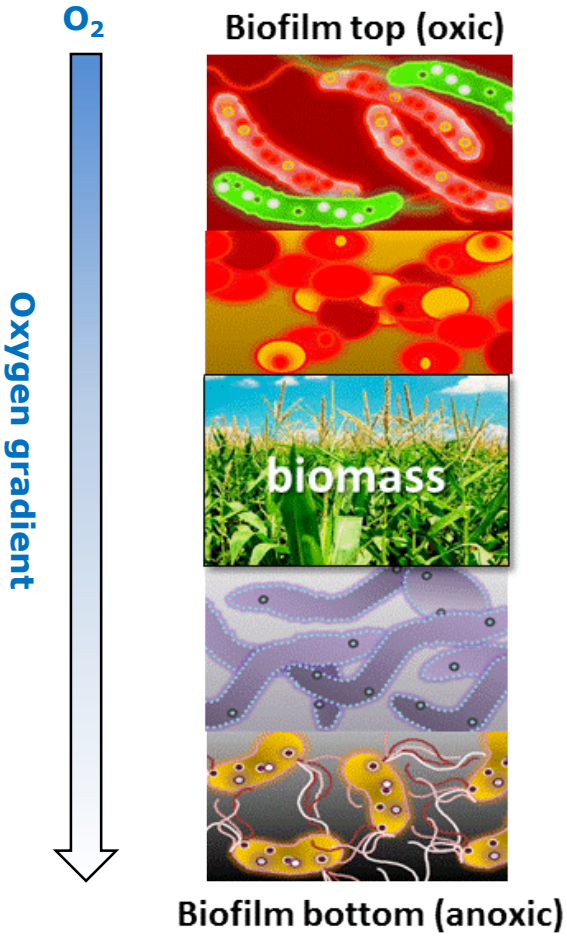
Cystic Fibrosis



Images from www.microbiologysociety.org, Filkins & O'Toole, PLOS Pathogen, 2015

Multispecies Biofilms

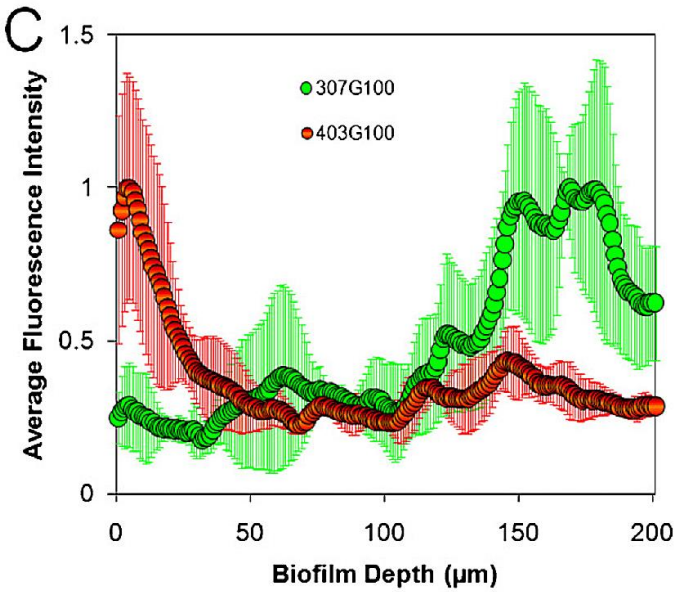




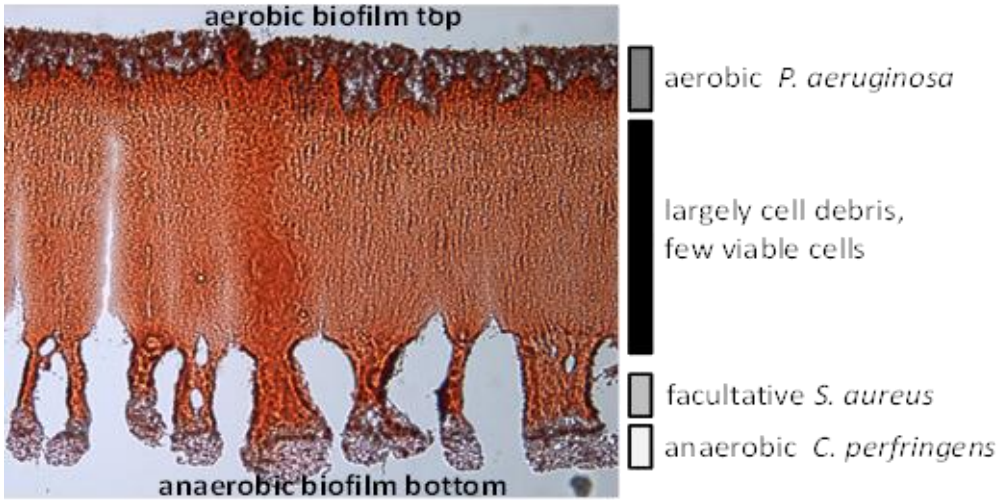
- Bacteria naturally grow as multispecies biofilms
- Chemical gradients create metabolic niches in the biofilm
- Slow and fast growing species naturally coexist
- Evolution has optimized different natural communities for specific environments

Effect of Nutrient Gradients in Multispecies Biofilms

Engineered *E. coli* biofilm



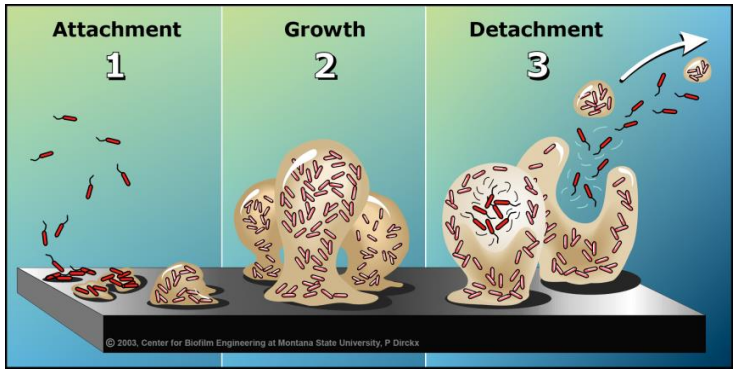
In vitro chronic wound biofilm



Bernstein et al., J. Biotechnology, 2012; James et. al., Wound Repair Regen., 2008

Biofilm Metabolic Models

- Multispecies biofilms are highly complex systems with a broad array of species and community level machinery
- Biofilm models focused on metabolic mechanisms are most tractable
- Biofilm metabolic models can account for:
  - Spatial organization
  - Nutrient competition
  - Byproduct cross feeding
  - Growth inhibition by toxins



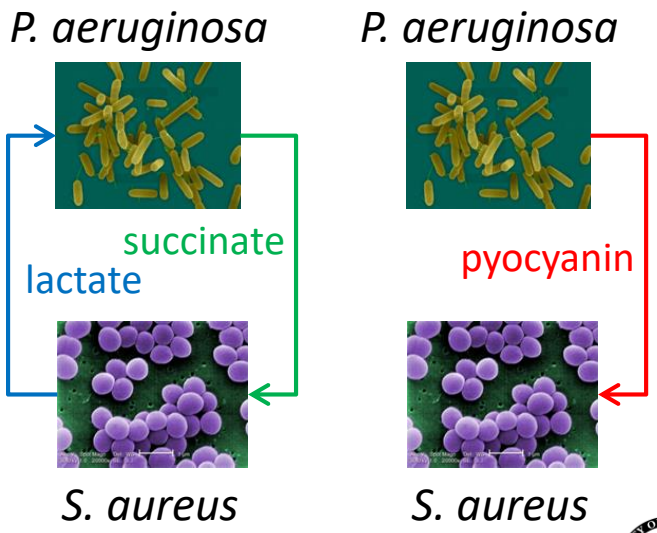
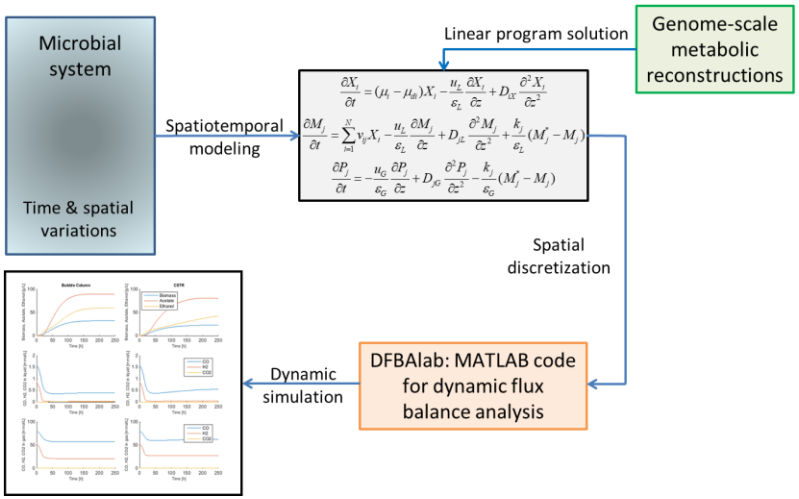


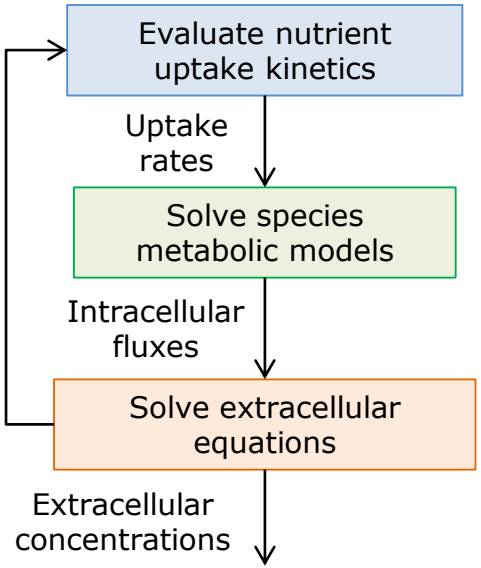
Image from Montanta State U.

2. Modeling Framework



Spatiotemporal Metabolic Modeling

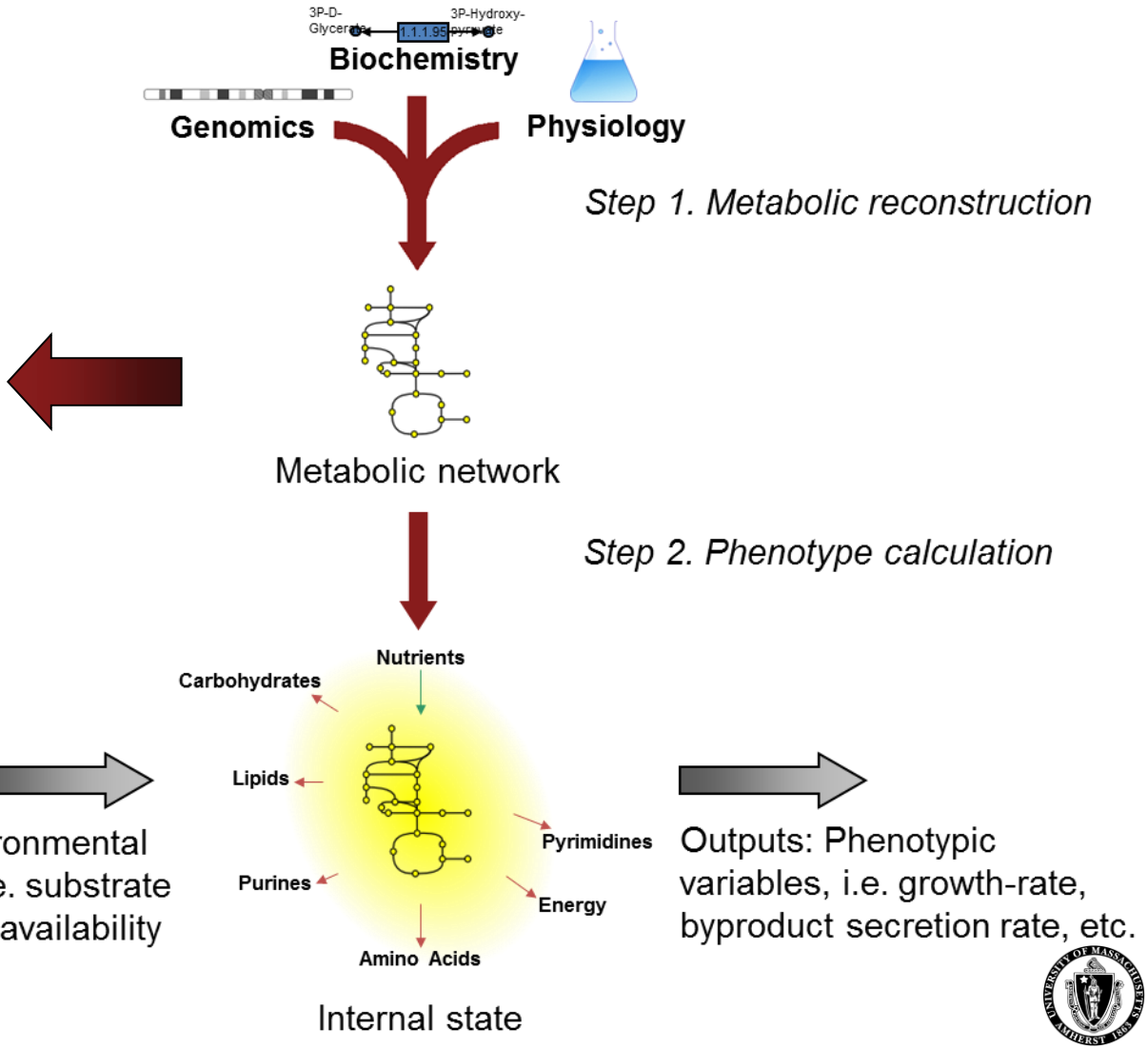
- Basic components
  - Substrate uptake kinetics based on local extracellular metabolite concentrations
  - Genome-scale reconstructions of metabolism
  - Transport equations describing spatiotemporal variations of the extracellular environment



