

OVERFLOW METABOLISM IN METHANOTROPHIC BACTERIA

C₁ Biocatalysis Lab

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SDSU

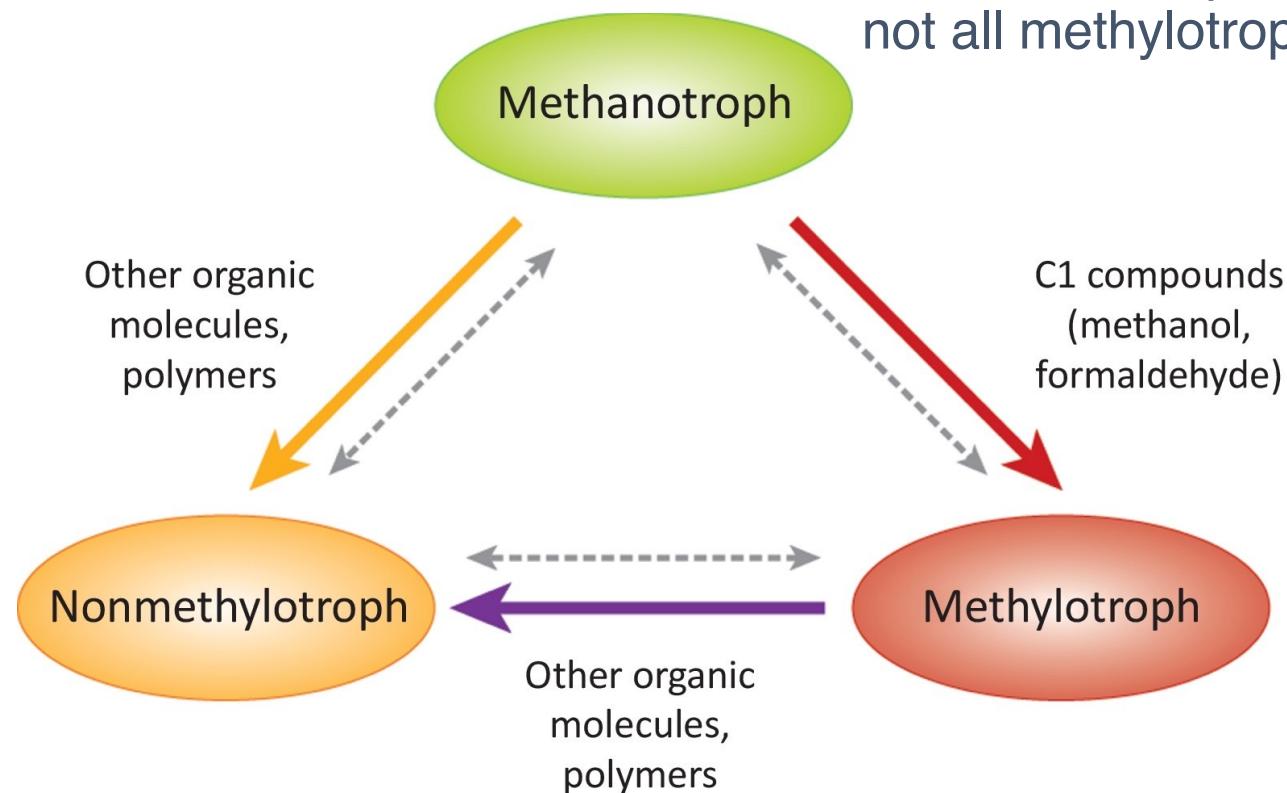
Committee: Dr. Marina Kalyuzhnaya (Chair, Biology),

Dr. Parag Katira (Engineering), and

Dr. Arun Sethuraman (Biology)

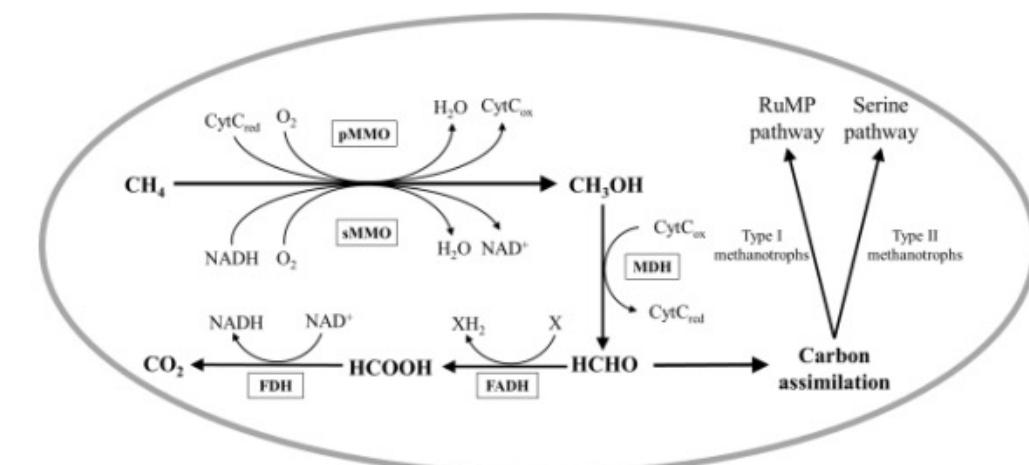
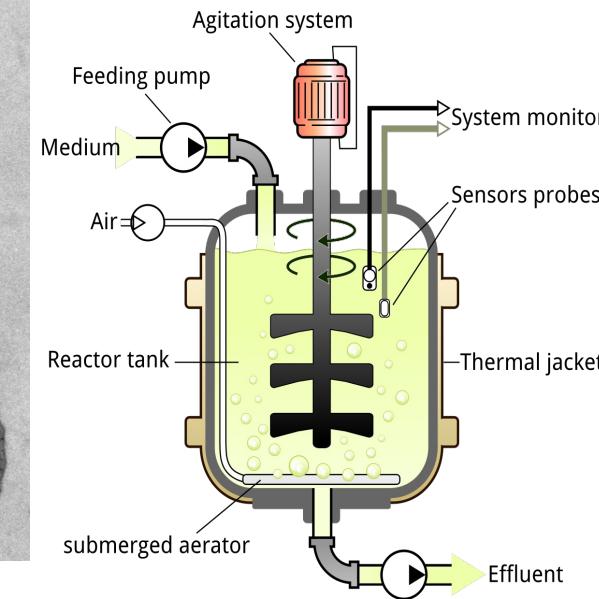
What is Methylotrophy/Methanotrophy?

- Methylotrophy is the ability to utilize reduced single-carbon compounds (i.e., methane, methanol, methylamine)
 - Methylotrophs are a diverse group of microorganisms which are capable of methylotrophy
- Methanotrophy is the specific ability of some methylotrophs to oxidize methane
 - Methanotrophs are a subset of methylotrophs which are capable of methanotrophy
 - All methanotrophs are methylotrophs, but not all methylotrophs are methanotrophs

