Fecal Sludge Biorefineries based on a Volatile Fatty Acid Platform

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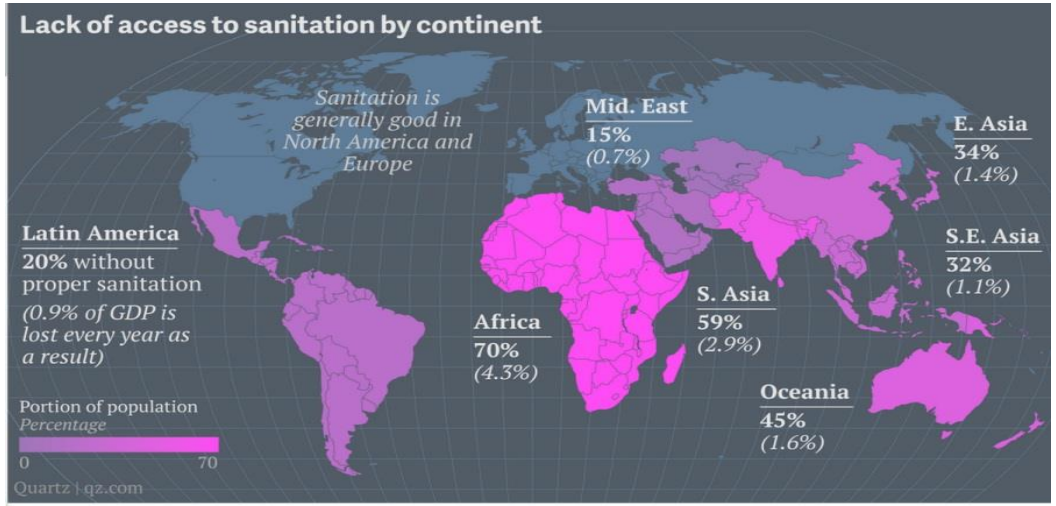
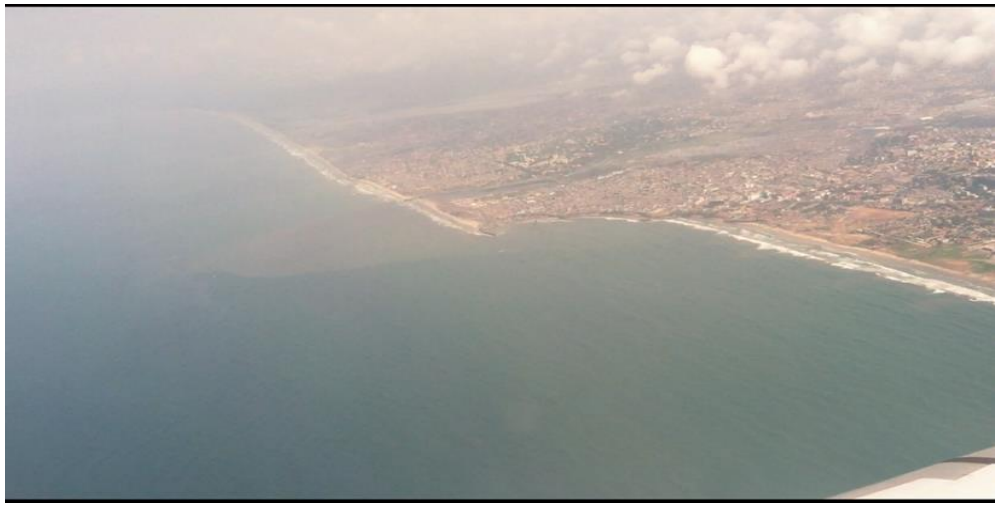




Lack of adequate sanitation is a global challenge

	Energy consumed annually (tera tons oe)	Energy consumed annually for water (assuming 3%, tera tons oe
USA	2.4	0.07
Ghana	0.01	?

Often limited by access to reliable energy inputs and chemicals

Is it possible to link sanitation with higher value chain biofuels and commodity chemicals?

??