- Several methods have been proposed
  - Table lookups of precomputed FBA solutions (Jayasinghe et al., *Biotechnol. J.*, 2014)
  - Lattice based descriptions of nutrient diffusion (Harcombe et al., *Cell Reports*, 2014)
- Based on assumption that intracellular dynamics are fast compared to extracellular dynamics

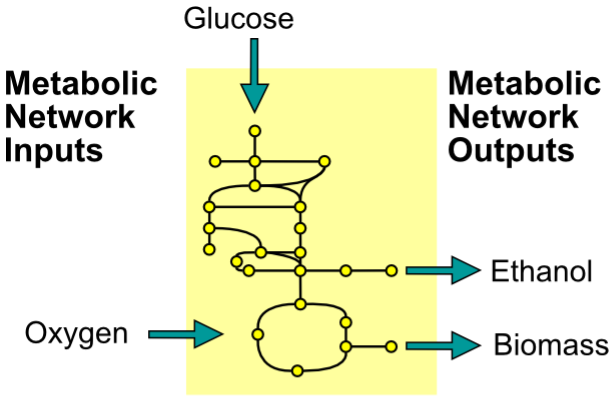
Intracellular Metabolism

- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Clostridium difficile*
- *Bacteroides thetaiotamicron*
- *Faecalibacterium prausnitzii*
- *Escherichia coli*
- *Clostridium phytofermentans*
- *Geobacter sulfurreducens*

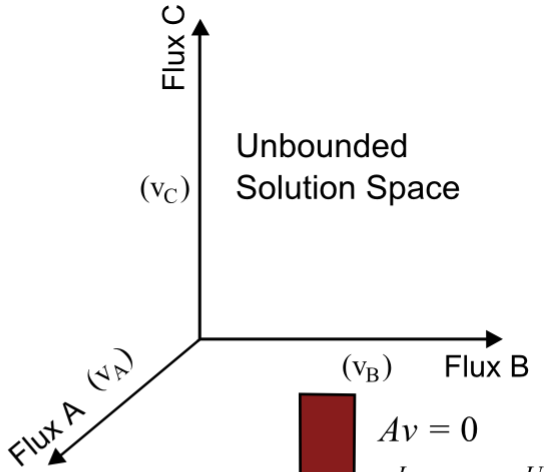


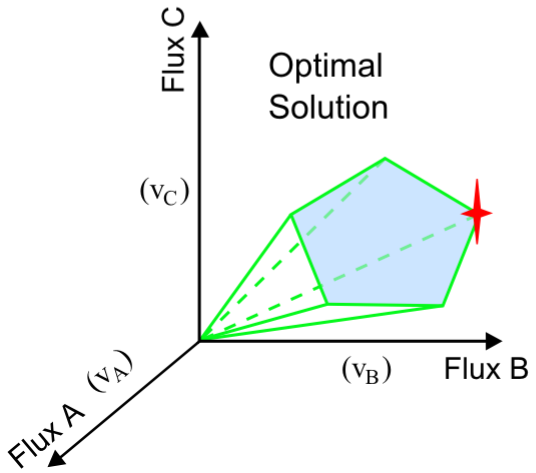
Thiele & Palsson, *Nature Protocols*, 2010; Milne et al., *Biotechnology Journal*, 2009

Flux Balance Analysis (FBA)



Phenotype Calculation: Determine Metabolic Flux Desitribution





Hypothesis: Evolutionary pressures enforce optimal resource utilization

Maximize Growth

$\text{Max} \quad w^T v$

$Av = 0$

$v^L < v < v^U$

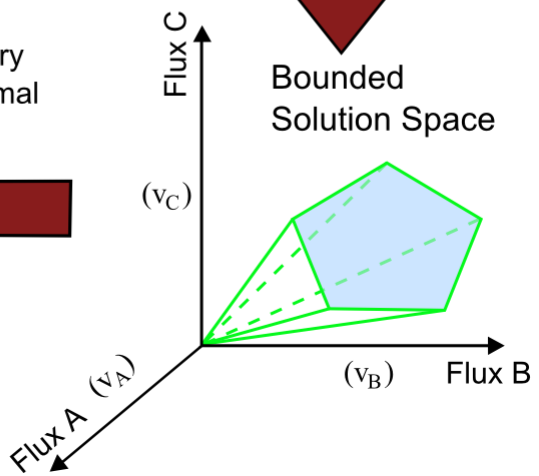


Figure courtesy for Krishna Mahadevan, U. Toronto; Price et al., *Nature Reviews Microbiology*, 2004

Biofilm Spatiotemporal Modeling Framework

Microbial system

Time & spatial variations

Spatiotemporal modeling

Linear program solution

Genome-scale metabolic reconstructions

Intracellular steady state

$$\frac{\partial X_i}{\partial t} = (\mu_i - \mu_{di})X_i - \frac{u_L}{\varepsilon_L}\frac{\partial X_i}{\partial z} + D_{iX}\frac{\partial^2 X_i}{\partial z^2}$$

$$\frac{\partial M_j}{\partial t} = \sum_{i=1}^{N} v_{ij} X_i - \frac{u_L}{\varepsilon_L}\frac{\partial M_j}{\partial z} + D_{jL}\frac{\partial^2 M_j}{\partial z^2} + \frac{k_j}{\varepsilon_L}(M_j^* - M_j)$$

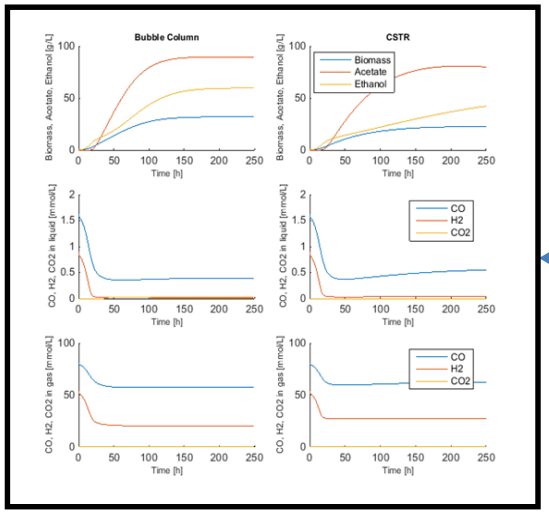
$$\frac{\partial P_j}{\partial t} = -\frac{u_G}{\varepsilon_G}\frac{\partial P_j}{\partial z} + D_{jG}\frac{\partial^2 P_j}{\partial z^2} - \frac{k_j}{\varepsilon_G}(M_j^* - M_j)$$

Extracellular dynamics

Spatial discretization

DFBAlab: MATLAB code for dynamic flux balance analysis

Dynamic simulation



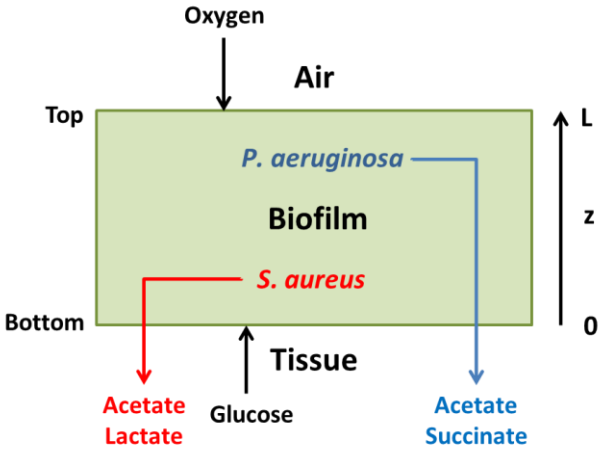
Chen et al., *BMC Systems Biology*, 2016; Gomez et al., BMC Bioinformatics, 2014

Current State of Modeling Effort

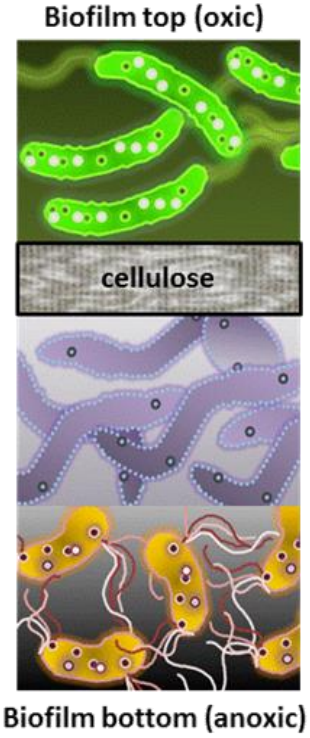
- Nutrient-dependent uptake kinetics
- Metabolite-dependent diffusion
- 1-D and 2-D spatial variations
- Nutrient competition
- Byproduct cross feeding
- Inhibitor secretion and diffusion
- Chemotaxis of motile species
- Biofilm expansion and erosion
- Antibiotic treatment