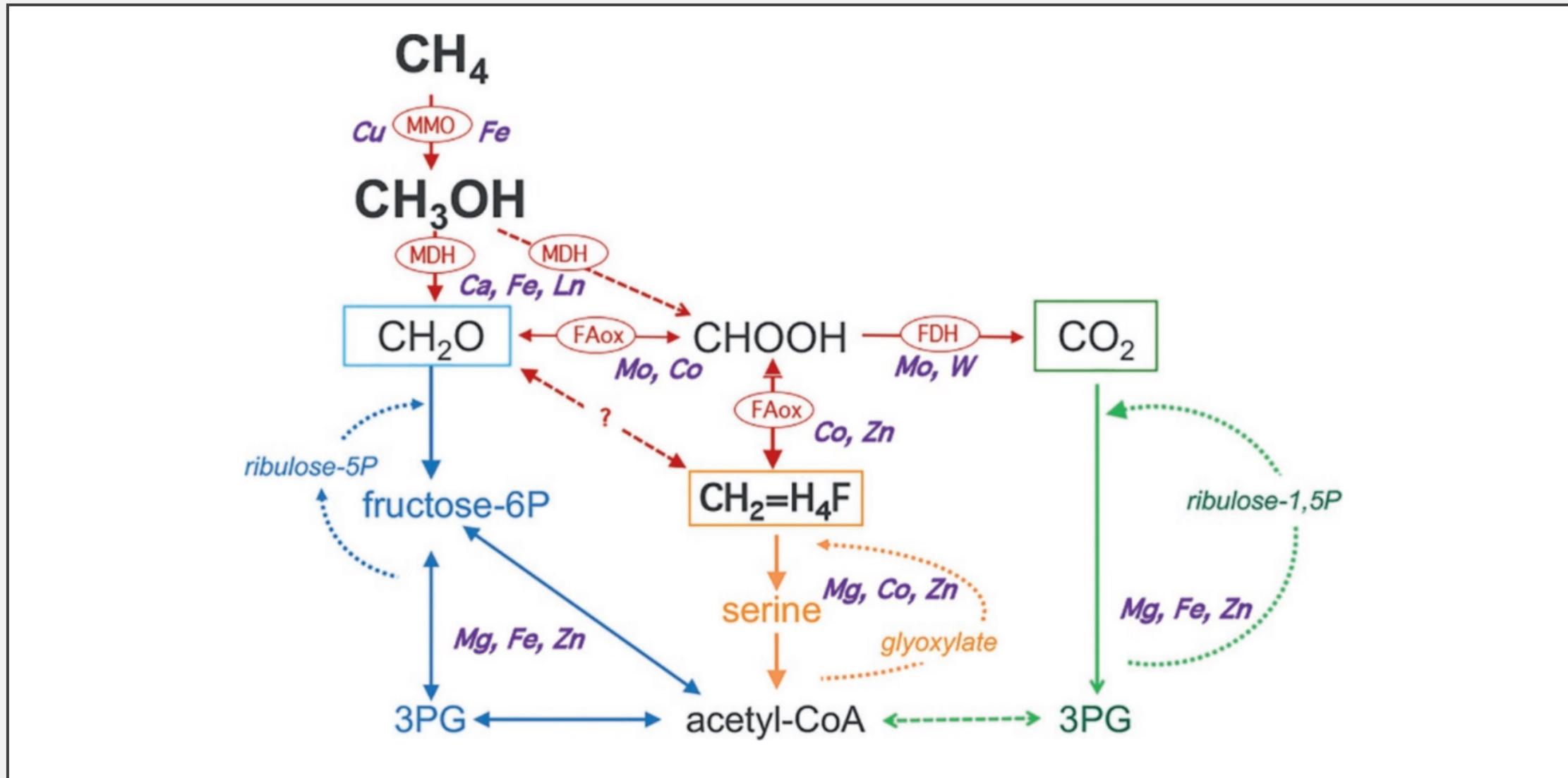


The field of methanotrophy

- Methane-oxidizing bacteria (MOB) are a diverse and highly specialized group of gram-negative bacteria which utilize single-carbon molecules as their main source of energy
 - Found and isolated from a variety of environments such as rice fields, forests, freshwater sources, swamps, and deserts
 - Cultured in a broad range of temperatures, pH, and saline concentrations
- In nature, methanotrophs can serve as bio-filters to remove CH₄ molecules from the environment
- Methane bio-utilization requires a set of dedicated enzymes:
 - Methane monooxygenase (MMO): oxidizes methane to methanol
 - Methanol dehydrogenase (MDH): converts methanol to formaldehyde
 - Formaldehyde activating enzyme (FAE): aids with formaldehyde use – formaldehyde can be used for the biosynthetic/anabolic pathways, or further oxidized to formic acid, then carbon dioxide
 - Formate dehydrogenase (FDH): oxidizes formic acid to carbon dioxide
- Methanotrophic bacteria represent a unique system for converting methane to bio-based materials



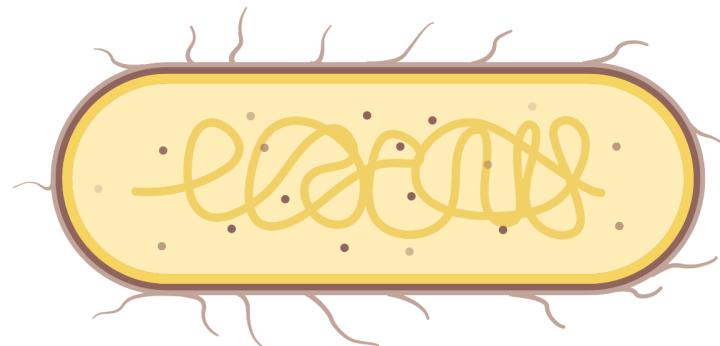
METAL REQUIREMENTS FOR CORE C₁ METABOLISM



What is Overflow Metabolism?

- Overflow metabolism is the biological phenomenon where fast growing cells wastefully excrete metabolic intermediates due to incomplete oxidation of their growth substrate.
- Overflow metabolism has been noted in all domains of life
- Common examples of overflow metabolites include acetate, formate, lactate, and ethanol.

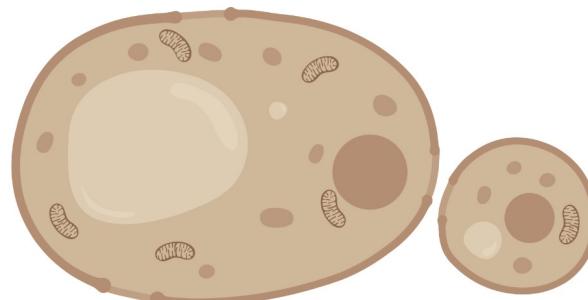
E. coli



Acetate

Vemuri, G. N., et al. (2006). Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. *Applied and environmental microbiology*, 72(5), 3653-3661.

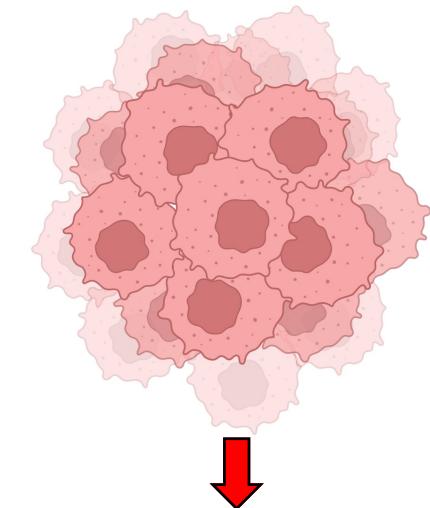
Yeast
Crabtree Effect



Ethanol

Perez-Samper, G. et al. (2018). The crabtree effect shapes the *Saccharomyces cerevisiae* lag phase during the switch between different carbon sources. *MBio*, 9(5), e01331-18.

Cancer
Warburg Effect



Formate + Lactate

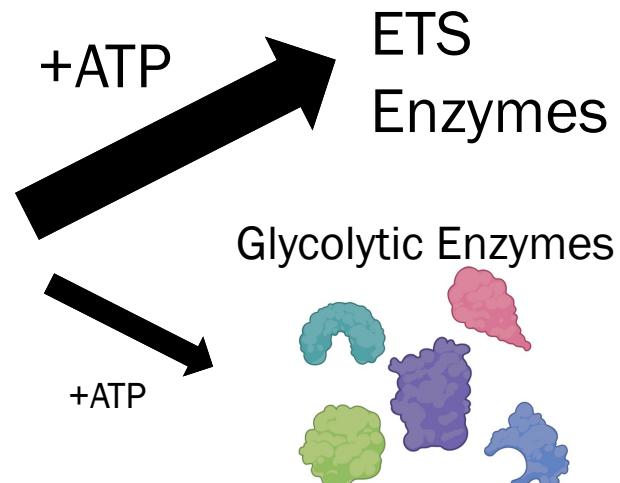
Vaupel, P., Schmidberger, H., & Mayer, A. (2019). The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. *International journal of radiation biology*, 95(7), 912-919.

What causes Overflow Metabolism to happen?

Main hypotheses

Protein Cost Hypothesis

The enzymatic cost of respiratory enzymes (i.e., ETS) exceeds that of fermentative enzymes (i.e., glycolytic)

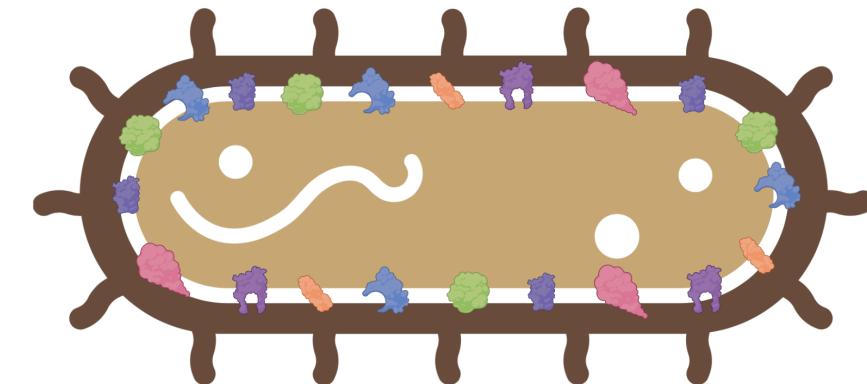


Veit, A., Polen, T., & Wendisch, V. F. (2007). Global gene expression analysis of glucose overflow metabolism in *Escherichia coli* and reduction of aerobic acetate formation. *Applied microbiology and biotechnology*, 74(2), 406-421.

Basan, M. et al. (2015). Overflow metabolism in *Escherichia coli* results from efficient proteome allocation. *Nature*, 528(7580), 99-104.

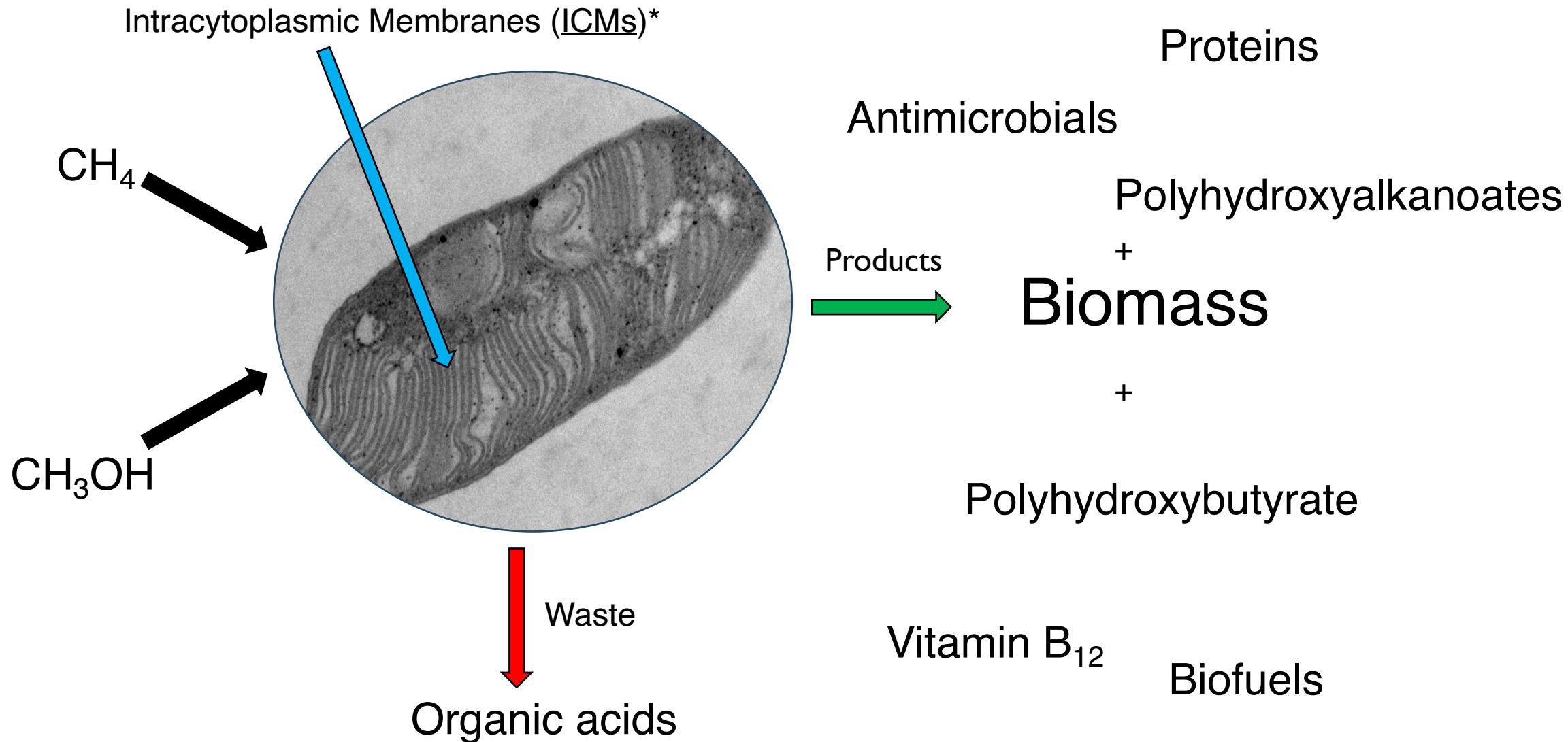
Membrane Real Estate Hypothesis

Fast-growing cells are saturated with membrane-bound machinery and lack the free space required to produce additional respiratory enzymes