Our approach

Domestic and Food waste

Faecal sludge

Municipal solid waste

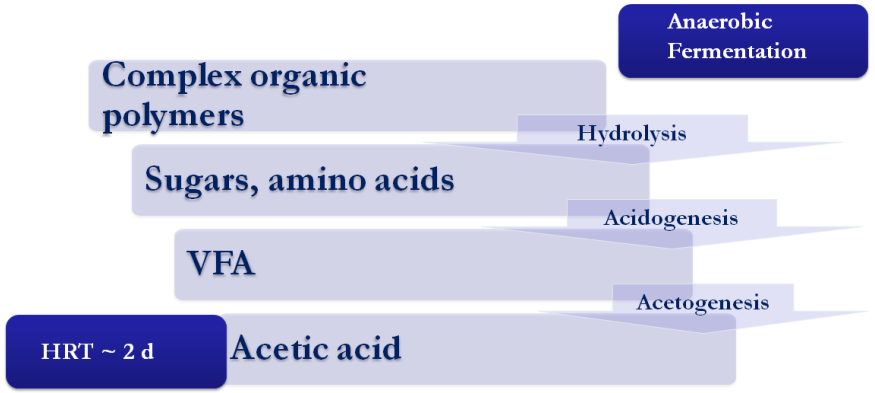
Animal by-product waste

Channel through fermentation platform

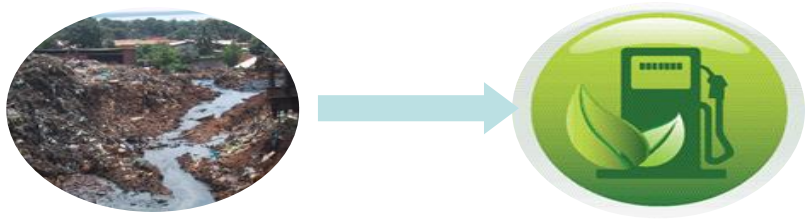
Commodity chemicals, lipids, biodiesel

BILL & MELINDA GATES foundation

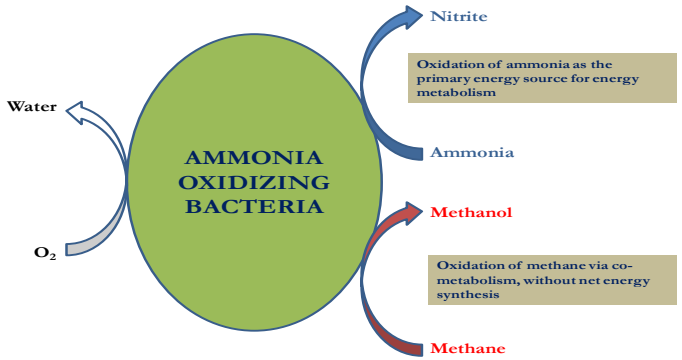
This presentation focuses on



Foundation for resource recovery through anaerobic C-conversions



Options for carbon recovery to fuels and chemicals



Resource efficient options for wastewater treatment and sanitation

Anaerobic Digestion

Complex organic polymers

Hydrolysis

Sugars, amino acids

Acidogenesis

VFA

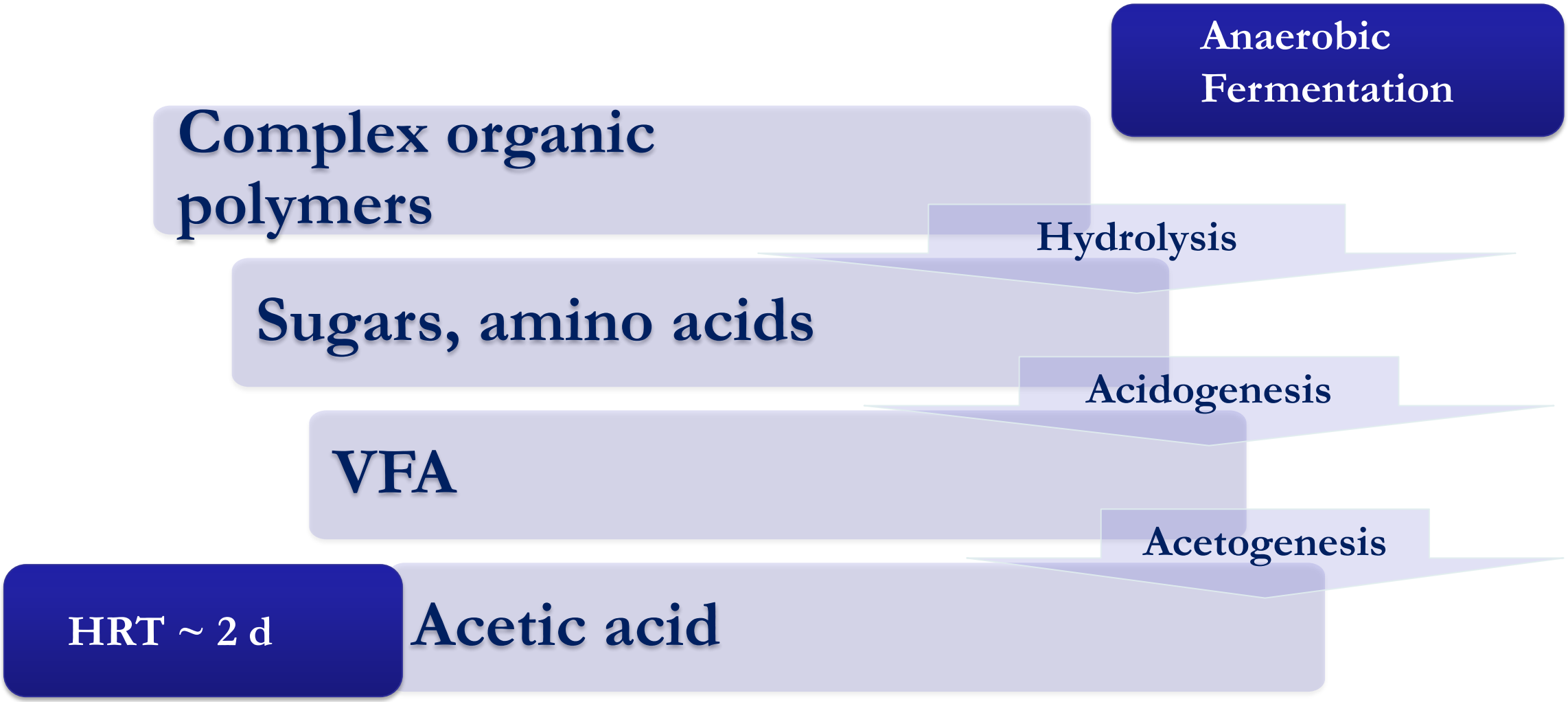
Acetogenesis