Current Research Projects

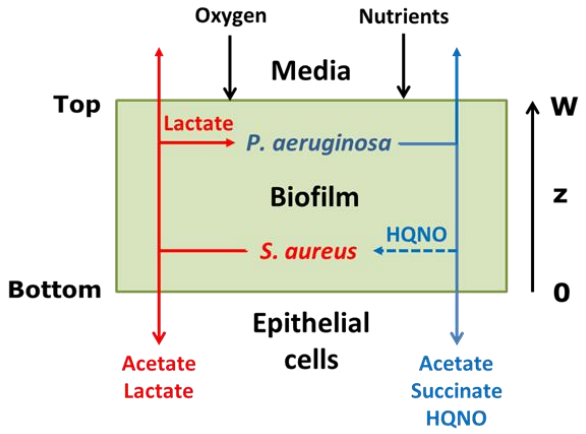
Chronic Wounds



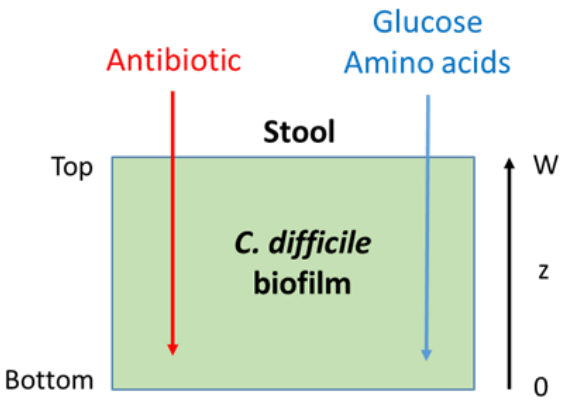
Engineered Systems



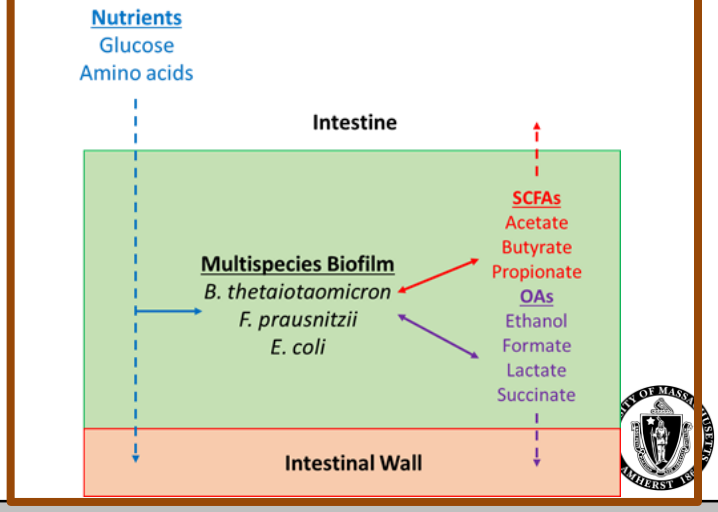
Cystic Fibrosis



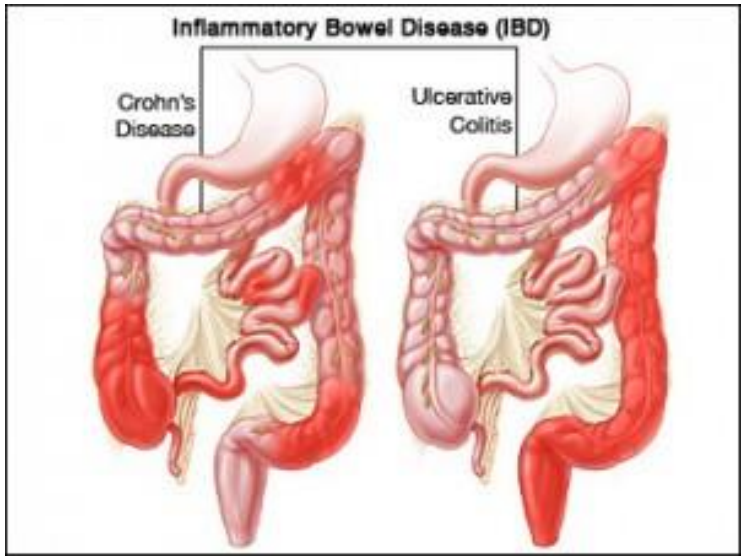
Clostridium difficile



Gut Microbiota

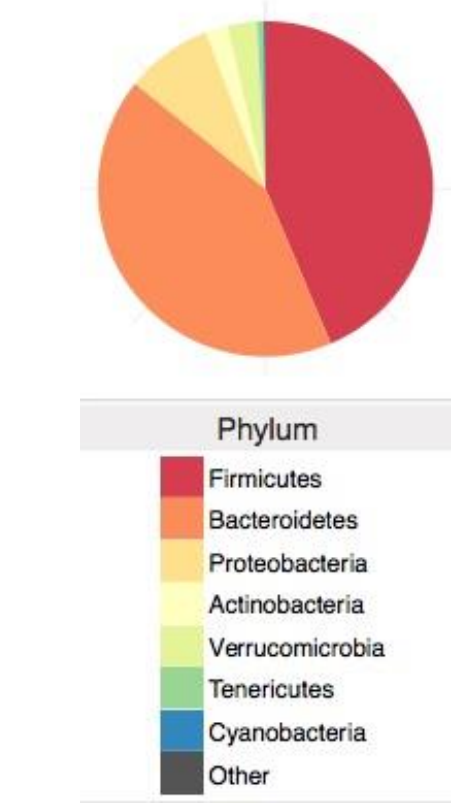


3. Application to Inflammatory Bowel Disease



The Human Gut Microbiome

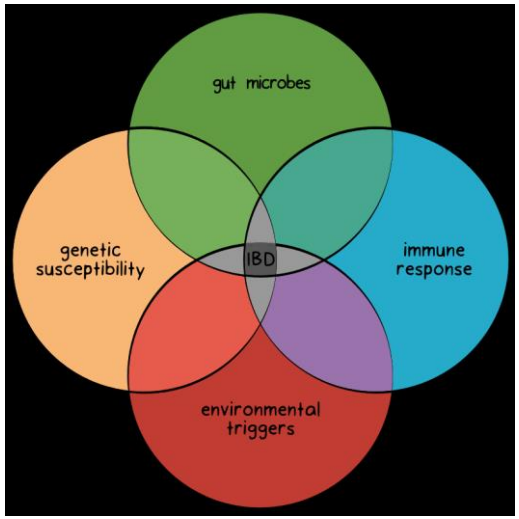
- A highly complex community consisting of approximately ~1,000 species in a typical human host
- Critical for fiber digestion and immune system function
- Commensal species normally provide resistance to colonization by gut pathogens
- Dysbiosis of the community is associated with gut infections, inflammatory bowel disease, obesity and diabetes
- Formation of multispecies biofilms has been demonstrated in germ-free mice and is likely in humans



Images from *Scientific America* and *Science News*

Inflammatory Bowel Disease (IBD)

- Severe reduction in butyrate-producing obligate anaerobes from the phylum Firmicutes (e.g. *Faecalibacterium prausnitzii*)
- Large increase in facultative anaerobes from the phylum Proteobacteria (e.g. *Escherichia coli*)
- Bacteroides have been shown to be nanoaerobes
- "Oxygen hypothesis" - chronic inflammation of intestine results in increased release of hemoglobin carrying O2 and ROS species into the lumen



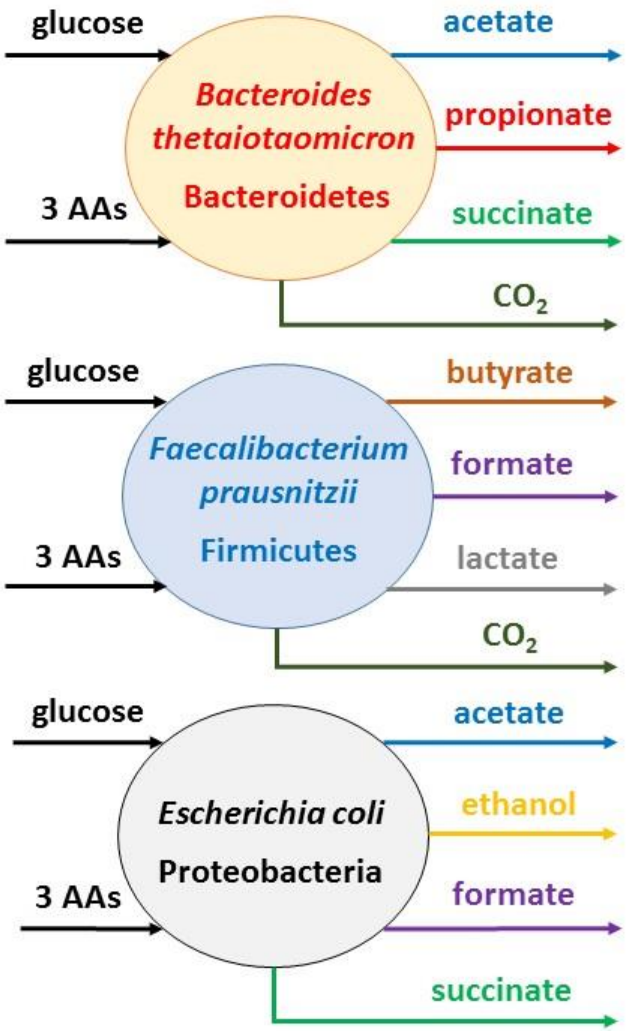
Scope of IBD in USA

- **Estimated prevalence1**
  - UC: 37-346:100,000
  - CD: 26-199:100,000
- **Physician visits: >700,000/year[2]**
- **Hospitalizations: 100,000/year[2]**
- **Annual direct costs: ~$4 billion[3]**

1. Lichtenstein G. 2012. *Goldman's Cecil Medicine*. 24th ed. . Philadelphia, PA: Elsevier Saunders; 2012:913-921.
2. CDC. http://www.cdc.gov/ibd/. 2015.
3. Lichtenstein GR. *Am J Gastroenterol*. 2016.[Abstract 682]

Images from www.khanacademy.org and www.annenberg.net

Minimal Species Model for IBD Progression



SINGLE SPECIES METABOLISM

Nutrients
Glucose
3 amino acids: *methionine, serine, tryptophan*

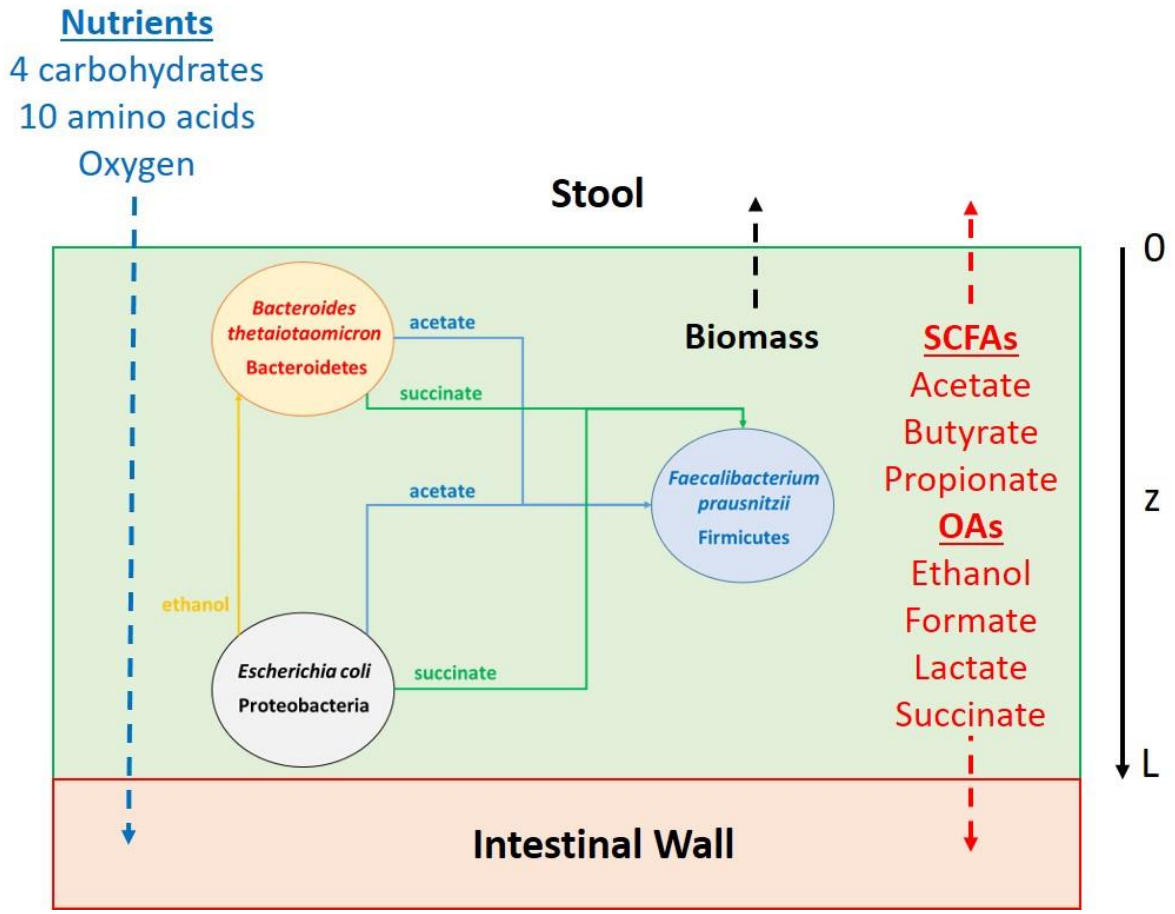
Short Chain Fatty Acids
Acetate
Butyrate
Propionate

Organic Acids
Acetate
Ethanol
Formate
Lactate
Succinate (BT biofilm)