Szenk, M., Dill, K. A., & de Graff, A. M. (2017). Why do fast-growing bacteria enter overflow metabolism? Testing the membrane real estate hypothesis. *Cell Systems*

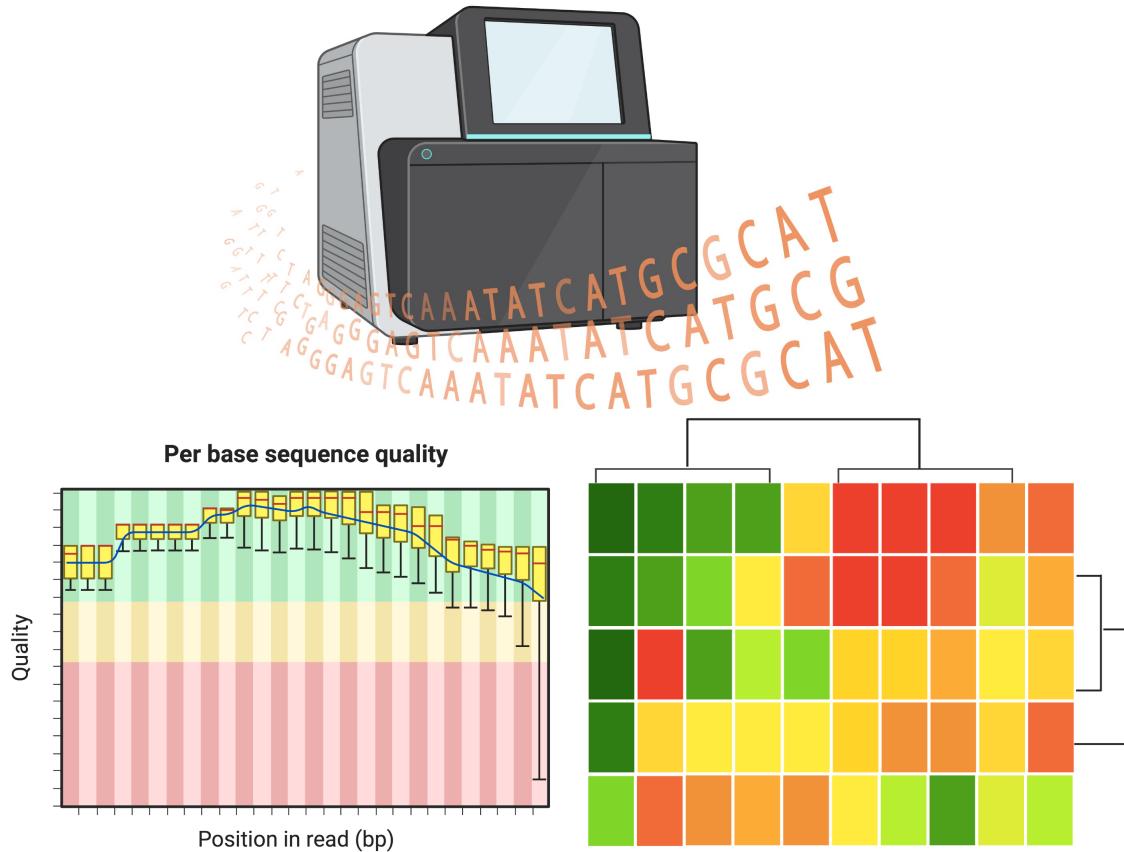
Overflow Metabolism in *Methylomicrobium alcaliphilum*



METHYLOMICROBIUM ALCALIPHILUM AS A MODEL SYSTEM

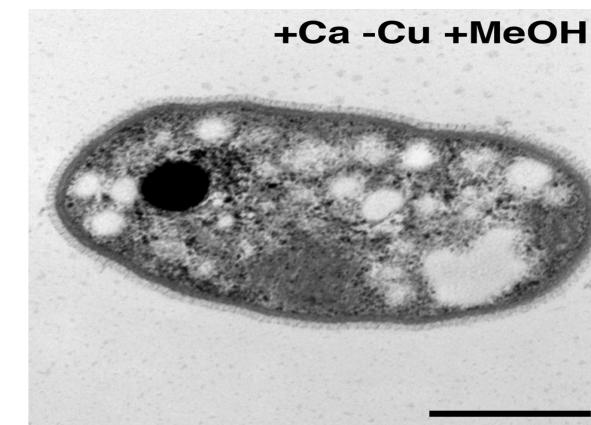
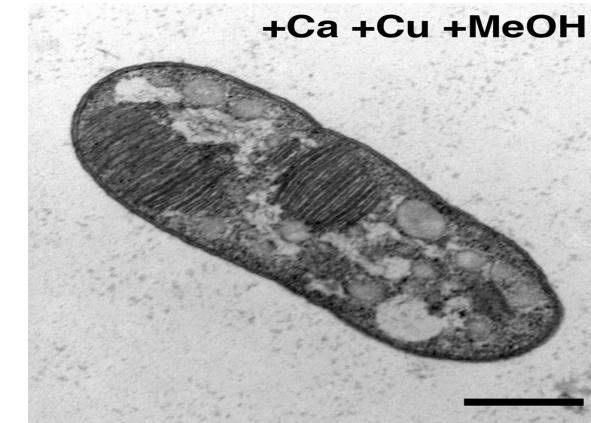
Protein Cost Hypothesis

-Omics data availability



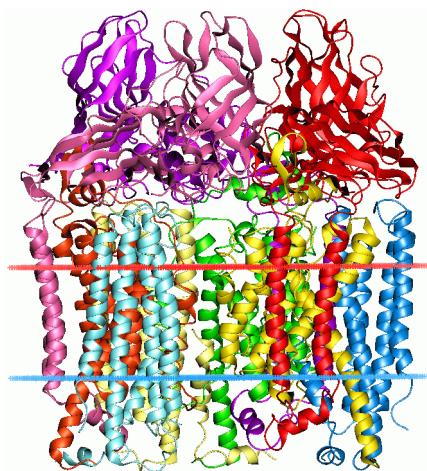
Membrane Real Estate Hypothesis

Physiology studies



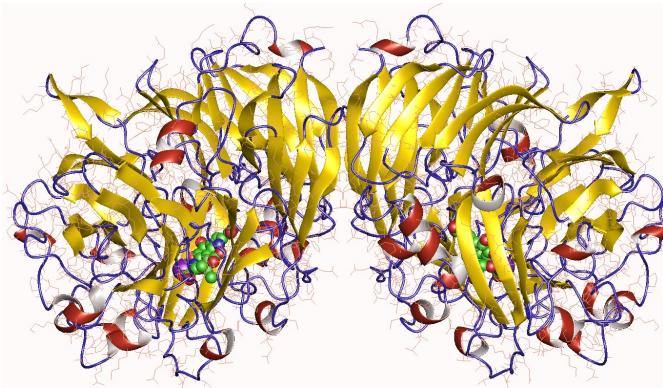
C_1 -Metabolism Machinery (*M. alcaliphilum*): Key Enzymes

Methane Monooxygenase



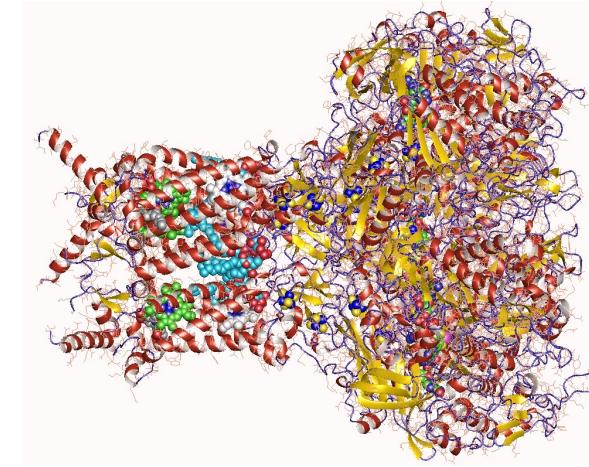
Copper (Cu)-dependent Particulate Methane Monooxygenase (pMMO)