Acetic acid

Methanogenesis

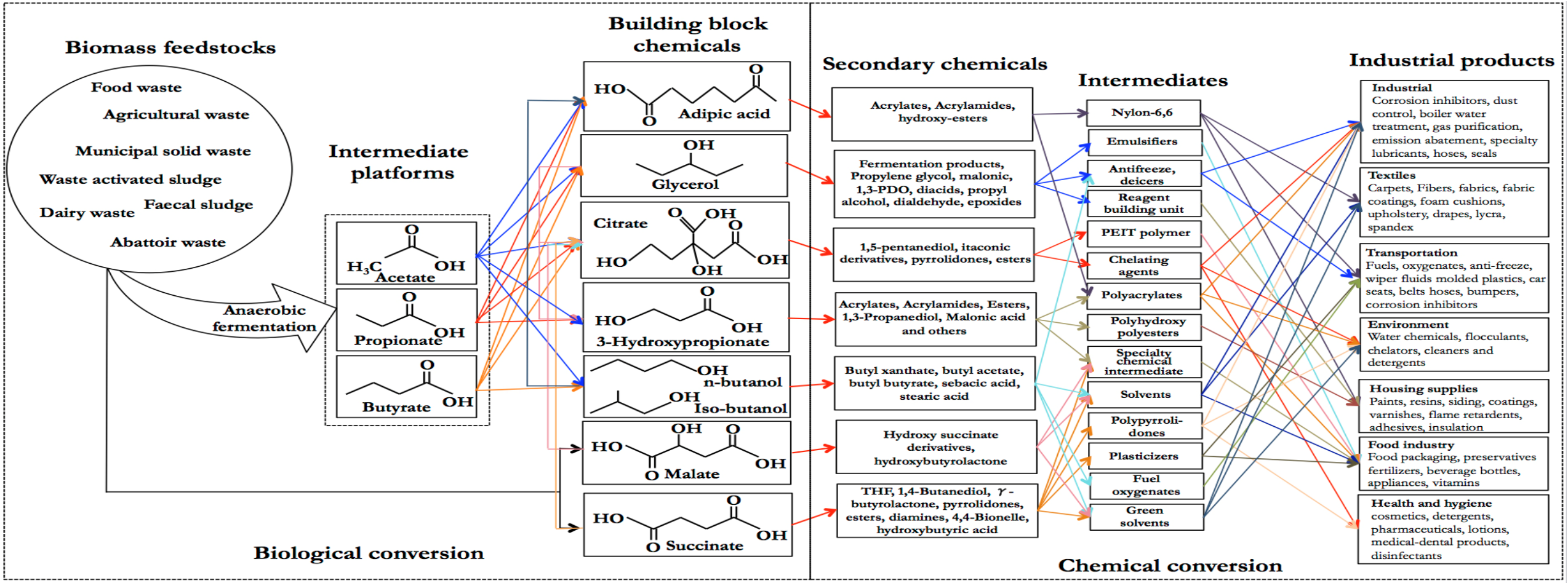
Methane

HRT > 10 d

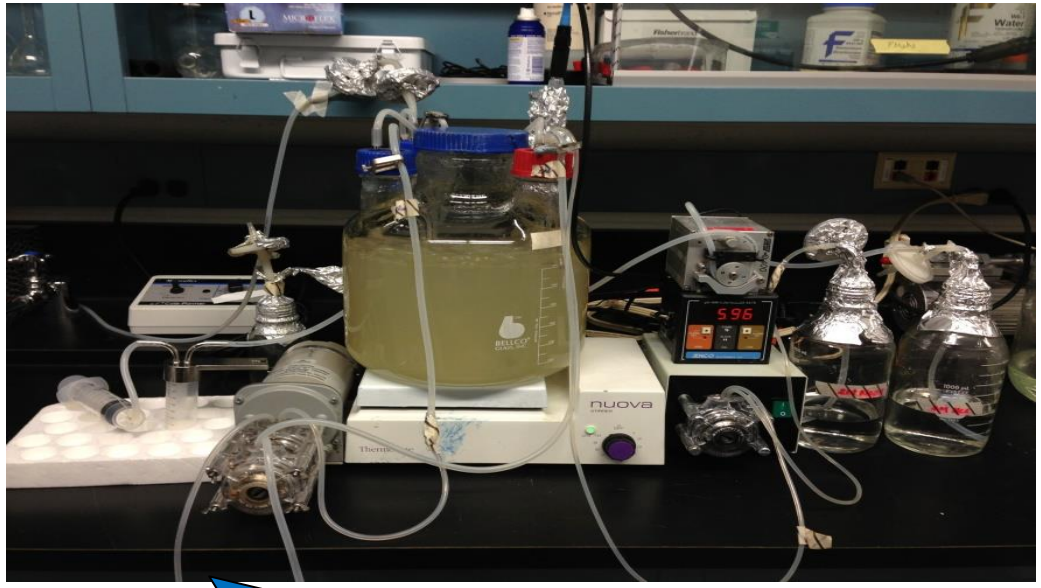


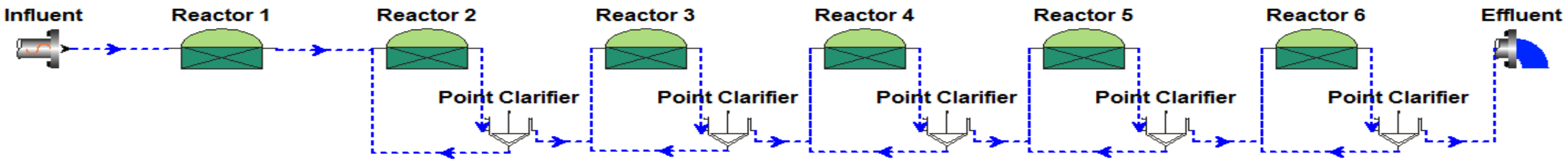
- Fermentation is more advantageous than just anaerobic digestion
- Fermentation can be incorporated into existing digestion processes

Fermentation as a platform for resource recovery



Overall Approach - Faecal Sludge to Chemical Products and Biodiesel



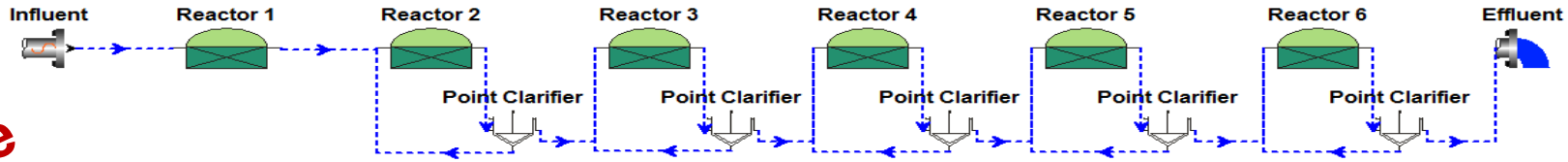


FS Fermentation and Digestion Model

Pit Latrines

Public Toilets

Private Septage



CH4

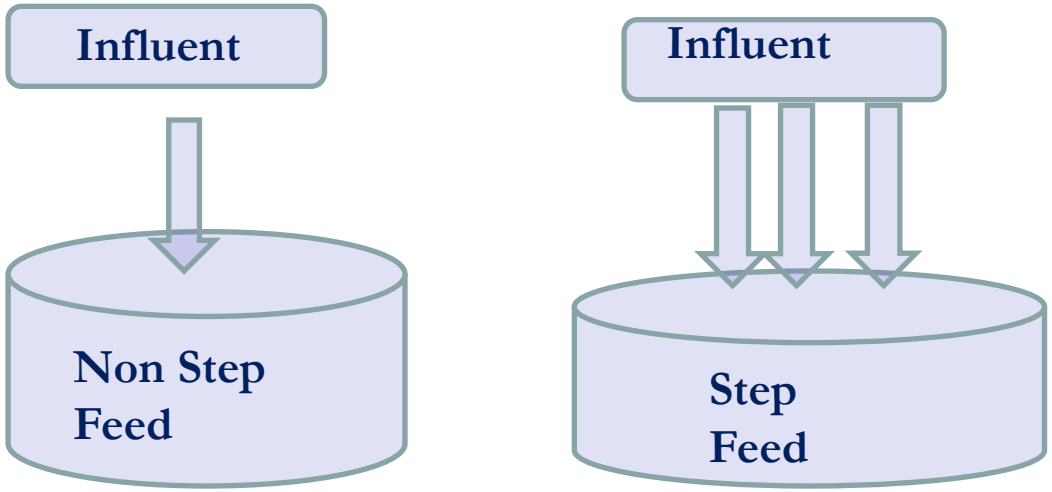
???

VFA

This model can be extended (and modified) for other fecal sludge processing operations