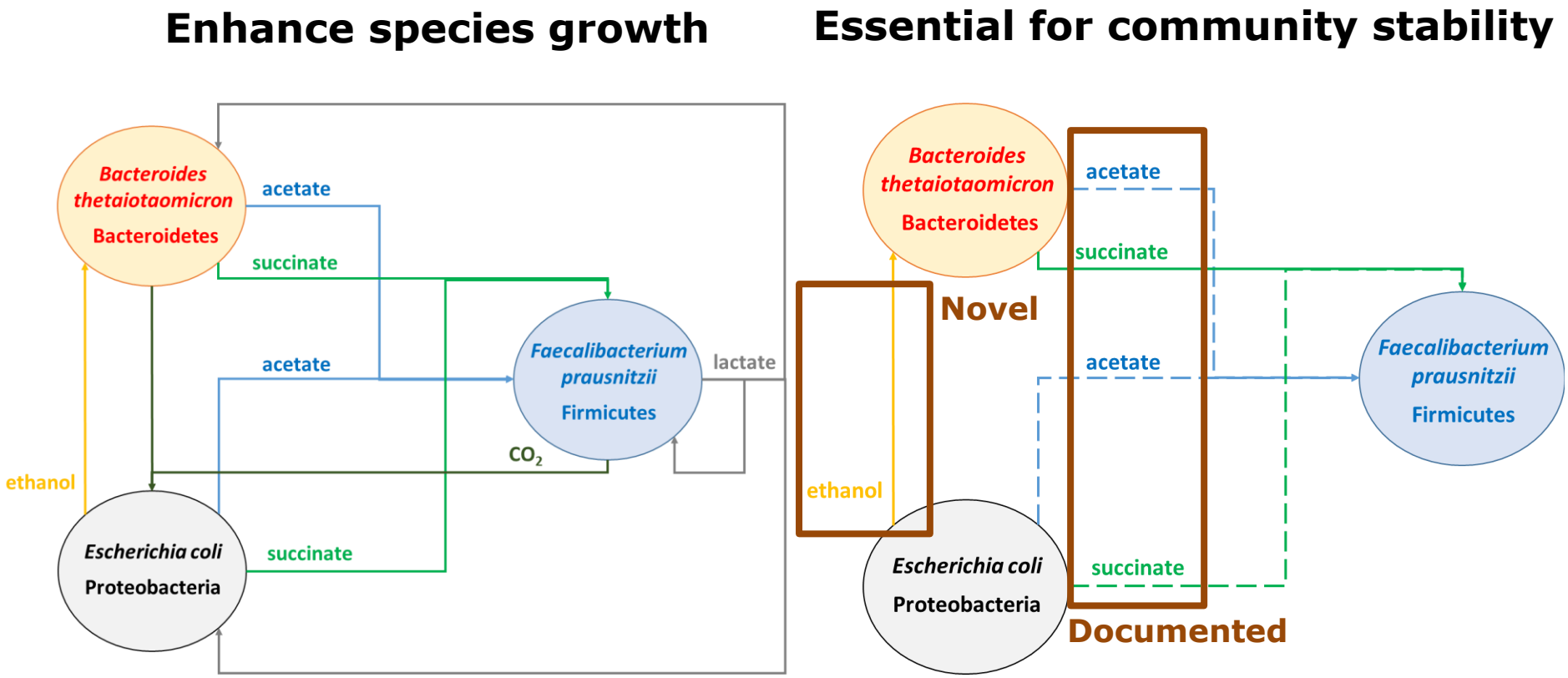
Other Byproducts
CO_2 (BT planktonic)

Multispecies Biofilm Metabolic Model

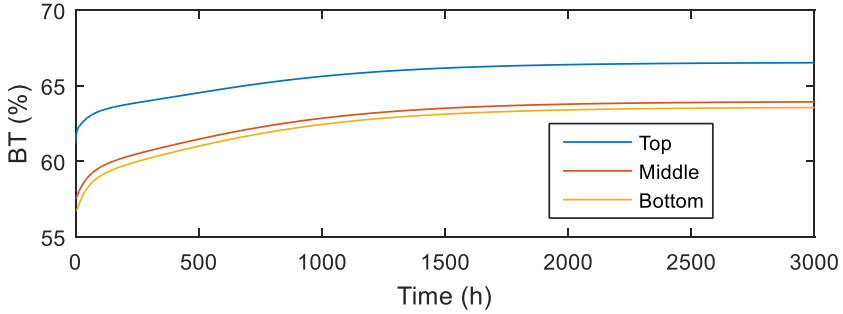
- 3 metabolic reconstructions
- Uptake kinetics for 15 nutrients
- 25 extracellular balances
- 40 μm fixed thickness
- 20 spatial node points
- 1440 LPs
- 520 nonlinear ODEs
- Efficiently solved in MATLAB with DFAlab

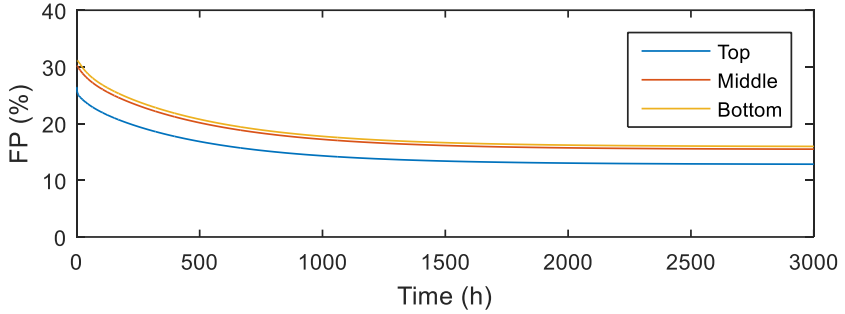


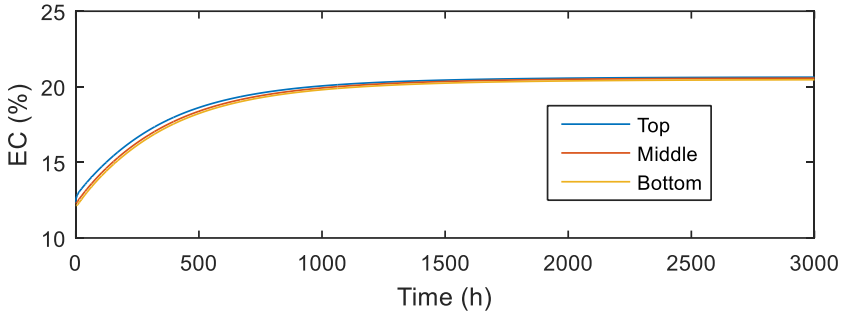
Discovery of Putative Crossfeeding Relationships