Methanol Dehydrogenases

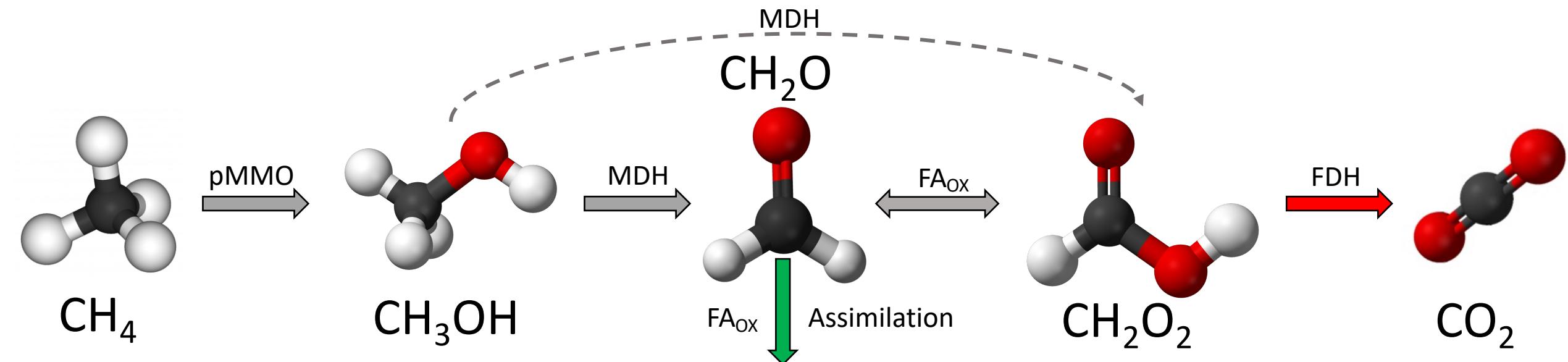


Calcium (Ca)-dependent methanol dehydrogenase (MxaF-MDH)
Lanthanum (La)-dependent methanol dehydrogenase (XoxF-MDH)

Formate Dehydrogenase



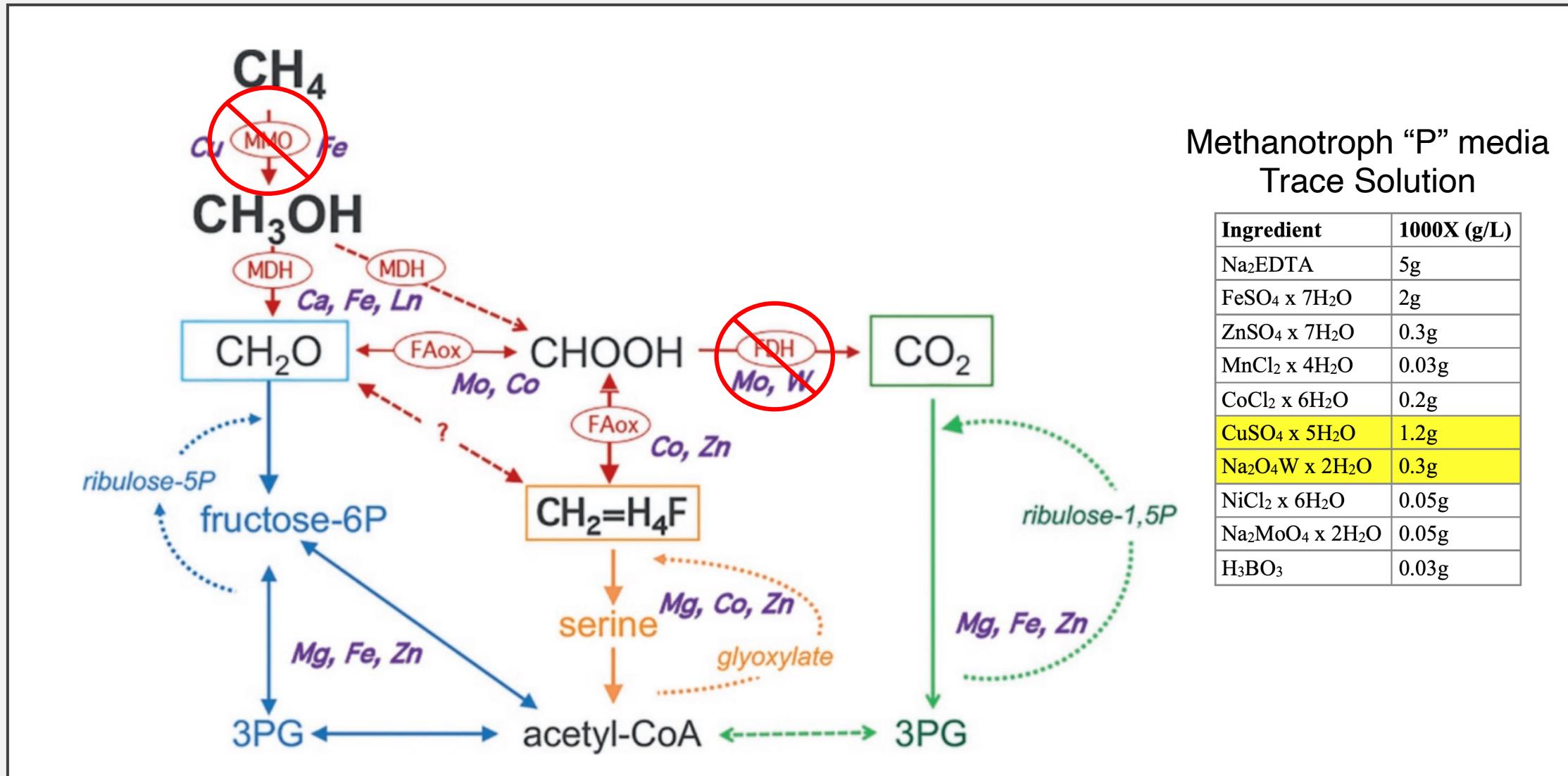
Tungsten (W)-dependent formate dehydrogenase (FDH)



METAL LIMITATION EXPERIMENTS TO ASSESS ORGANIC ACID OVERFLOW IN *METHYLOMICROBIUM* *ALCALIPHILUM*

Master's Thesis: Aim 1

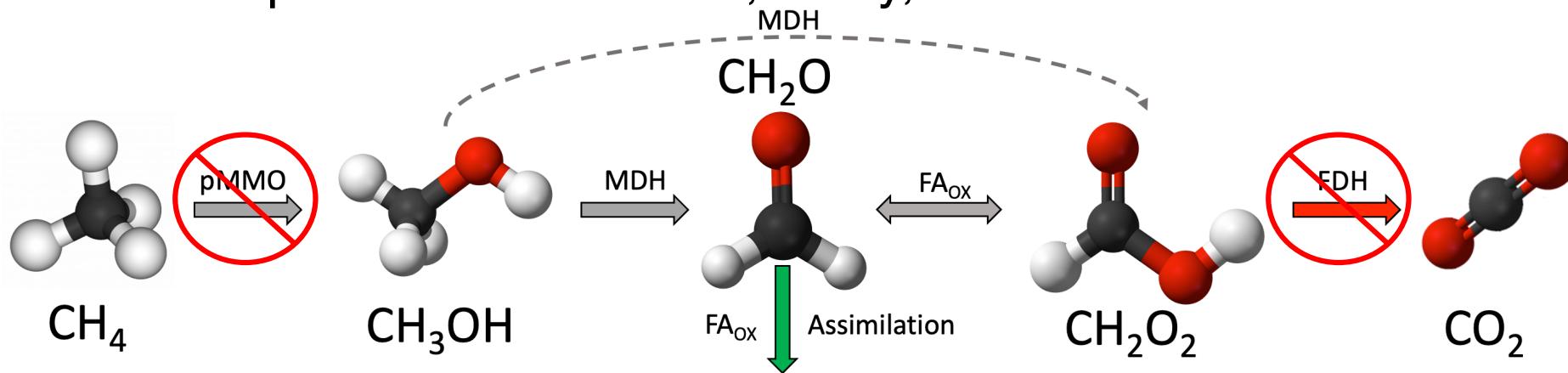
ASSESSING C1 OVERFLOW METABOLISM – METAL LIMITATION EXPERIMENTS



Aim 1 Hypothesis

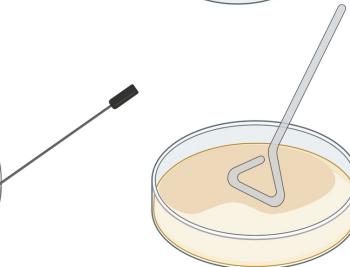
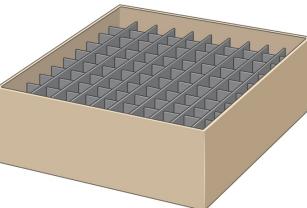
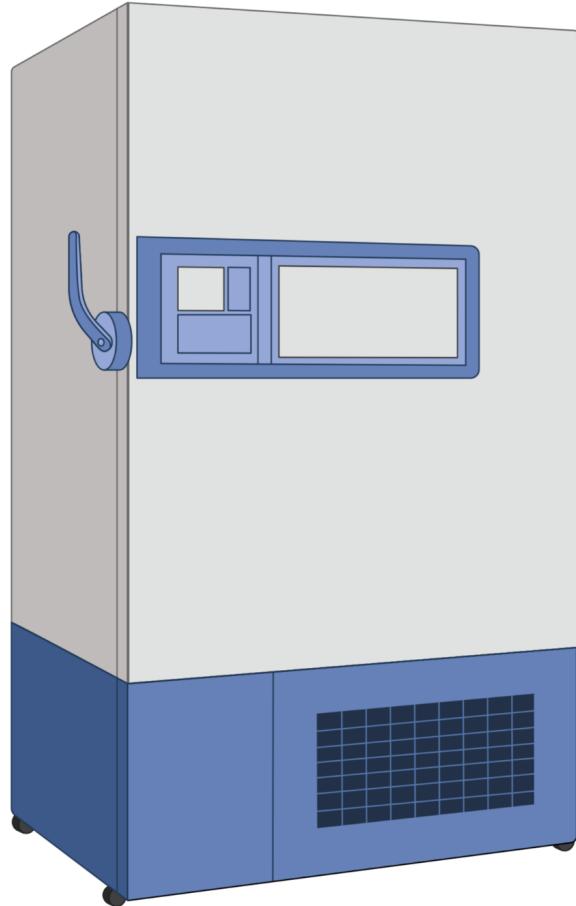
- Copper (Cu) supplemented cells have the capacity to form ICMs, increases affinity for cellular respiration, and increases growth rate
- Tungsten (W) supplemented cells have the capacity to oxidize formic acid to carbon dioxide via formate dehydrogenase

Cells grown in **+Cu/-W** supplemented conditions should exhibit the strongest Overflow Metabolism. Conversely, cells grown in **-Cu/+W** supplemented conditions are expected to show little, if any, Overflow Metabolism.