Microbial pathways involved in VFA production

1. Continuous lab-scale systems fed with food waste
2. Impact of reactor configuration on process and microbiology

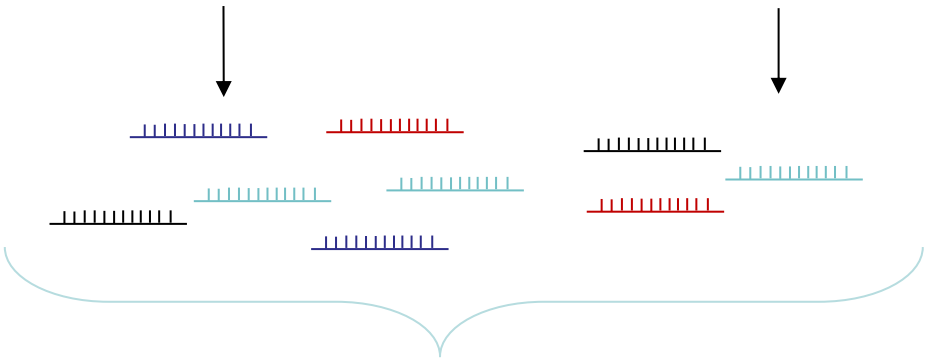


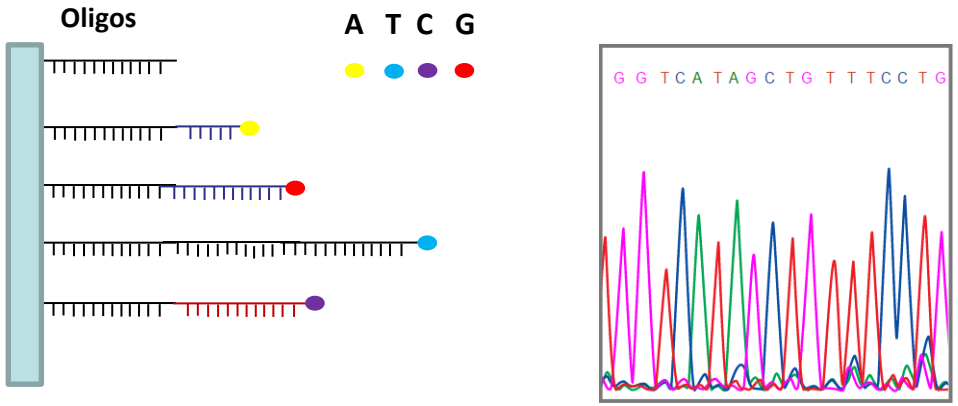
Near identical performance (VFA yield, speciation obtained from both syste designs

Martin *et al., 2015, 2017,* unpublished

Library Preparation/Sequencing

Mixed microbial community DNA





Illumina ® Flow Cell

Bioinformatics

ATGCTGAANTGCGATCTATGCTGNATGC
GTCANGTGTACGGGTGACGTAGNNCGTAATGCA
ATNCAGTCGTAGACTAGCNTAGCCGTTTTACGATC

Raw sequences

GTCANGTGTACGGGTGACGTAGNNCGTAATGCA
ATNCAGTCGTAGACTAGCNTAGCCGTTTTACGATC

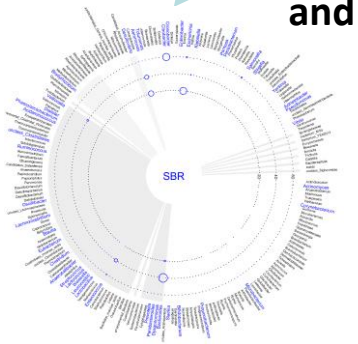
Trimming/Quality Control
(by size, # ambiguous bases, homopolymeric regions)

GTCANGTGTACGGGTGA
CAGTCCACATGCCCACTACGTACGTATGCATG

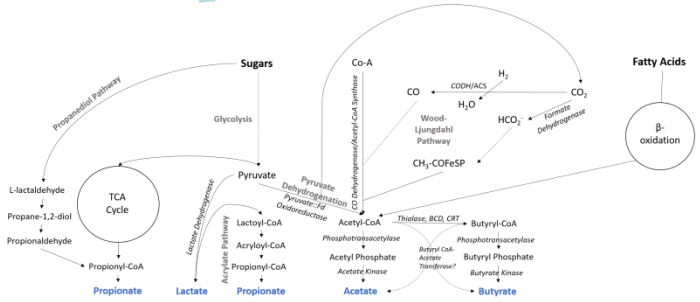
Alignment to Reference Database (Protein Coding Sequences)

Note: Coding regions of in-house annotated anammox species and recently published anammox and comammox species were manually appended to NCBI non-redundant (nr) protein database (ver. 123)

Data Processing and Visualization



Community Structure: Which organisms are contributing to the reactor's potential protein products?



Functional Potential: Which potential functional pathways is each sample capable of producing, and which organisms are likely to produce these proteins?

Notwithstanding identical process performance, microbial populations entirely different