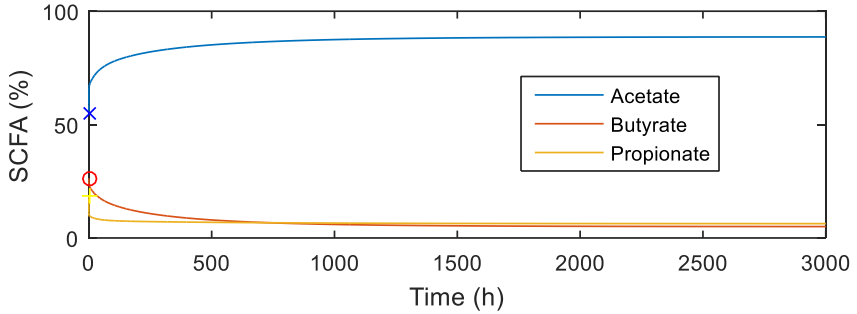


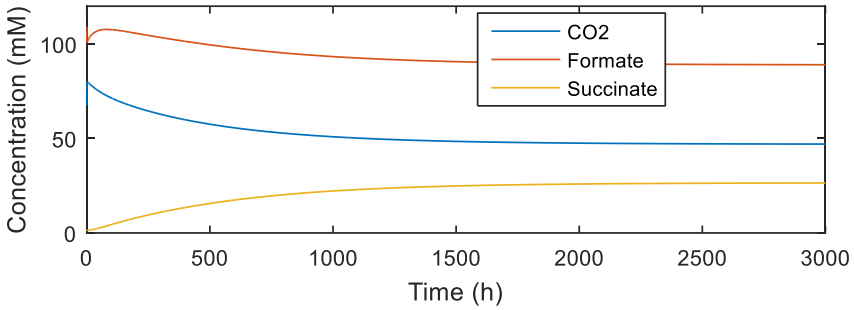
Oxygen Induces Dysbiosis Dynamics

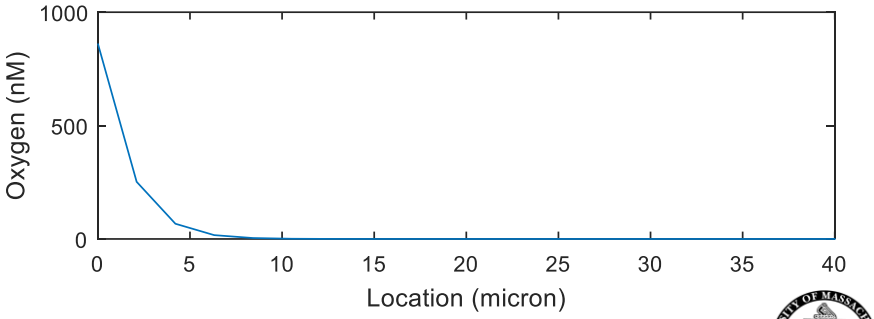




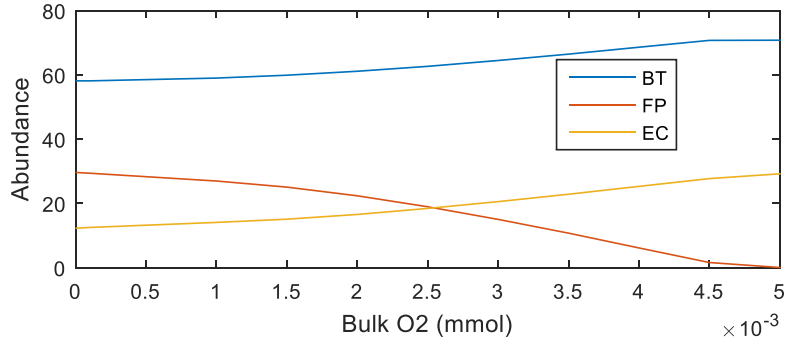


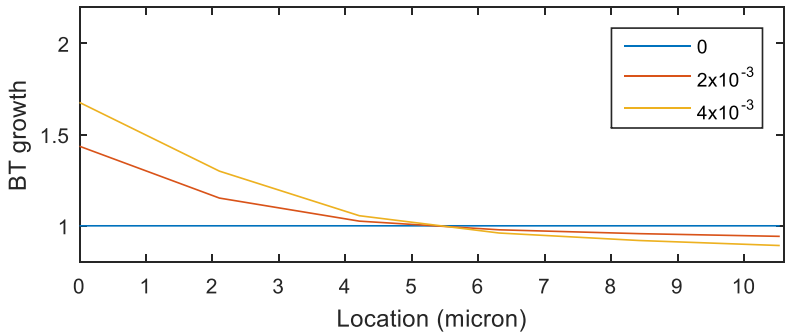


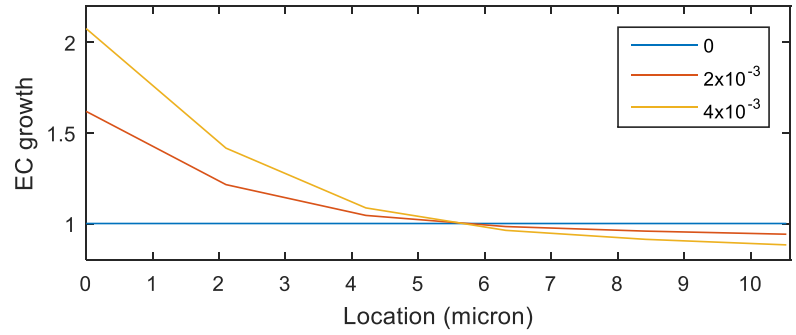


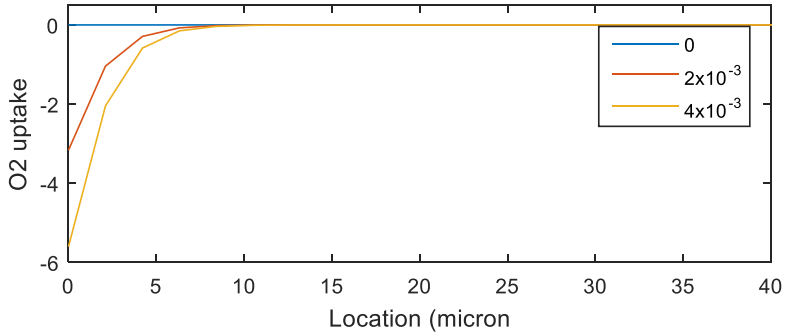


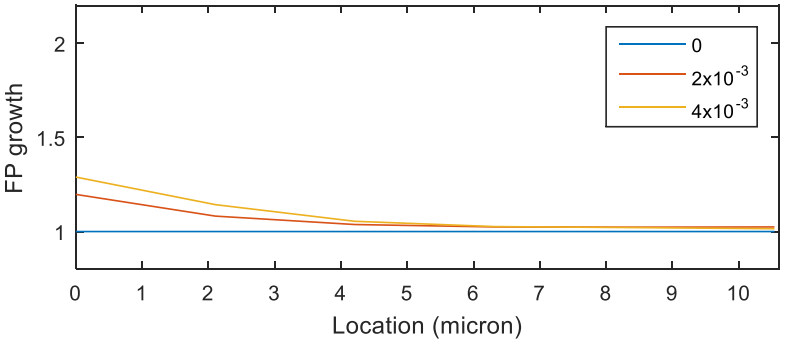
Higher Oxygen Levels Increase Dysbiosis



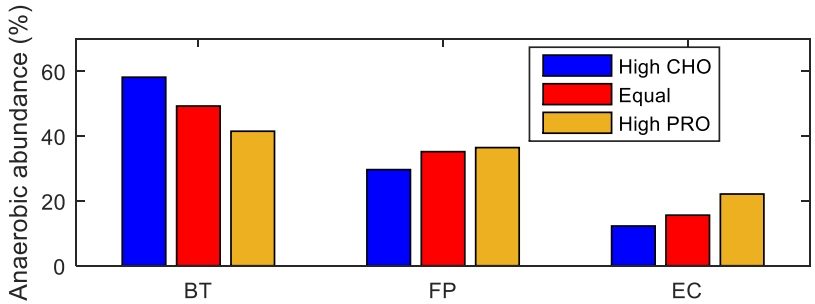


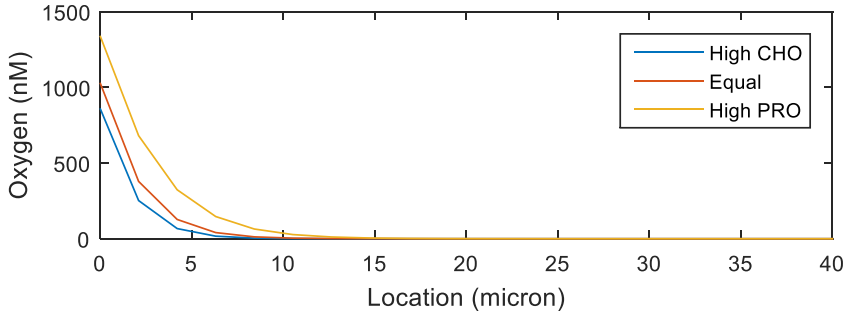


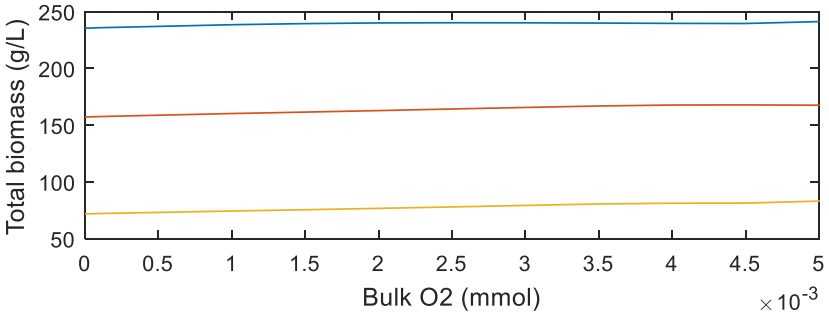


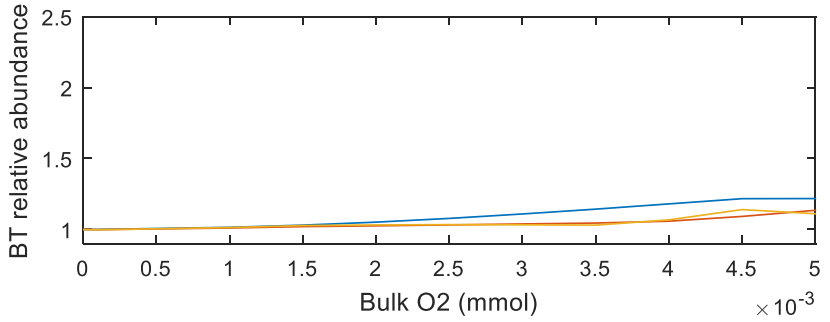


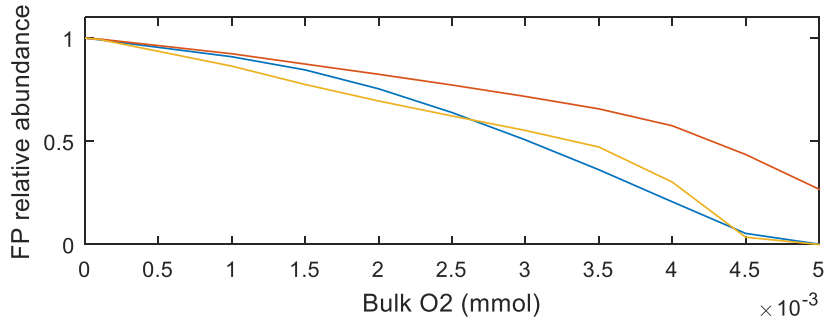
Oxygen Sensitivity Depends on Diet

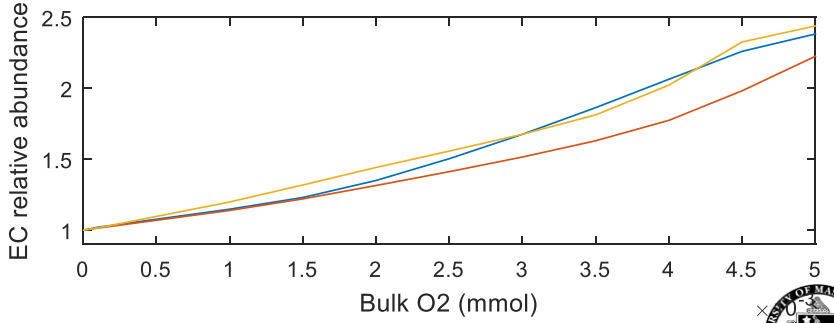






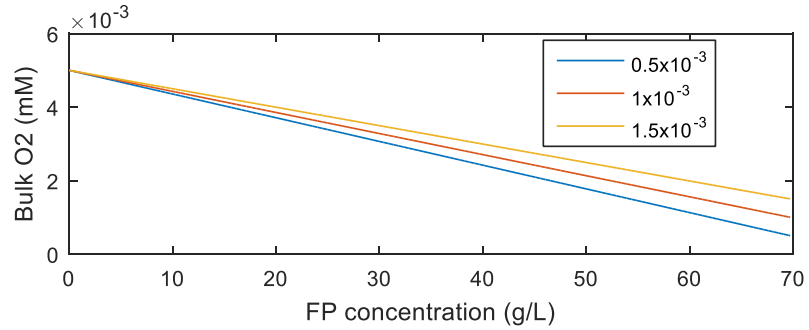


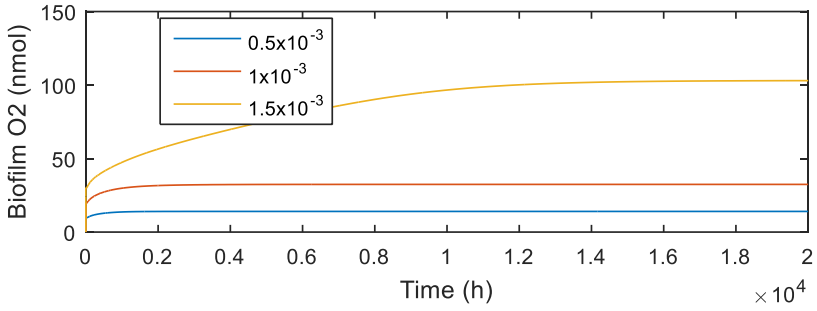


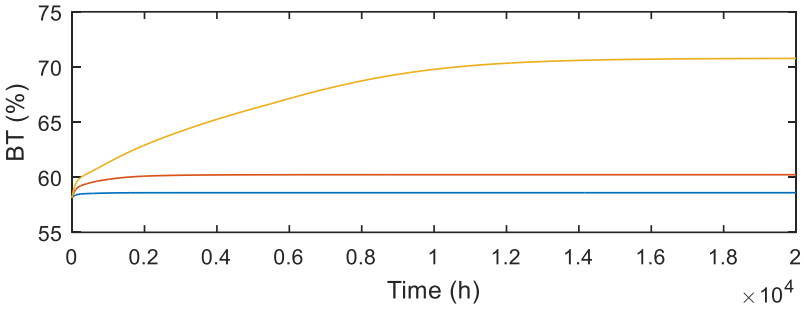


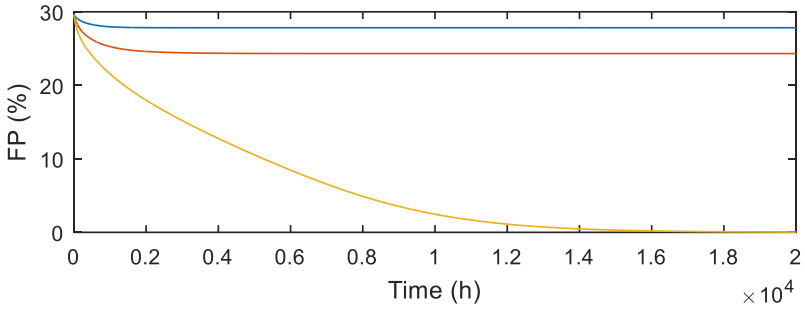
Henson & Phalak, *BMC Systems Biology*, 2017

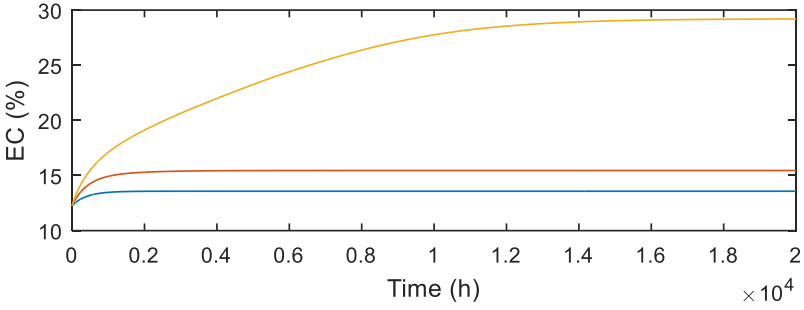
Host-Microbiota Feedback Leads to Slow Dysbiosis

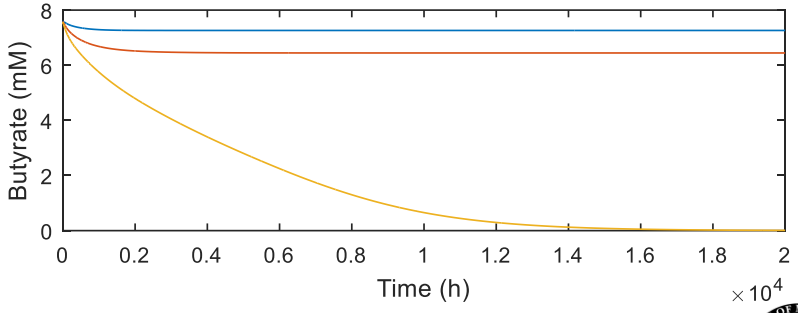












4. Application to *Clostridium difficile* infection

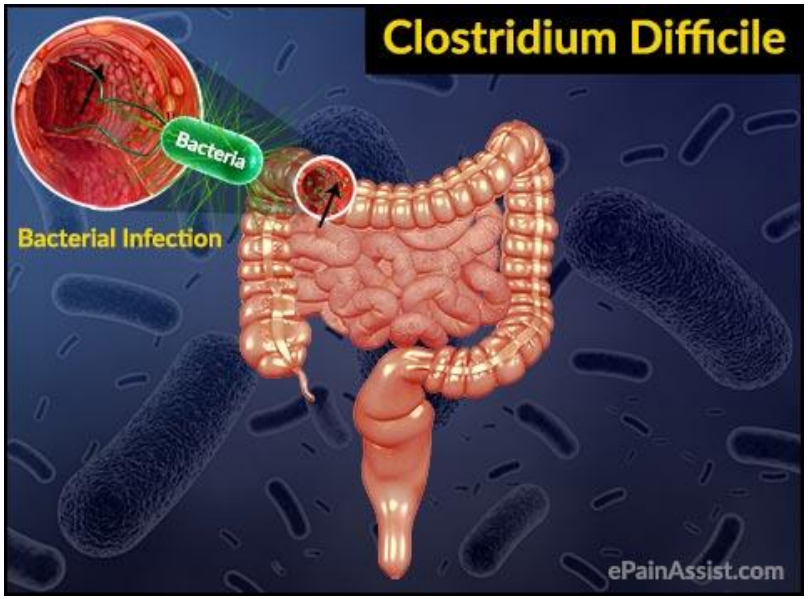


Image from www.epainassist.com

Clostridium difficile Infection (CDI)