Metal Limitation Experiments to assess Overflow Metabolism

Prepare samples



Inoculate cultures



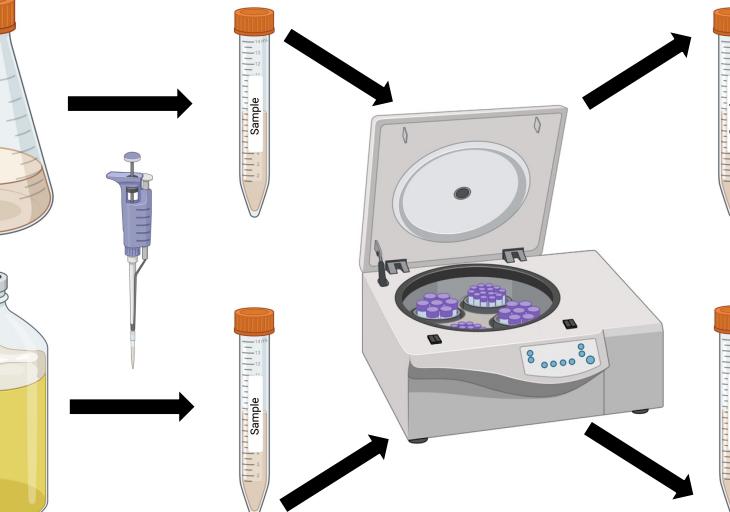
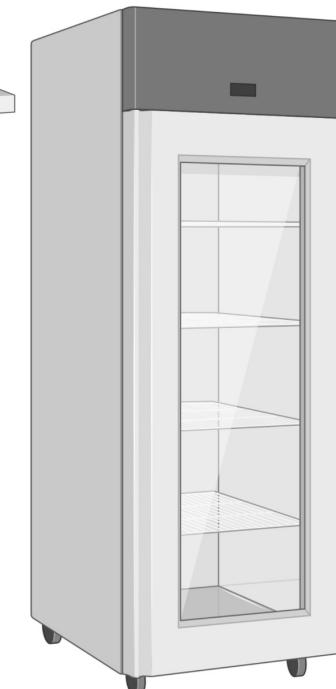
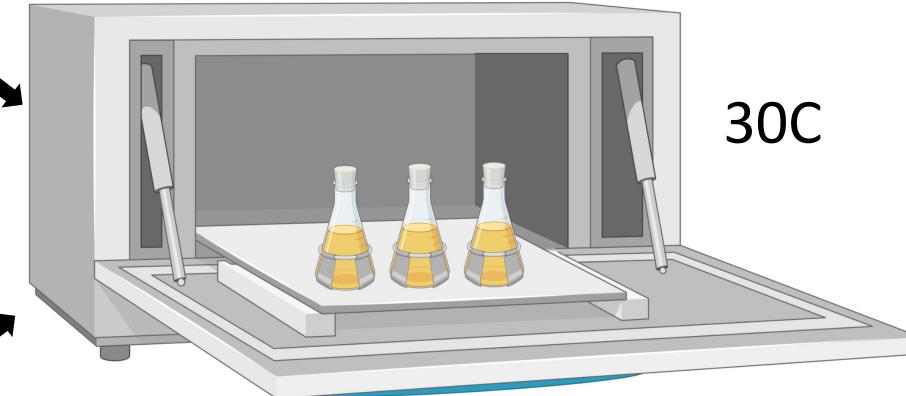
Ingredient	1000X (g/L)
Na ₂ EDTA	5g
FeSO ₄ x 7H ₂ O	2g
ZnSO ₄ x 7H ₂ O	0.3g
MnCl ₂ x 4H ₂ O	0.03g
CoCl ₂ x 6H ₂ O	0.2g
CuSO ₄ x 5H ₂ O	1.2g
Na ₂ O ₄ W x 2H ₂ O	0.3g
NiCl ₂ x 6H ₂ O	0.05g
Na ₂ MoO ₄ x 2H ₂ O	0.05g
H ₃ BO ₃	0.03g

+Cu/+W, 0.1% MeOH
+Cu/-W, 0.1% MeOH
-Cu/+W, 0.1% MeOH
-Cu/-W, 0.1% MeOH

Incubate and extract

Collect supernatant at 3 O.D. ranges:

- Early-exponential
- Mid-exponential
- Late-stationary

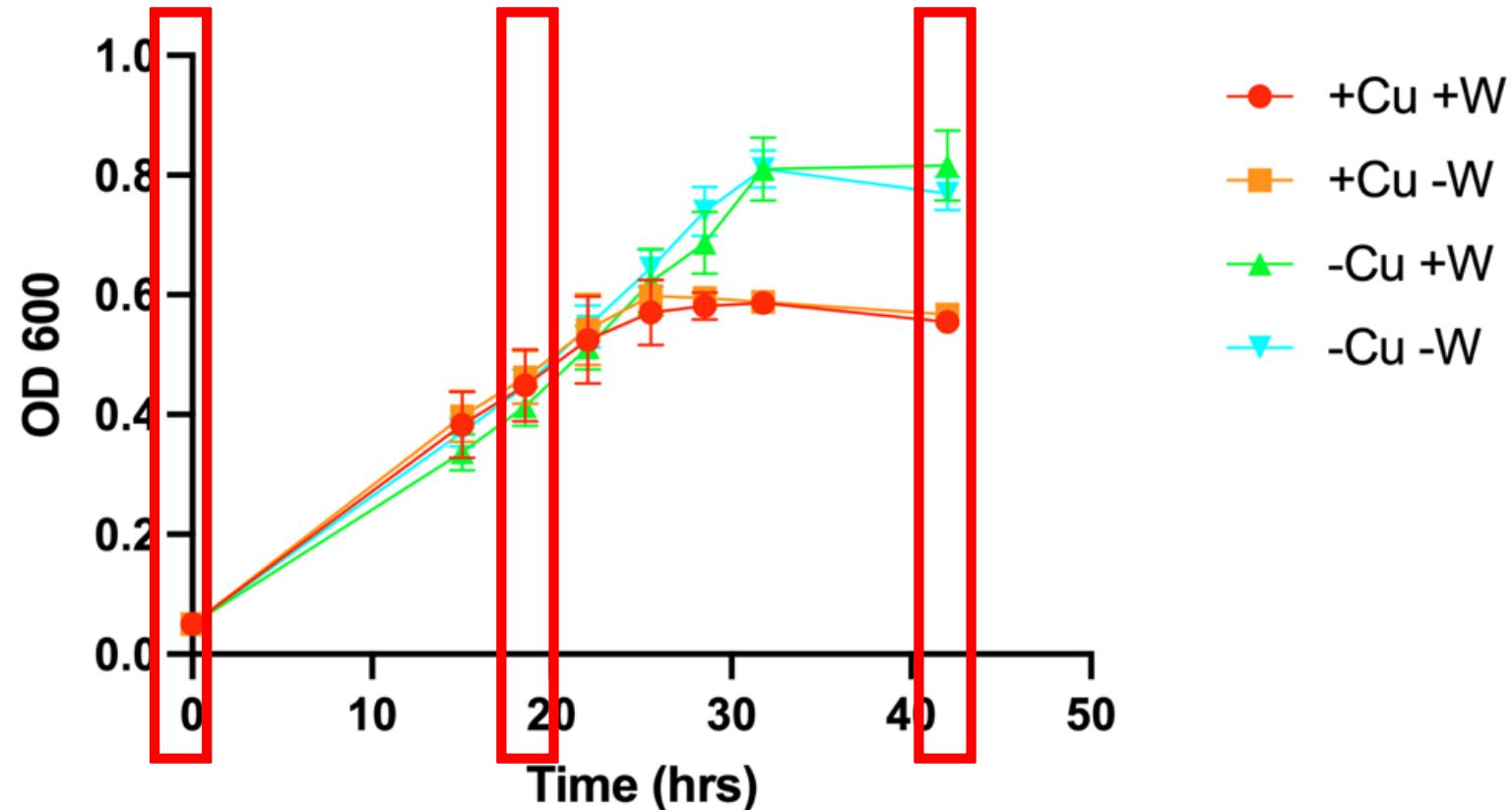


Cold storage

EXPERIMENT RESULTS

Date	Time out	Time in	Hours	+Cu/+W BR1	+Cu/+W BR2	+Cu/+W BR3	+Cu/-W BR1	+Cu/-W BR2	+Cu/-W BR3	-Cu/+W BR1	-Cu/+W BR2	-Cu/+W BR3	-Cu/-W BR1	-Cu/-W BR2	-Cu/-W BR3	Remaining
2022-09-08	-	16:00	0	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	100 mL
2022-09-09	07:00	07:45	15	0.368	0.336	0.444	0.369	0.376	0.447	0.307	0.336	0.367	0.354	0.360	0.397	~97.5 mL
2022-09-09	10:30	11:00	18.5	0.430	0.400	0.516	0.432	0.441	0.513	0.384	0.409	0.448	0.430	0.431	0.480	~95 mL
2022-09-09	14:00	14:30	22	0.502	0.465	0.606	0.509	0.507	0.610	0.477	0.508	0.551	0.530	0.524	0.587	~92.5 mL
2022-09-09	17:35	18:10	25.5	0.564	0.520	0.628	0.584	0.581	0.629	0.575	0.605	0.684	0.630	0.623	0.680	~90 mL
2022-09-09	20:30	21:10	28.5	0.588	0.556	0.600	0.586	0.596	0.601	0.643	0.674	0.745	0.729	0.705	0.786	~87.5 mL
2022-09-09	23:45	01:15	31.75	0.578	0.587	0.593	0.578	0.592	0.595	0.761	0.805	0.866	0.780	0.808	0.842	~85 mL
2022-09-10	10:10	10:45	42	0.552	0.536	0.575	0.559	0.564	0.579	0.860	0.770	0.838	0.741	0.770	0.797	~82.5