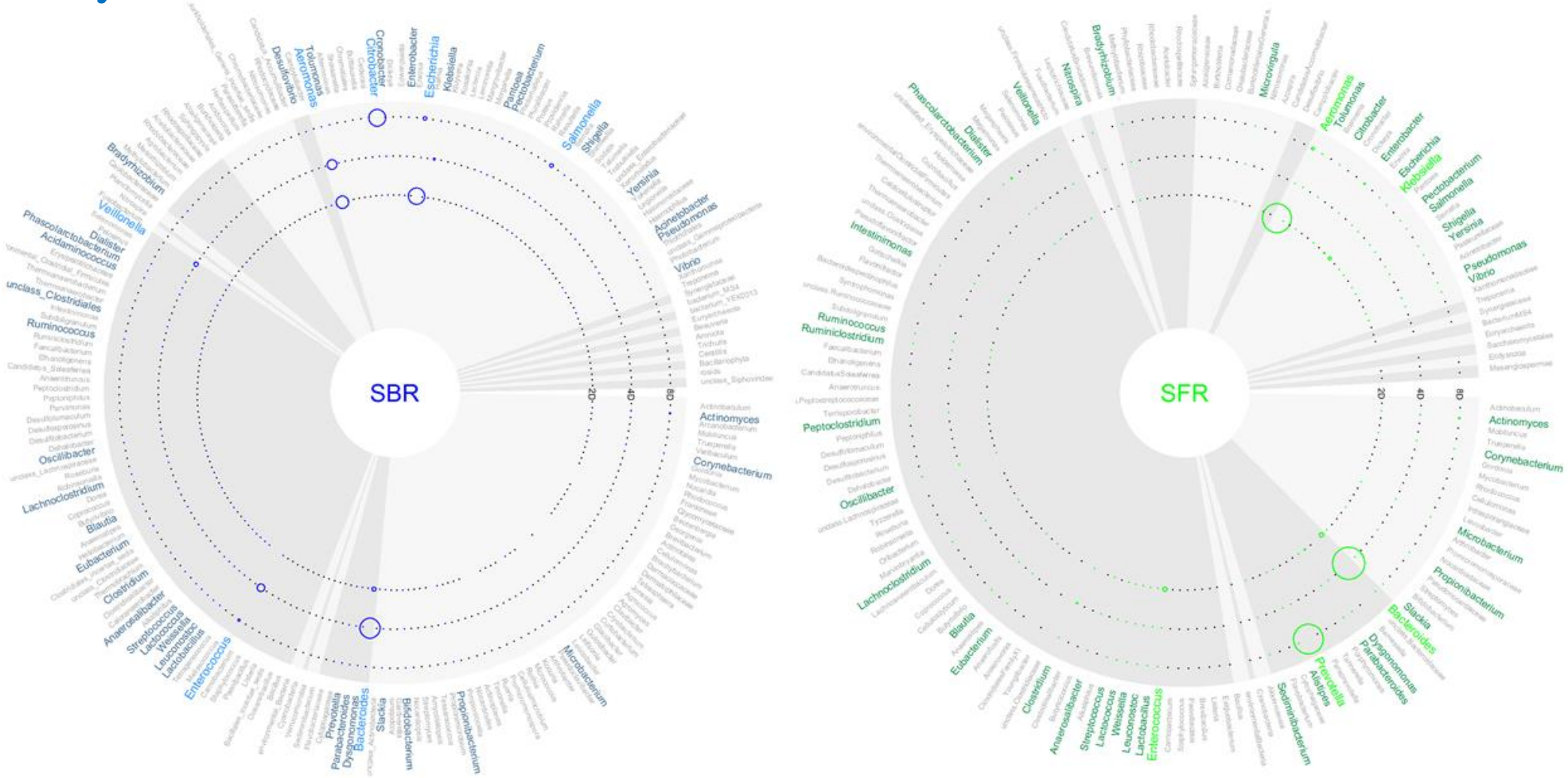
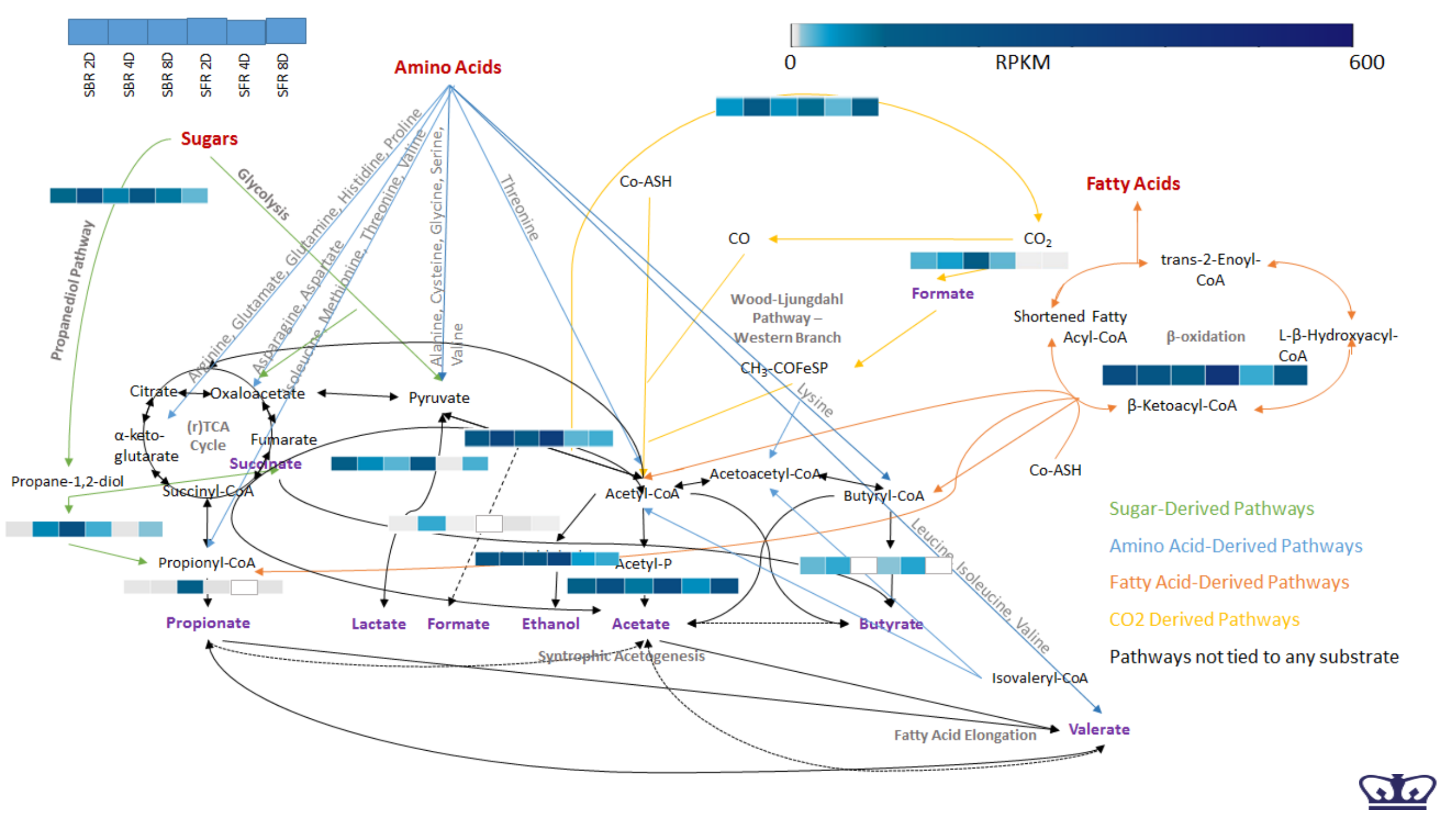
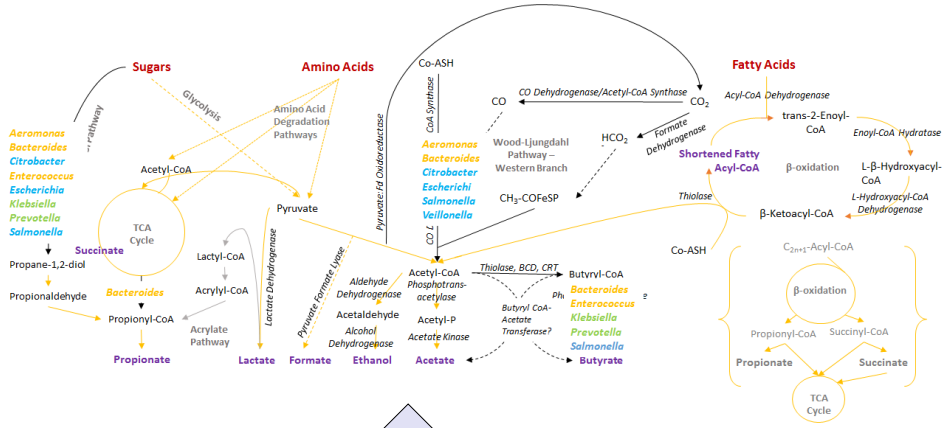


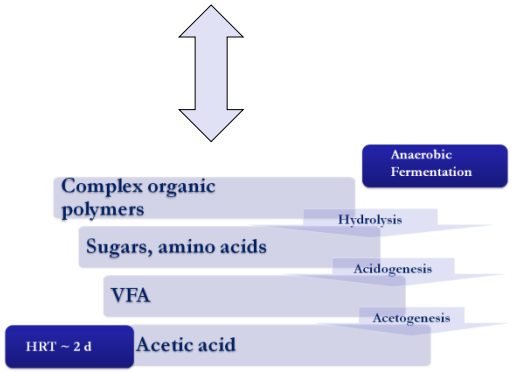
Figure 1. Comparison of microbial community profiles in both SBR and SFR at 8d, 4d, and 2d HRT; genera included have been assigned >= 50 reads per million (RPM) for at least one HRT; dark blue or green color indicates assignment of >= 1,000 RPM; light blue or green color indicates assignment of >= 50,000 RPM; dot size scaled to RPM values; shading reflects phylum-level relationships