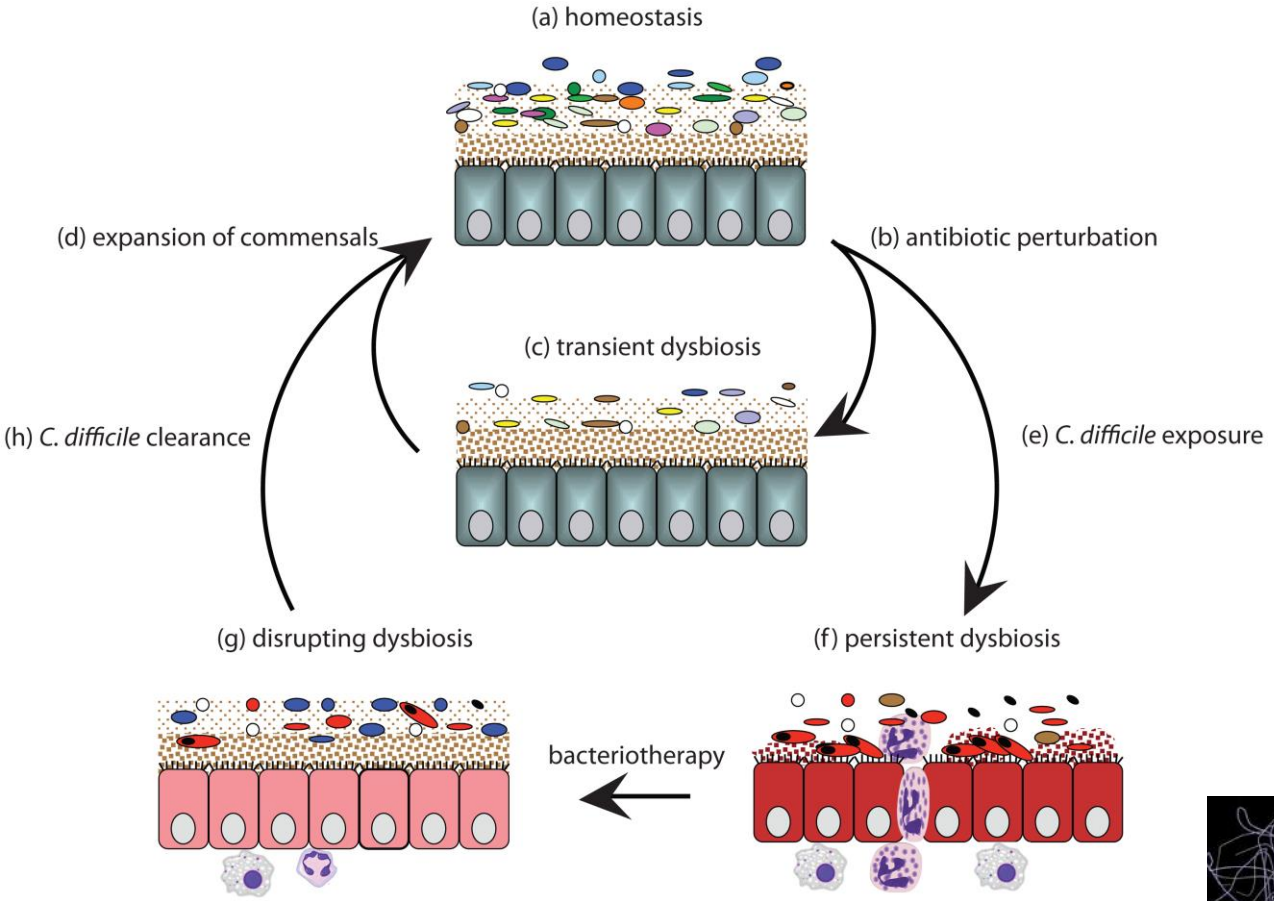


Table 2

Possible Complications of *C difficile* Infection[2,3,7]

Complications
Dehydration
Pseudomembranous colitis
Impaired renal function leading to renal failure
Ileus
Hypotension
Anasarca
Intestinal perforation
Peritonitis
Toxic megacolon
Organ failure
Death

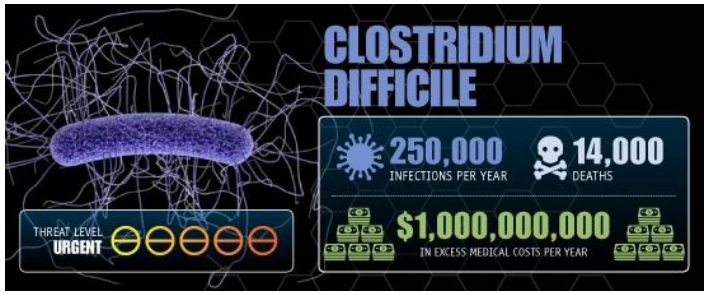
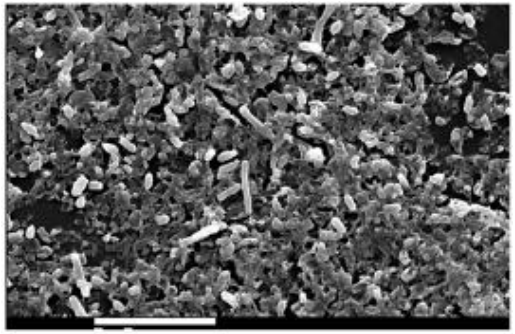
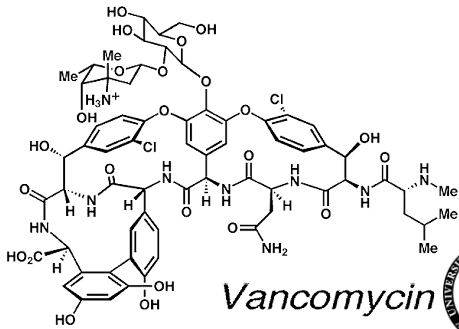


Image from Lawley et al., *PLoS Pathogens*, 2012; Advance Healthcare Network; CDC

C. difficile Metabolism and Treatment

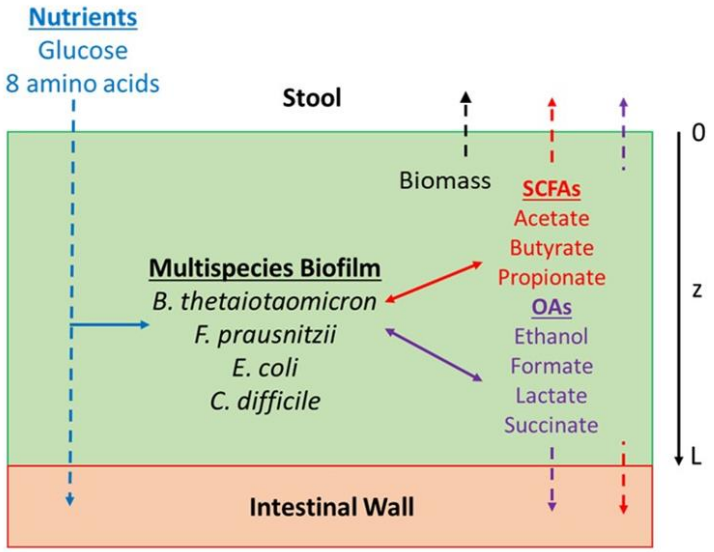
- Nutritional capabilities
  - Versatile carbon source utilization (e.g. glucose, fructose, xylose, succinate, etc.)
  - Requires 6 amino acids (cysteine, isoleucine, leucine, proline, tryptophan, valine)
- Biofilm formation has been demonstrated *in vitro* and observed in germ-free mice
- Estimated that 3-15% of healthy adults are asymptomatically colonized with *C. difficile*
- Oral vancomycin is commonly used for moderate to severe infections





Images from CDC; Dawson et al., *PLoS One*, 2012; Scripps Research Institute

Multispecies Biofilm Metabolic Model



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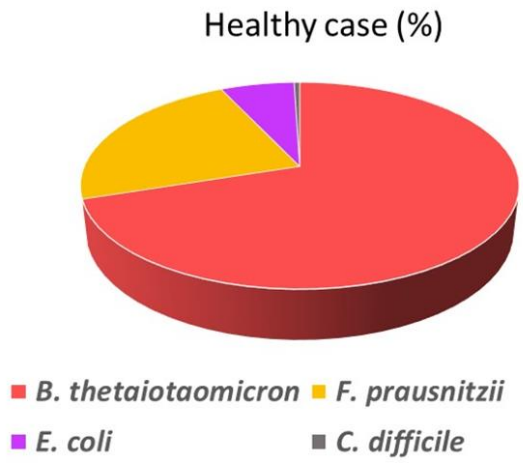
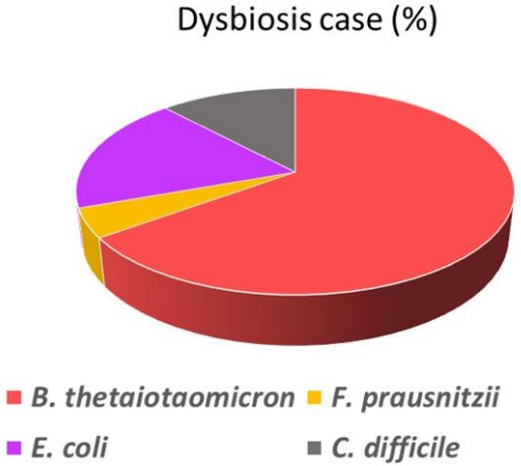


Host-microbiota perturbation

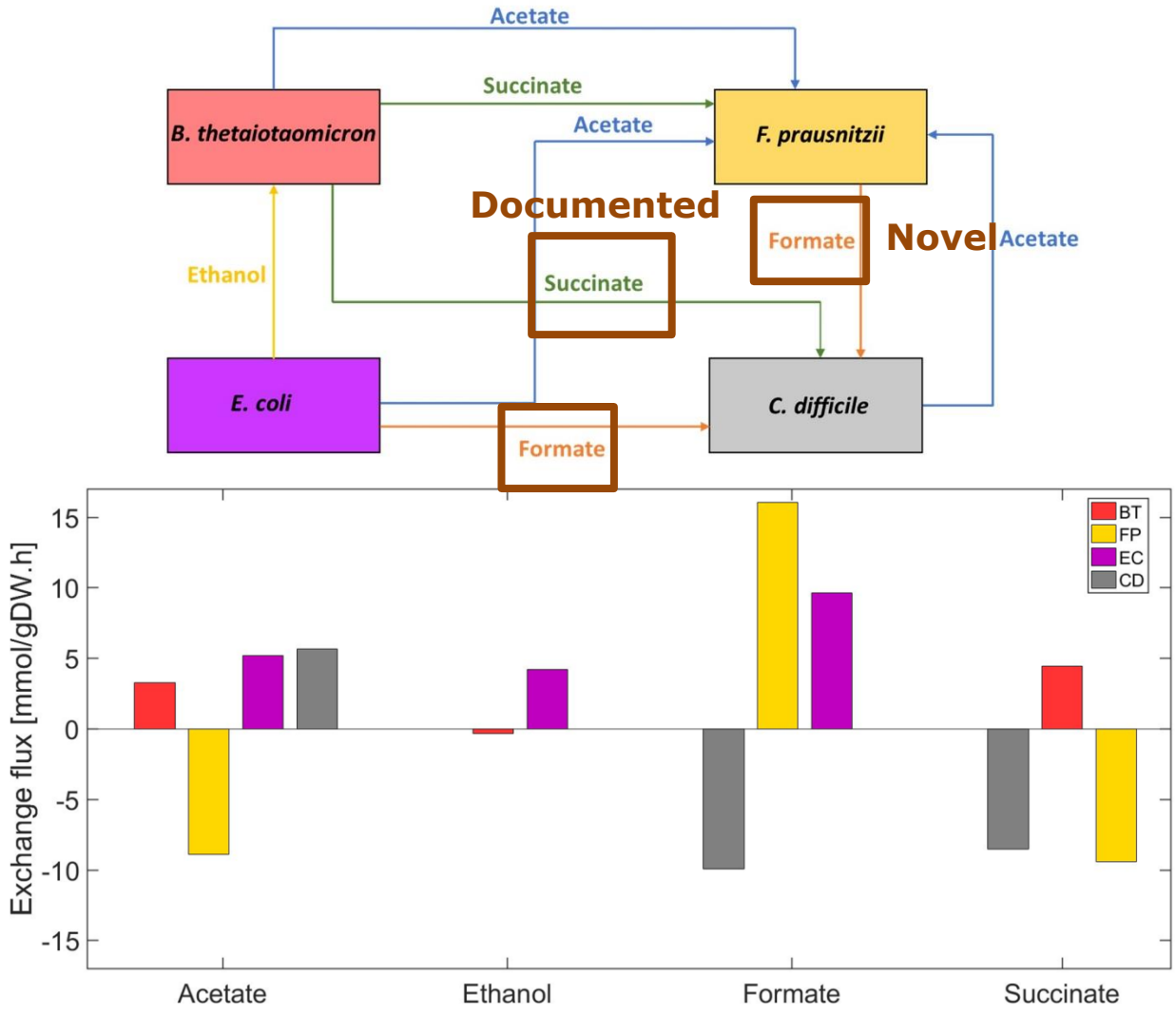
Decreased glucose
Increased amino acids
Increased primary bile acids
Increased nitrate