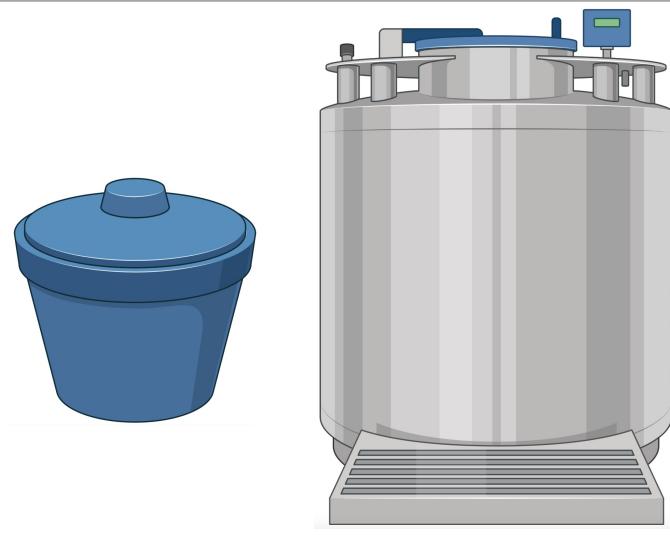
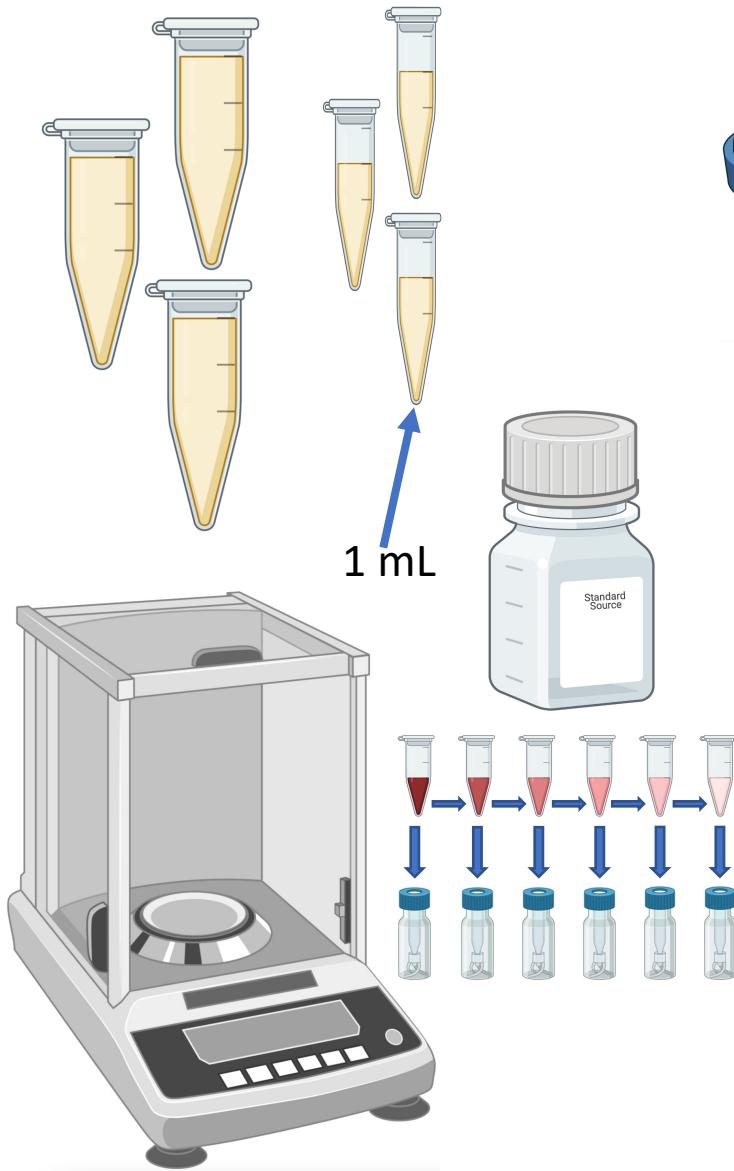
Metal Limitation Growth



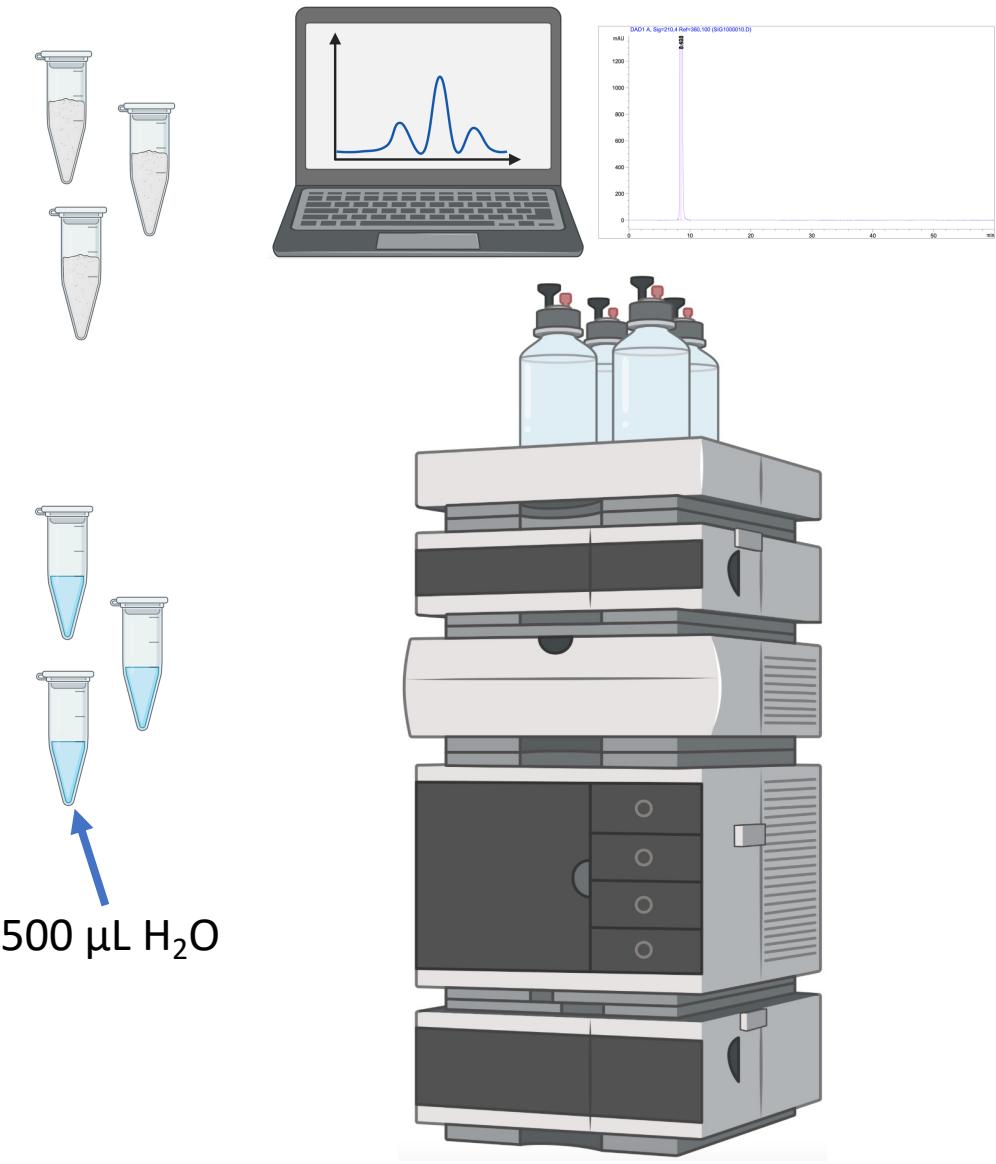
Assessing organic acid production – HPLC processing

Prepare samples/standards Flash freeze & lyophilization H₂O rehydration Process & assess