SBR 2D
SBR 4D
SBR 8D
SFR 2D
SFR 4D
SFR 8D
0
RPKM
600
Amino Acids
Sugars
Glycolysis
Propanediol Pathway
Arginine, Glutamate, Glutamine, Histidine, Proline
Asparagine, Aspartate
Isoleucine, Methionine, Threonine, Valine
Alanine, Cysteine, Glycine, Serine, Valine
Threonine
Co-ASH
CO
CO_2
Formate
Wood-Ljungdahl Pathway – Western Branch
CH_3-COFeSP
Lysine
Fatty Acids
trans-2-Enoyl-CoA
Shortened Fatty Acyl-CoA
β-oxidation
L-β-Hydroxyacyl-CoA
β-Ketoacyl-CoA
Co-ASH
Citrate
Oxaloacetate
Pyruvate
α-keto-glutarate
(r)TCA Cycle
Fumarate
Succinate
Succinyl-CoA
Propane-1,2-diol
Acetyl-CoA
Acetoacetyl-CoA
Butyryl-CoA
Propionyl-CoA
Acetyl-P
Leucine, Isoleucine, Valine
Propionate
Lactate
Formate
Ethanol
Acetate
Butyrate
Syntrophic Acetogenesis
Isovaleryl-CoA
Fatty Acid Elongation
Valerate
Sugar-Derived Pathways
Amino Acid-Derived Pathways
Fatty Acid-Derived Pathways
CO2 Derived Pathways
Pathways not tied to any substrate

Significance

- As we attempt to move away from ‘digestion’ or ‘fermentation’ towards carbon recovery and biorefining, we need more structured information
- It becomes beneficial to link
  - Process configurations and operating conditions with microbial ecology, metabolic function
    - and in turn with product yield and speciation
  - Feedstocks (can import or mine added ones) with products
- Not needed for every case
  - Synthesized approaches needed to enhance translation





Conversion of VFA to Lipids

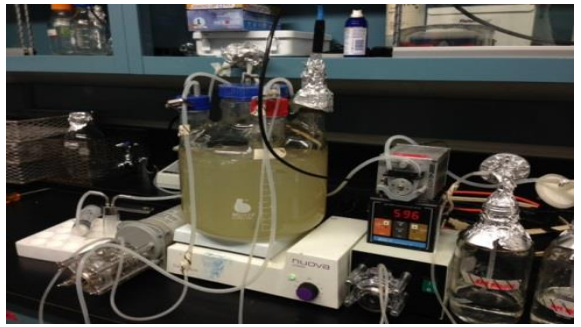
- Different COD sources
  - VFA from food waste fermentation
  - Synthetic VFA
  - Glucose
- Different initial VFA concentrations

6:1:3 acetate, propionate, butyrate. 2 day HRT

- Different initial N concentrations
  - Excess N: COD:N = 5:1
  - Limiting N: COD:N = 25:1, 50:1, 125:1, 250:1
  - Stoichiometric COD:N supply =33:1