B



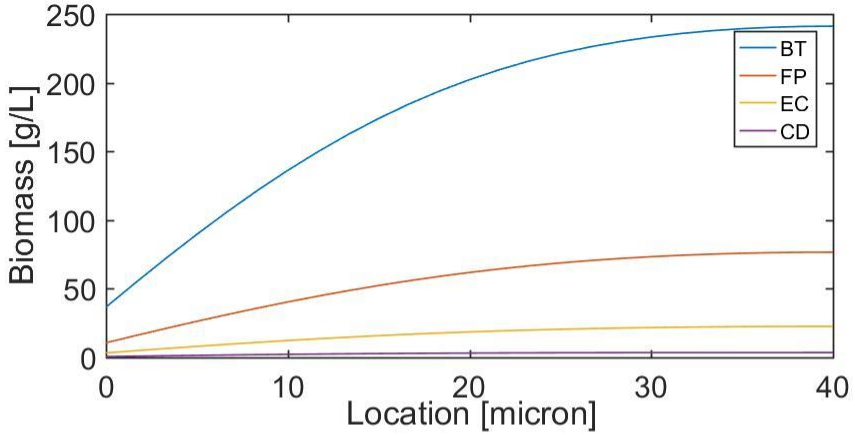
Phalak & Henson, submitted, 2018

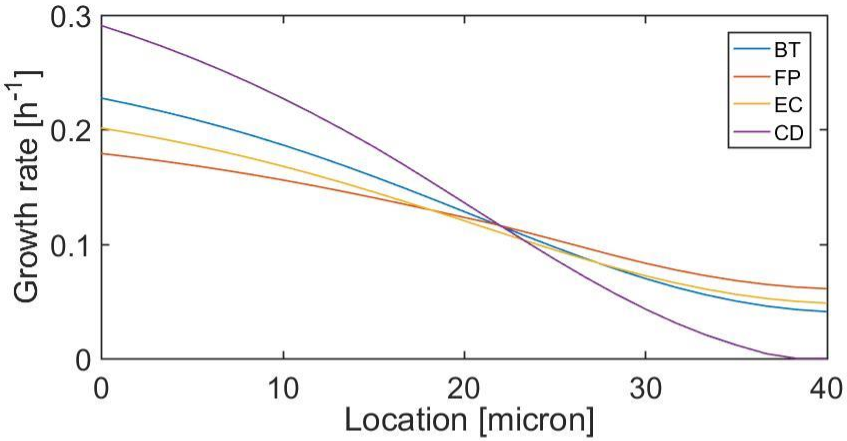
Predicted Crossfeeding Relationships

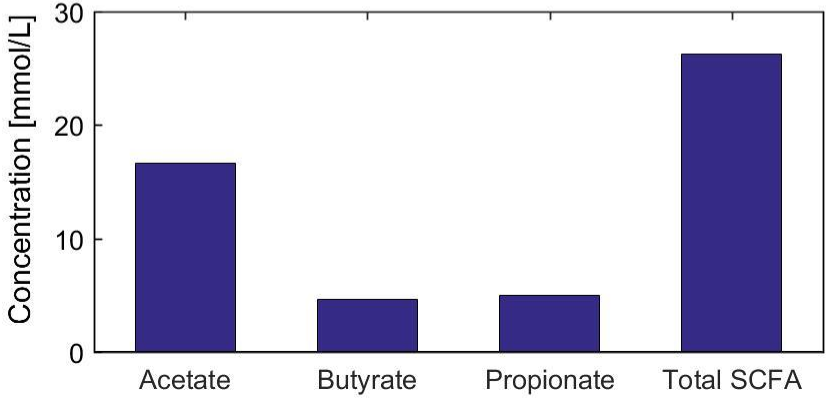


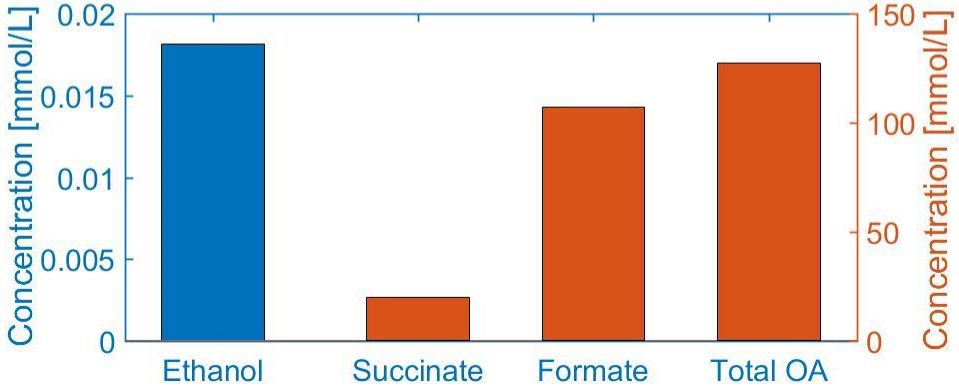
Phalak & Henson, submitted, 2018

Healthy State: No Host-Microbiota Perturbation

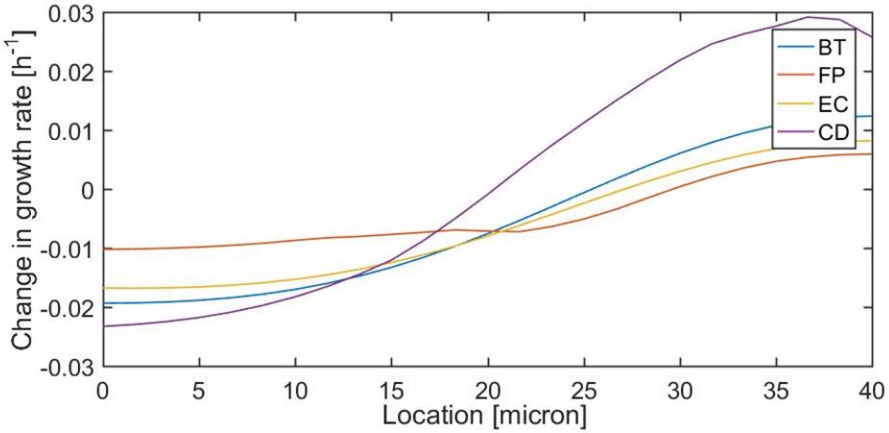


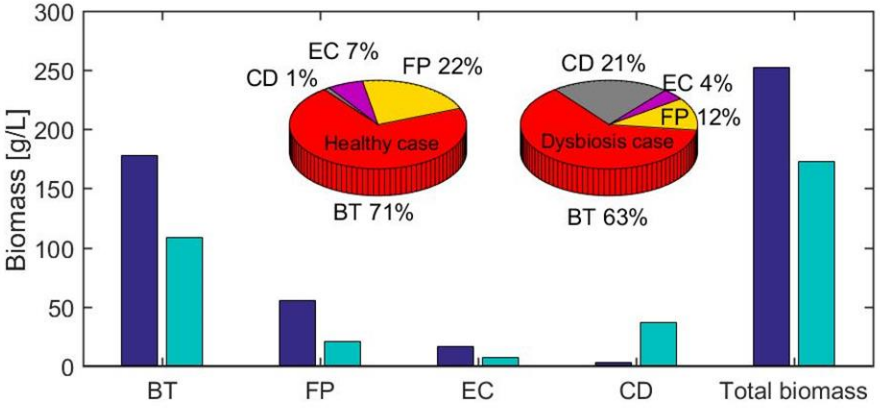


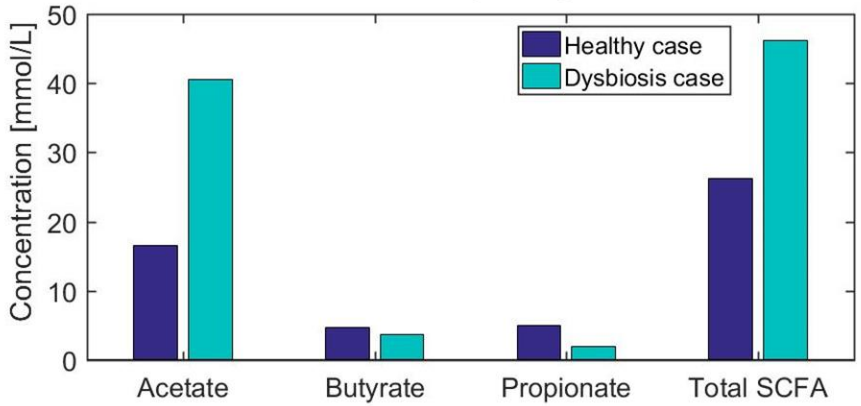


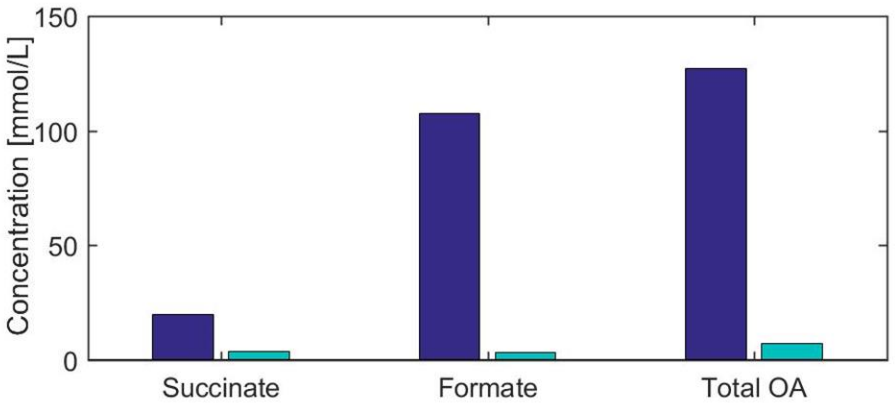


Glucose & Amino Acid Perturbations Induce Dysbiosis

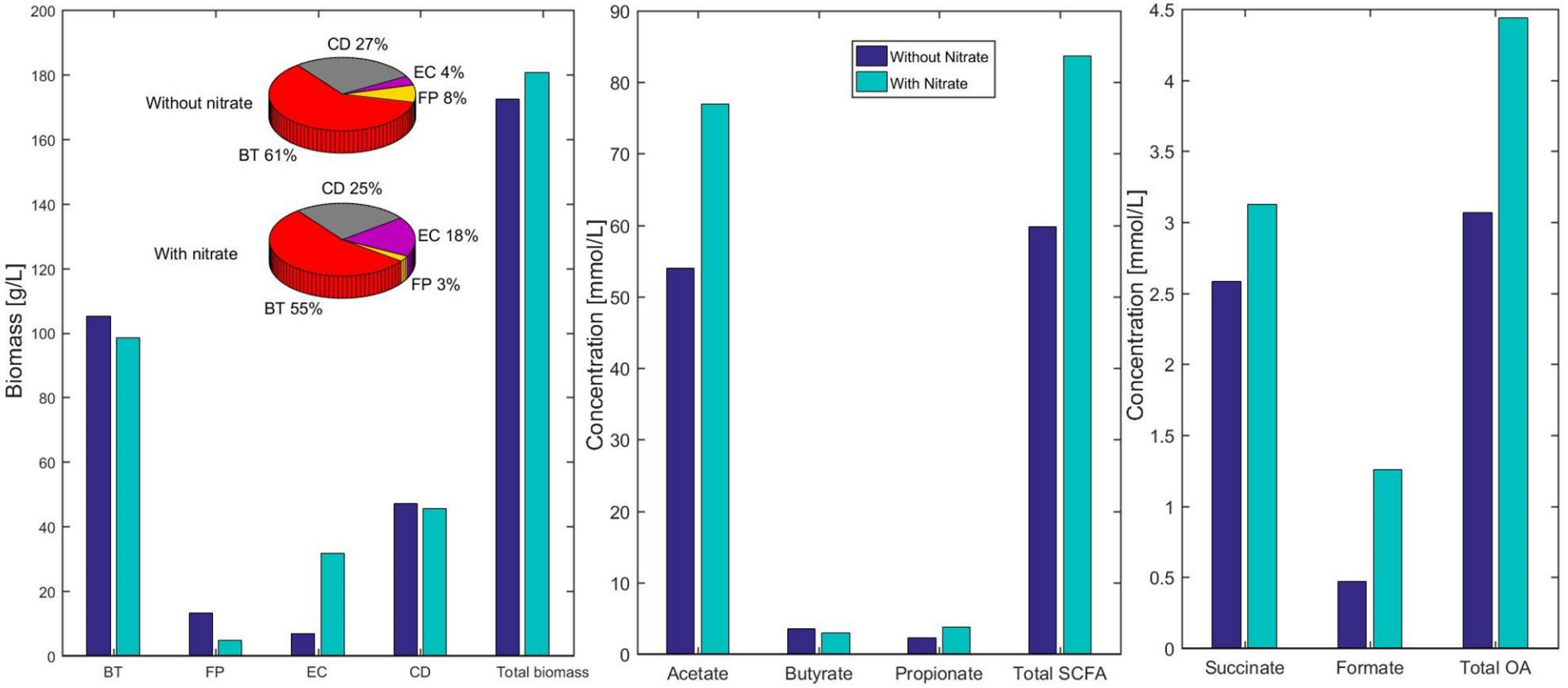




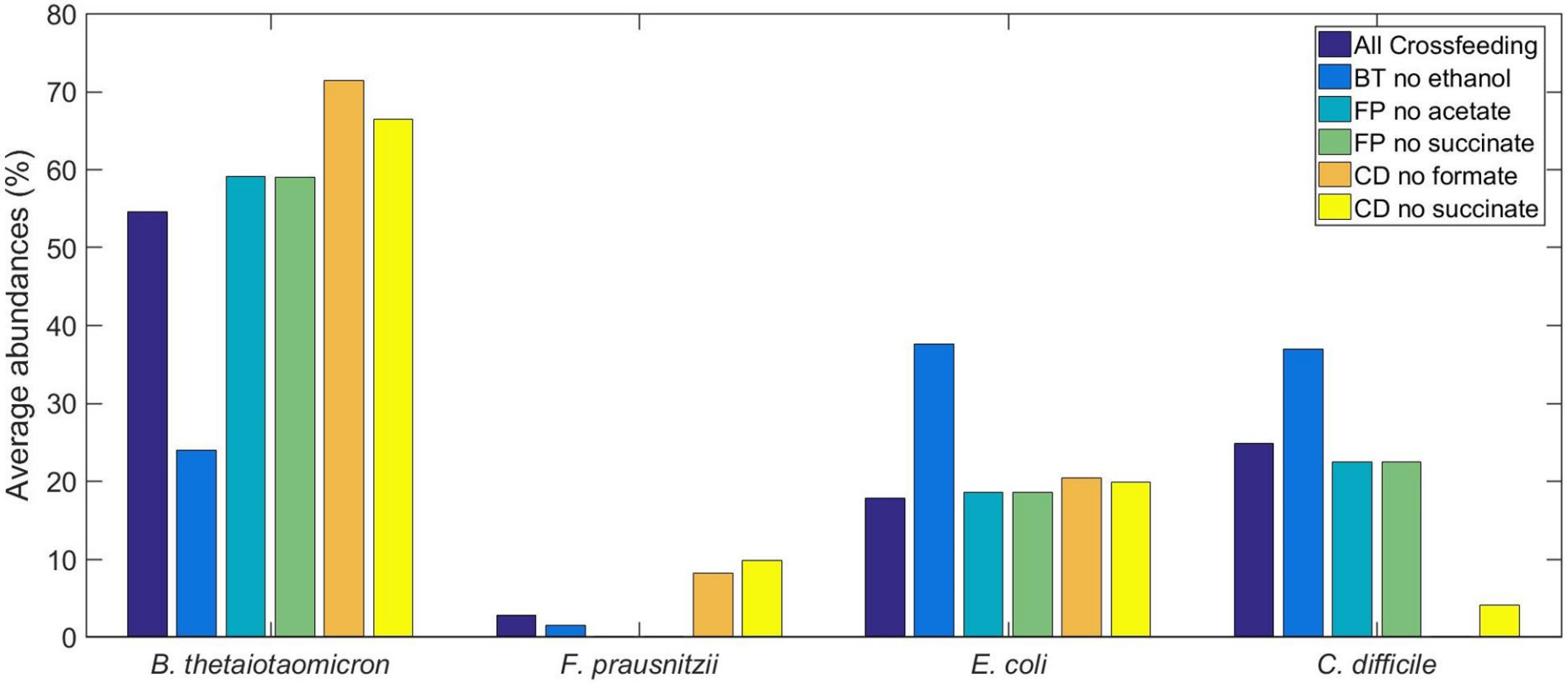




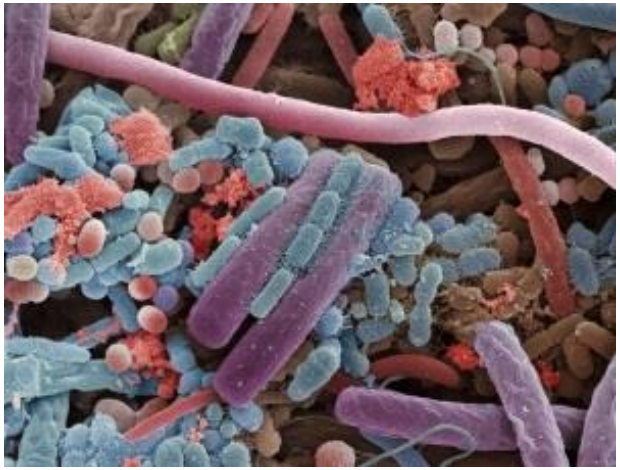
Additional Nitrate Perturbation Increases *E. coli*



C. difficile Expansion Requires Formate & Succinate

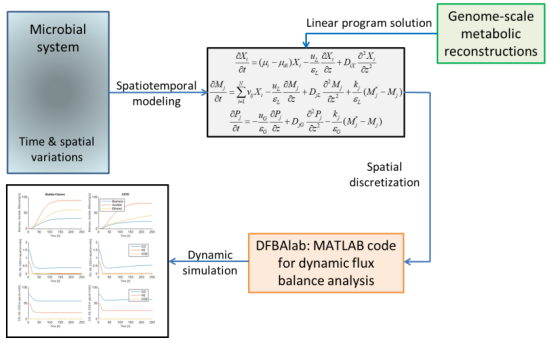


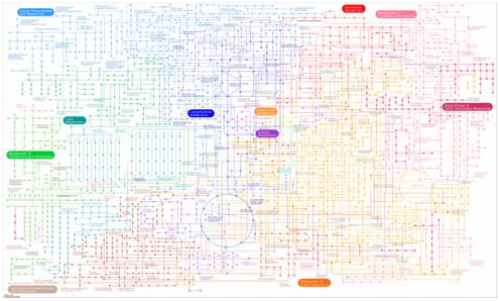
5. Concluding Remarks