Spent supernatant

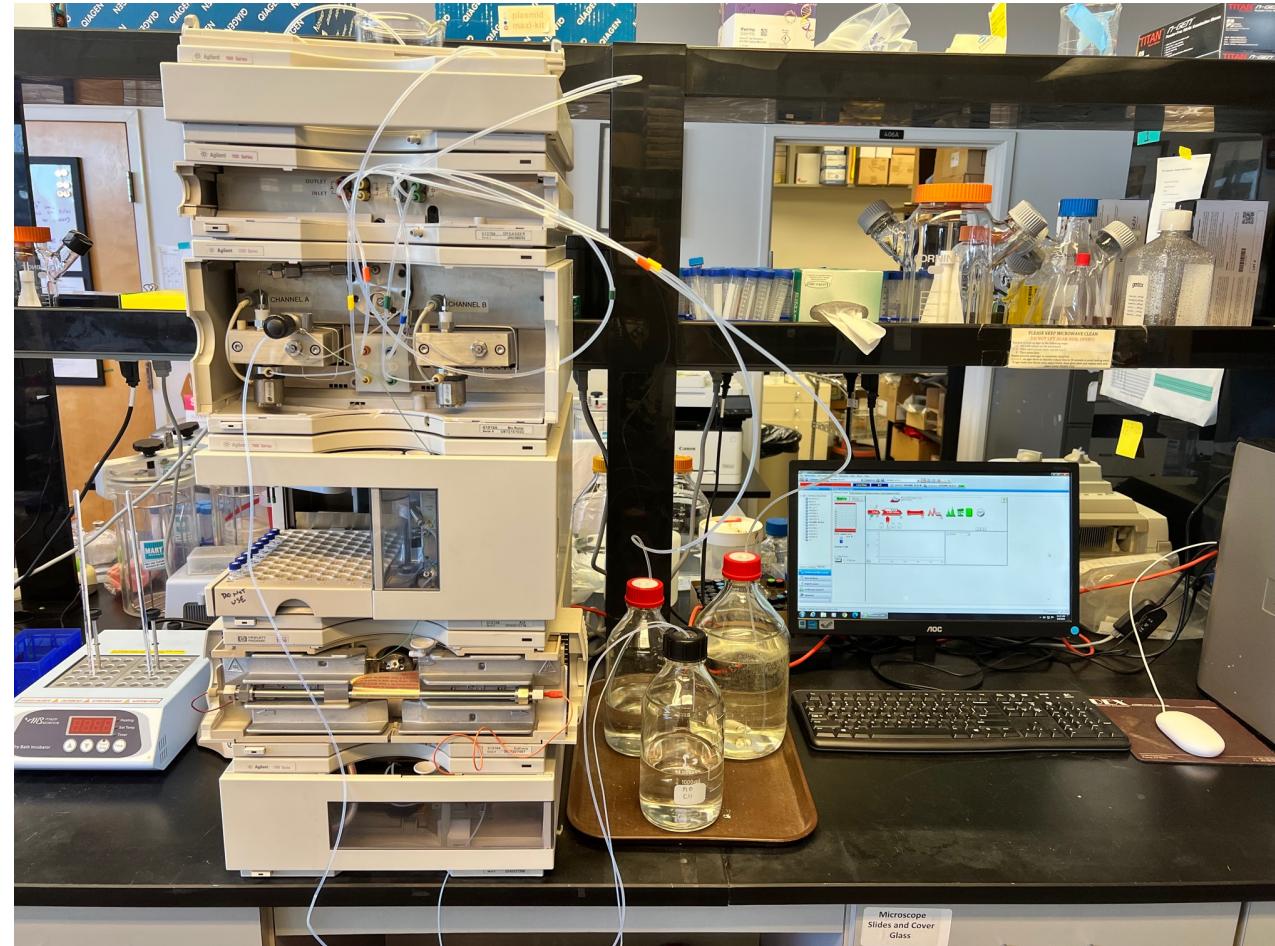


HPLC organic acid analysis



HPLC Organic Acid Assay

- Machine: Agilent 1100 Series
- HPLC Column: “HPLC Organic Acid Column” (Aminex HPX-87H Ion Exclusion Column, 300 mm x 7.8 mm)
- Column Temperature: 25C
- Buffer: 0.005 N H₂SO₄
- Flow rate: 0.5 mL/min
- Run time: 45-minute/sample



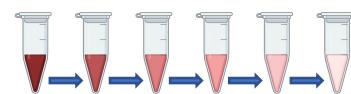
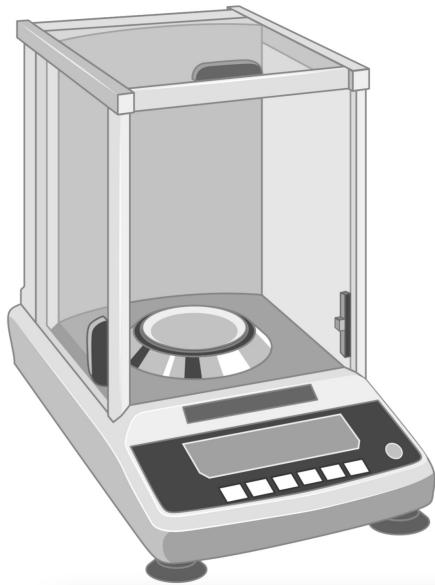
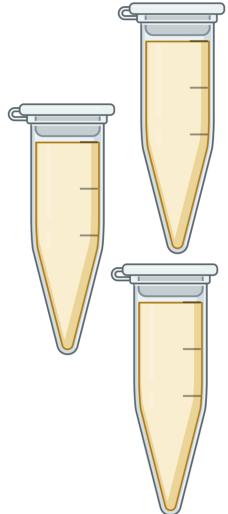
Assessing organic acid production – H-NMR processing

Prepare samples/standards

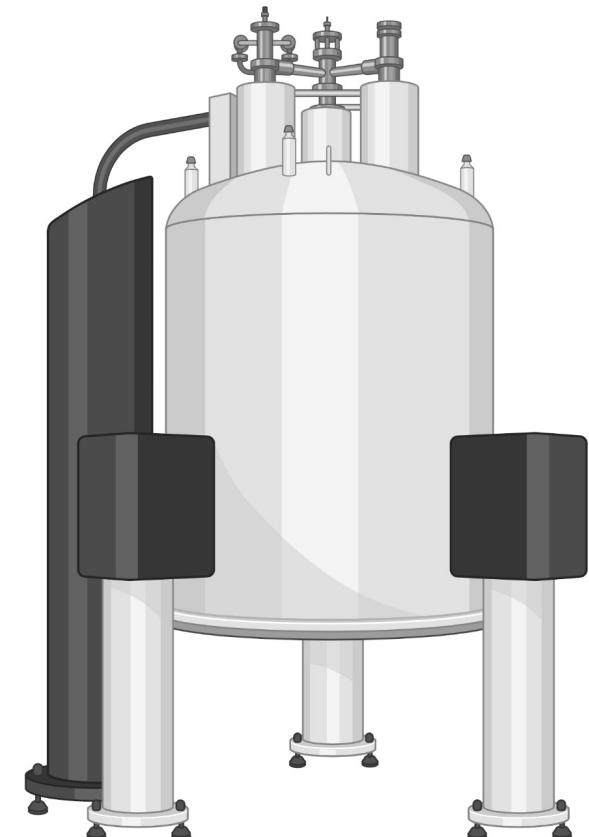
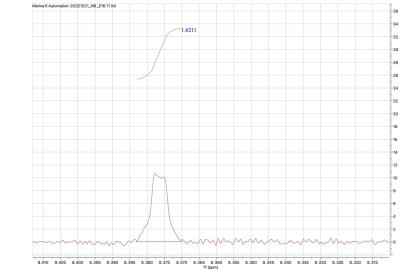
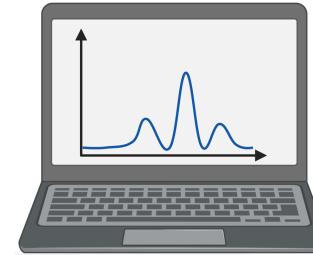
10% D₂O preparation

Process & assess

Spent supernatant



¹H-NMR organic acid analysis

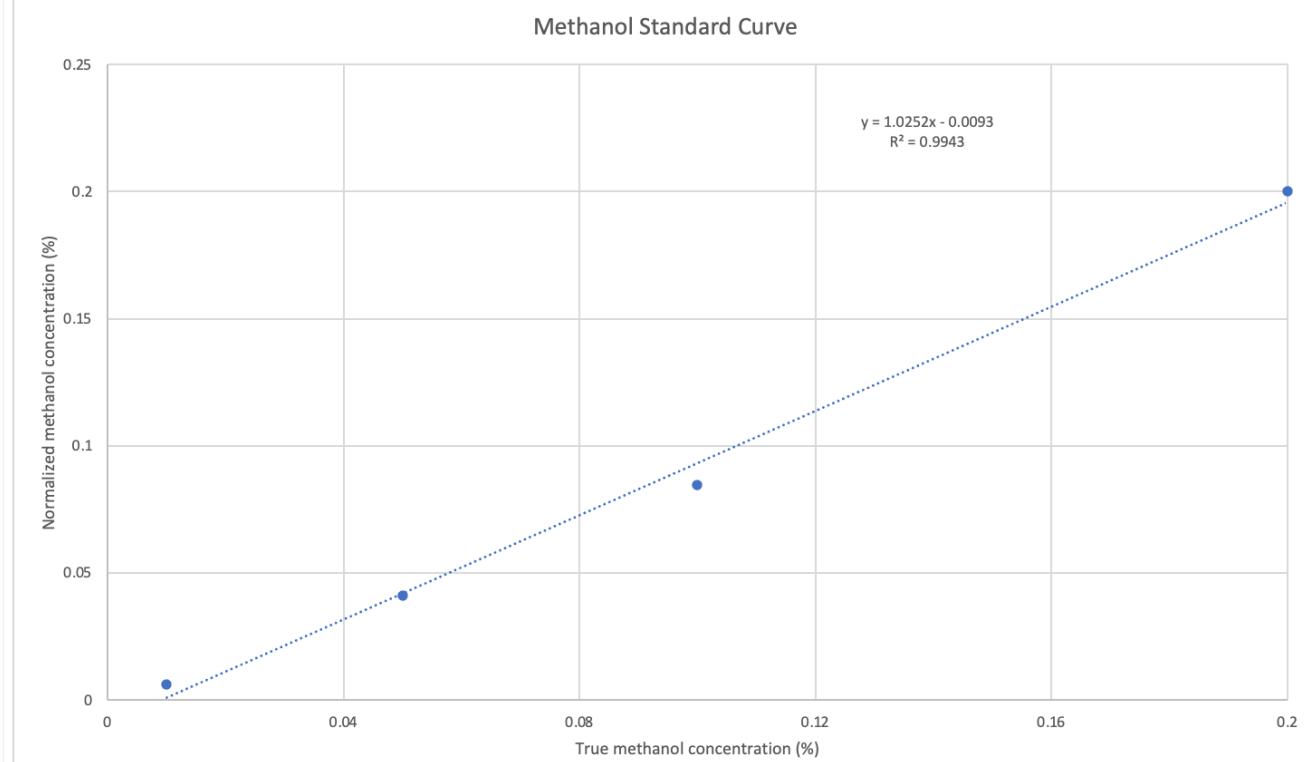
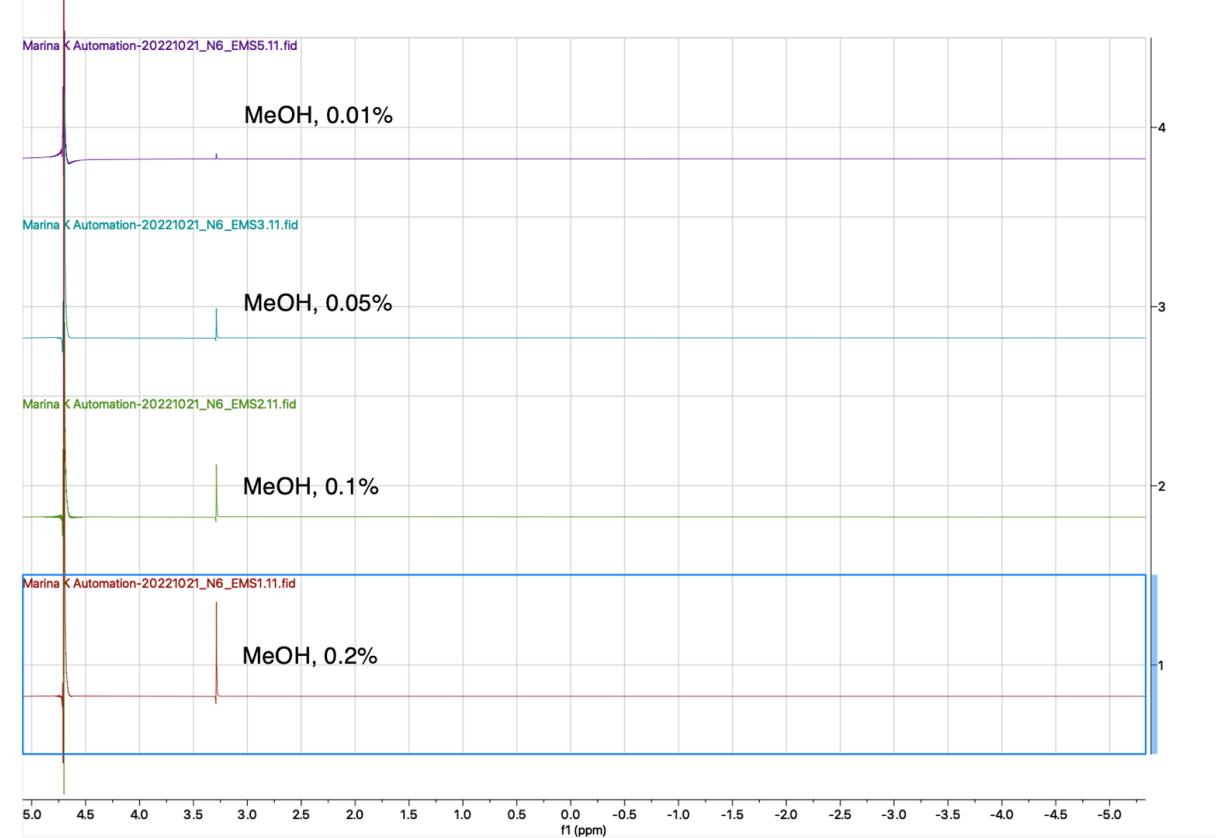