Lipid content of *C. albidus*

Batch reactor

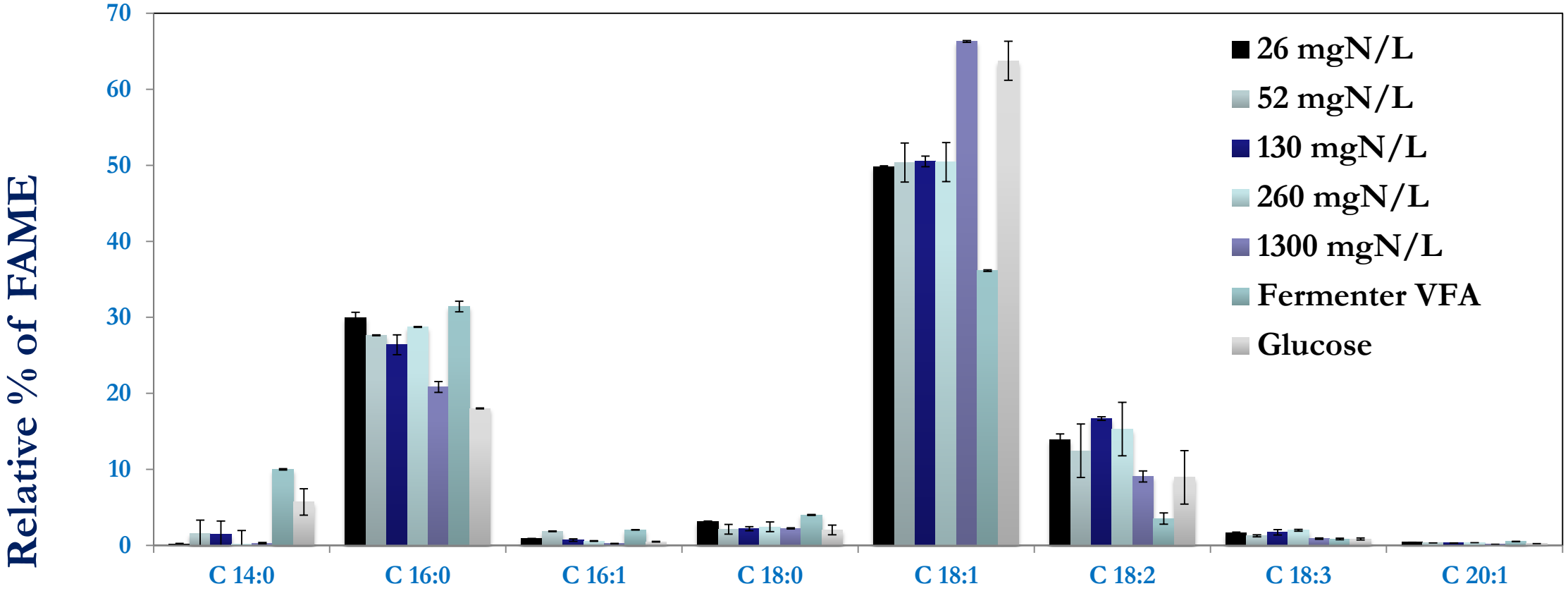


Chemostat

Vajpeyi and Chandran, 2015

Vajpeyi and Chandran, 2015

Lipid Composition

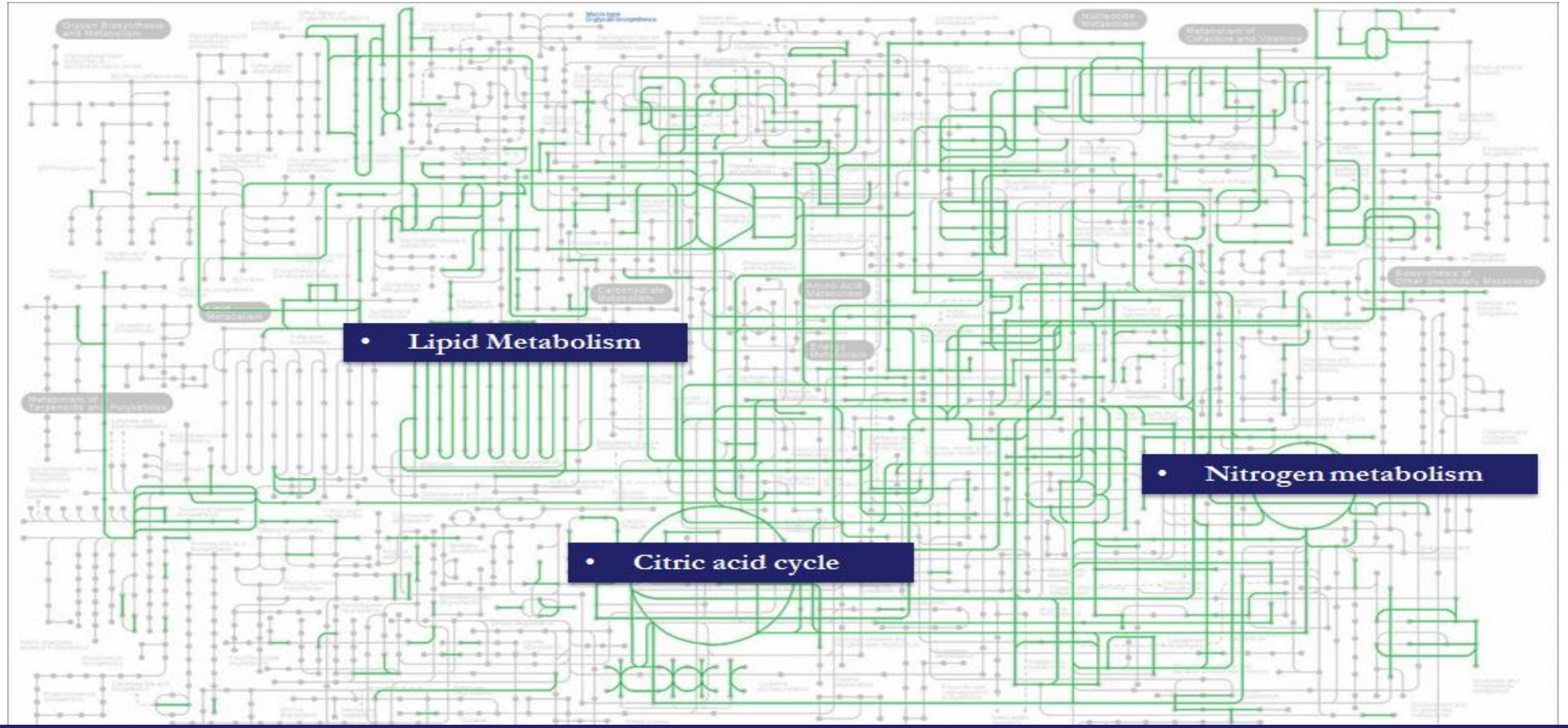


Major fatty acids accumulated are palmitic (C16:0), oleic (C18:1), and linoleic acid (C18:2)

Similar to soybean oil and jatropha oil, which are used as feedstock for biodiesel production in the US and the EU

What else can *C. albidus* accumulate (or do)?

Under what conditions?

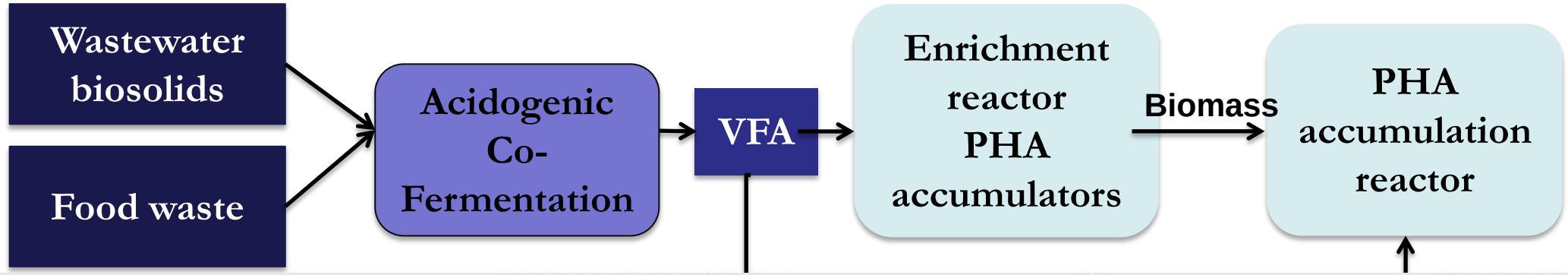


Total size ~ 25 Mbp, Genbank accession number LKPZ 00000000.1

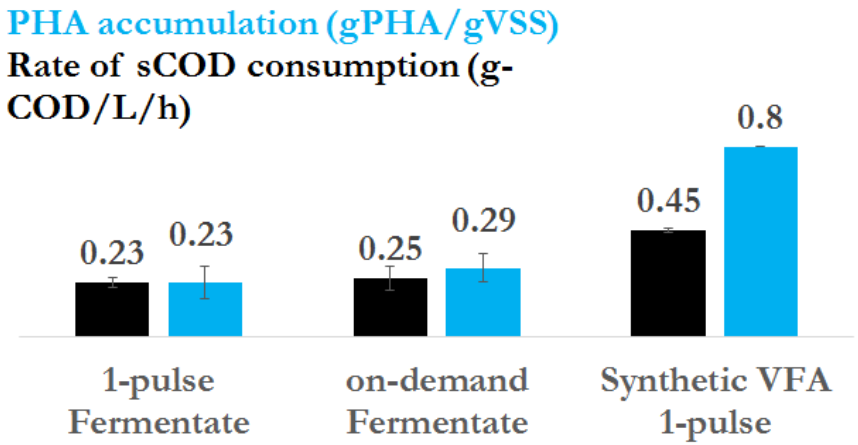
Vajpeyi and Chandran, 2016

Conversion of VFA to Bioplastics

Our study:



Reference	Wastewater type	f_{VFA} (g-COD_{VFA}/g-COD_{sCOD})		Max PHA content (gPHA/gVSS)
Morgan-Sagastume et al., 2010	Waste sludge stream	60%	HRT=5d	21%
Reddy and Mohan 2012	Food waste	53%	HRT=3d	40%
Pavlakis E. et al. (this study)	Food waste	44%	HRT=2d	N/A
	Wastewater biosolids + Food waste	35%	HRT=2d	23%