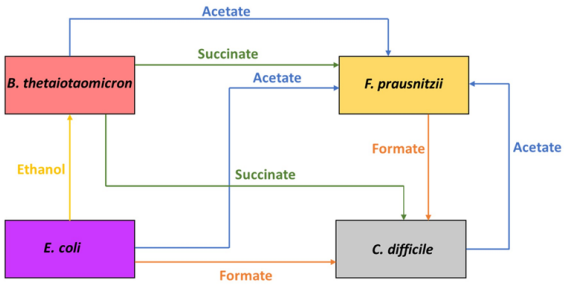


Key Points

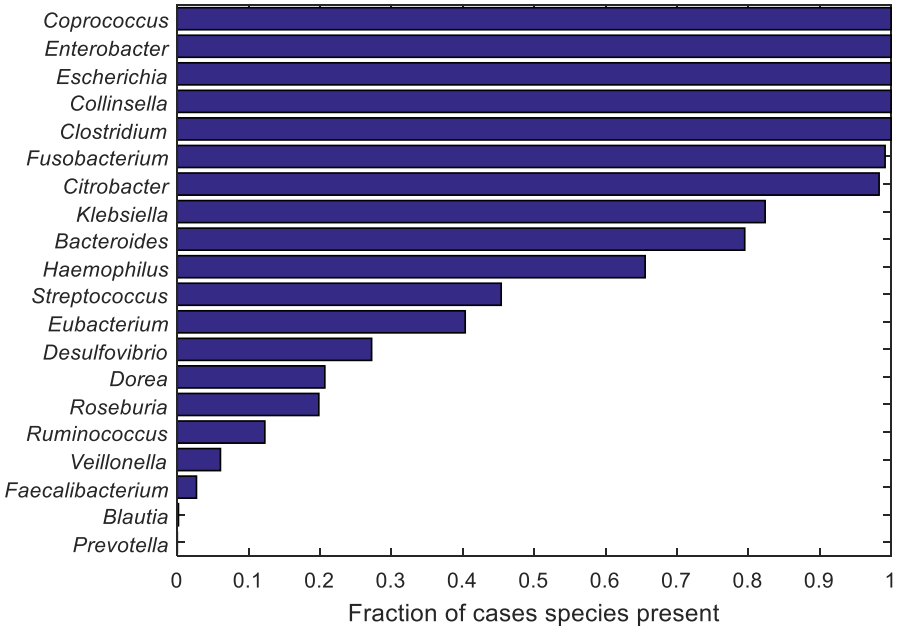
- Our biofilm metabolic modeling framework provides predictions of species interactions in heterogeneous environments
- Available genome-scale metabolic reconstructions can be incorporated directly
- Putative crossfeeding relationships can be discovered rather than assumed *a priori*
- Prediction of both temporal and spatial behavior can be computationally expensive

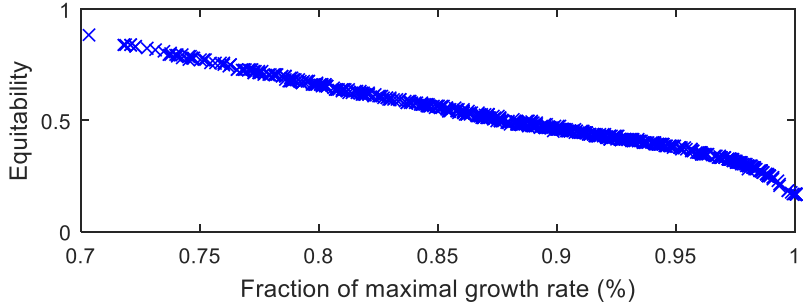


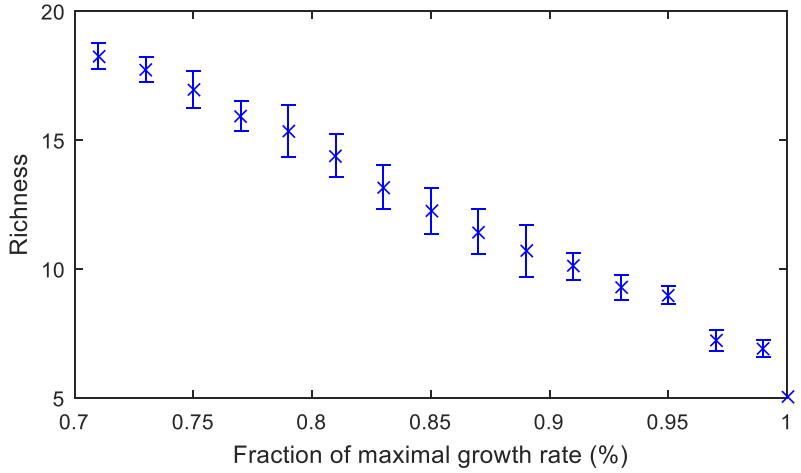


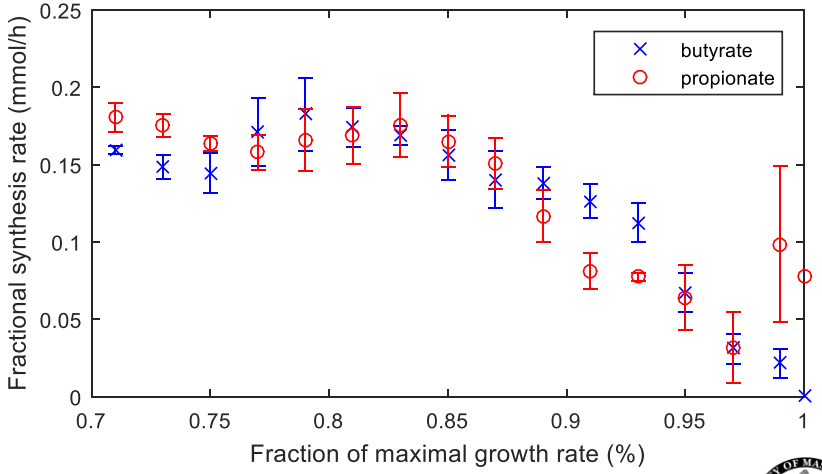


Future Direction – More Complex Communities









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  - Ross Carlson (Montana State U.) – chronic wounds
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Dr. Jin Chen

Questions?

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Poonam Phalak