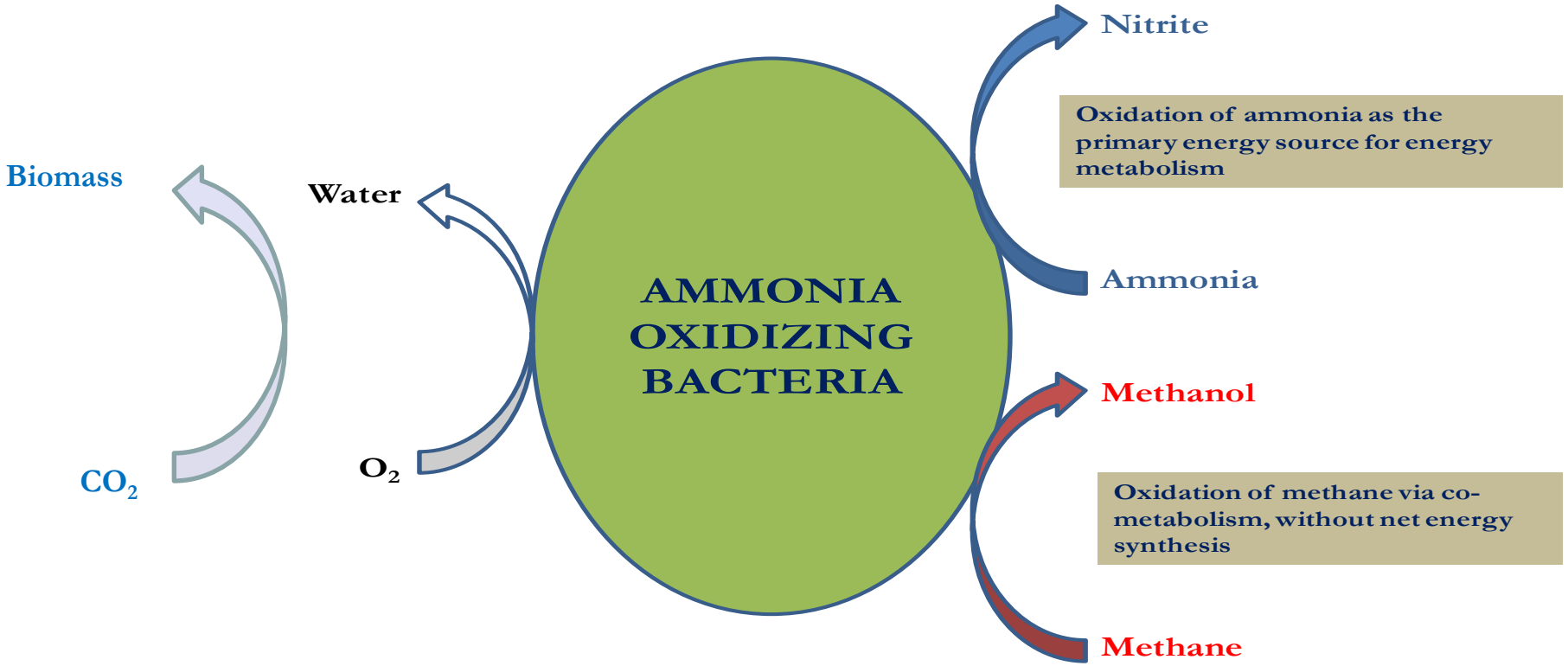
Water Quality-Energy Implications



- Another version of carbon refining
  - Separation of N-P and C
  - Converting the carbon to the appropriate form for meeting WQ objectives

Org-N and NH_3
Org-N and NH_3
Org-N and NH_3

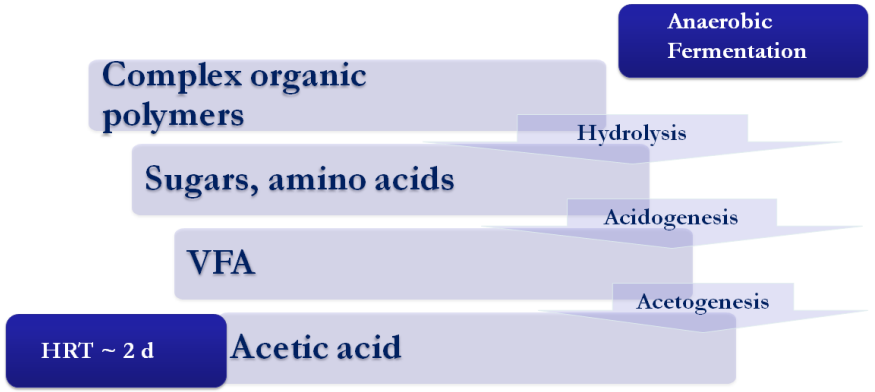
III. C. From Greenhouse Gas to Green Fuel



- Concomitant oxidation of CH_4 and CO_2 fixation
- Prospect of combining C &N cycles

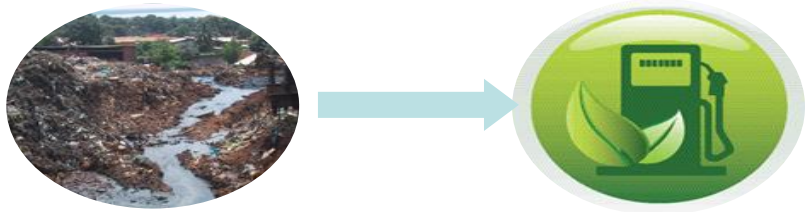
Taher and Chandran, ES&T, 2013

Concluding remarks



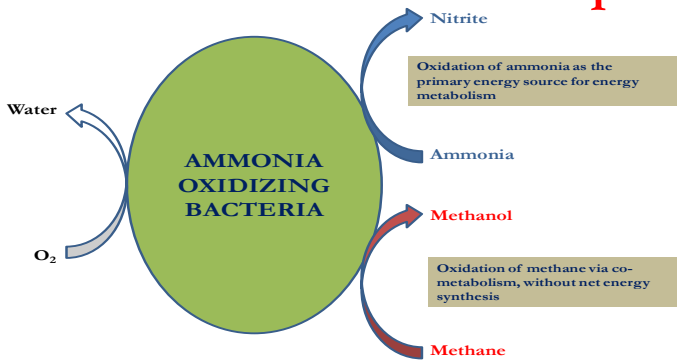
Channeling anaerobic C-conversions through SC-FA offers attractive flexible prospects for resource recovery

Detailed understanding of microbial structure and function in conjunction with reductionist approaches needed to advance implementation



Wide variety of endpoints (chemicals, fuels..) possible

Disrupting conventional agro- or fossil-based pathways



Links to other applications needed and possible

Resource efficient options for FSM and sanitation

Acknowledgements: Shashwat Vajpeyi, Medini Annavajhala, Justin Shih, Ato Fanyin Martin, Edris Taher, Yu-Chen Su