^1H -NMR Organic Acid Assay

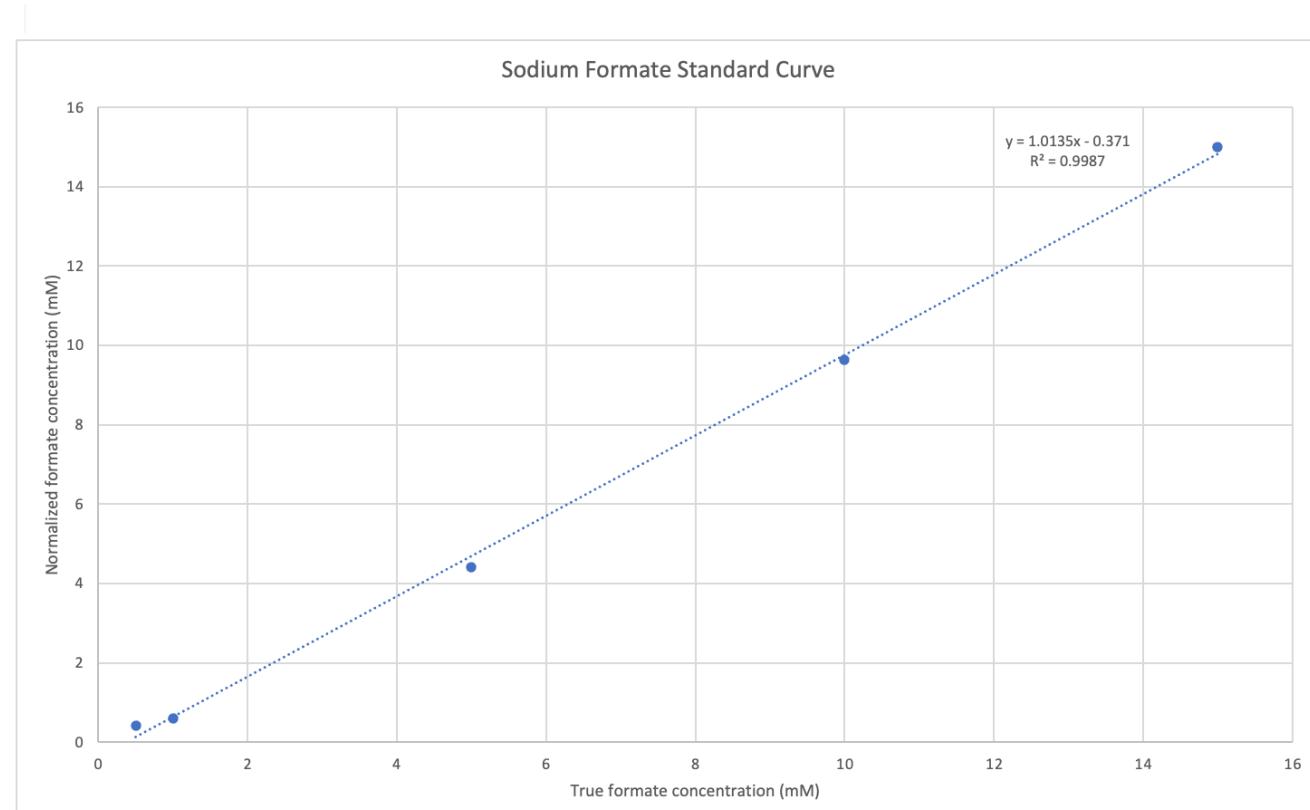
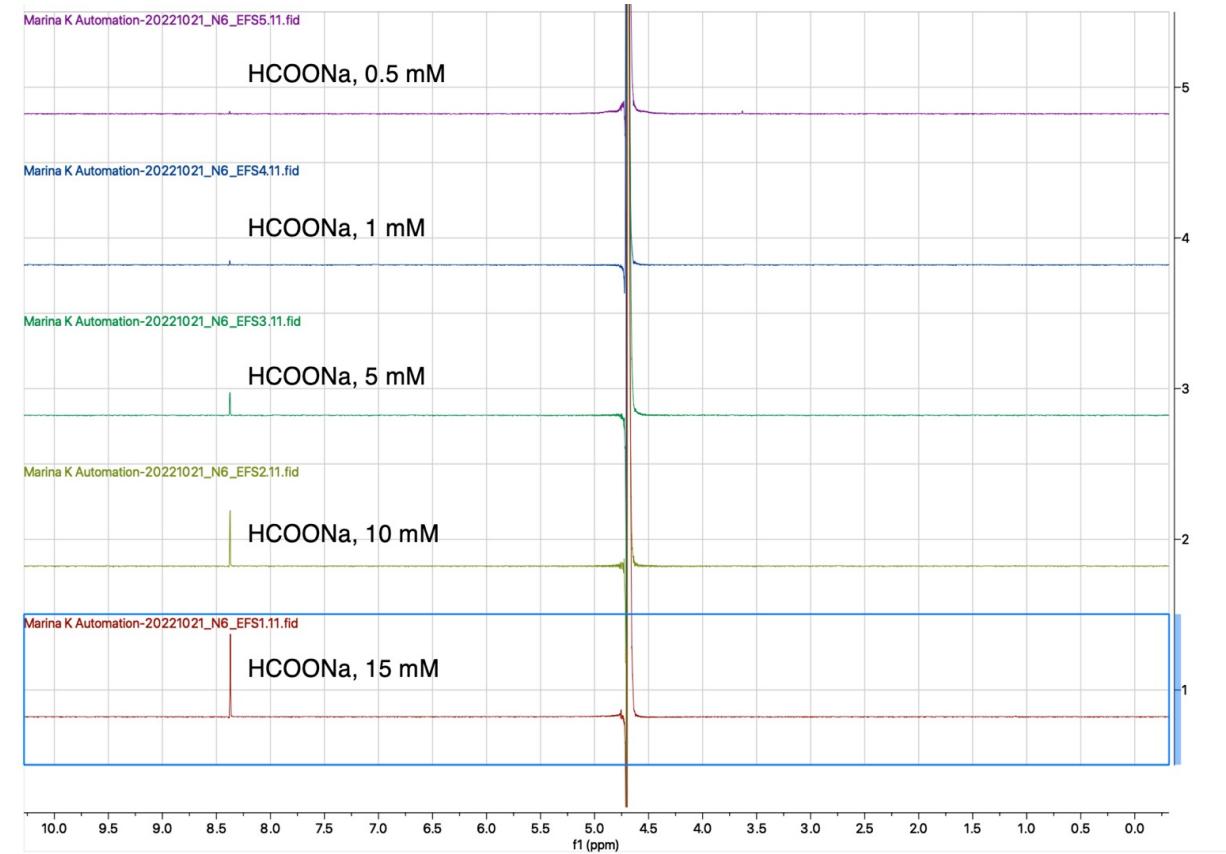
- Machine: Varian 600 MHz H-NMR
- Probe temperature: room temperature
- Number of data points: 32,000
- Spectral width: 10,000 Hz, 6s relaxation delay
- Data processing tools: MestReNova



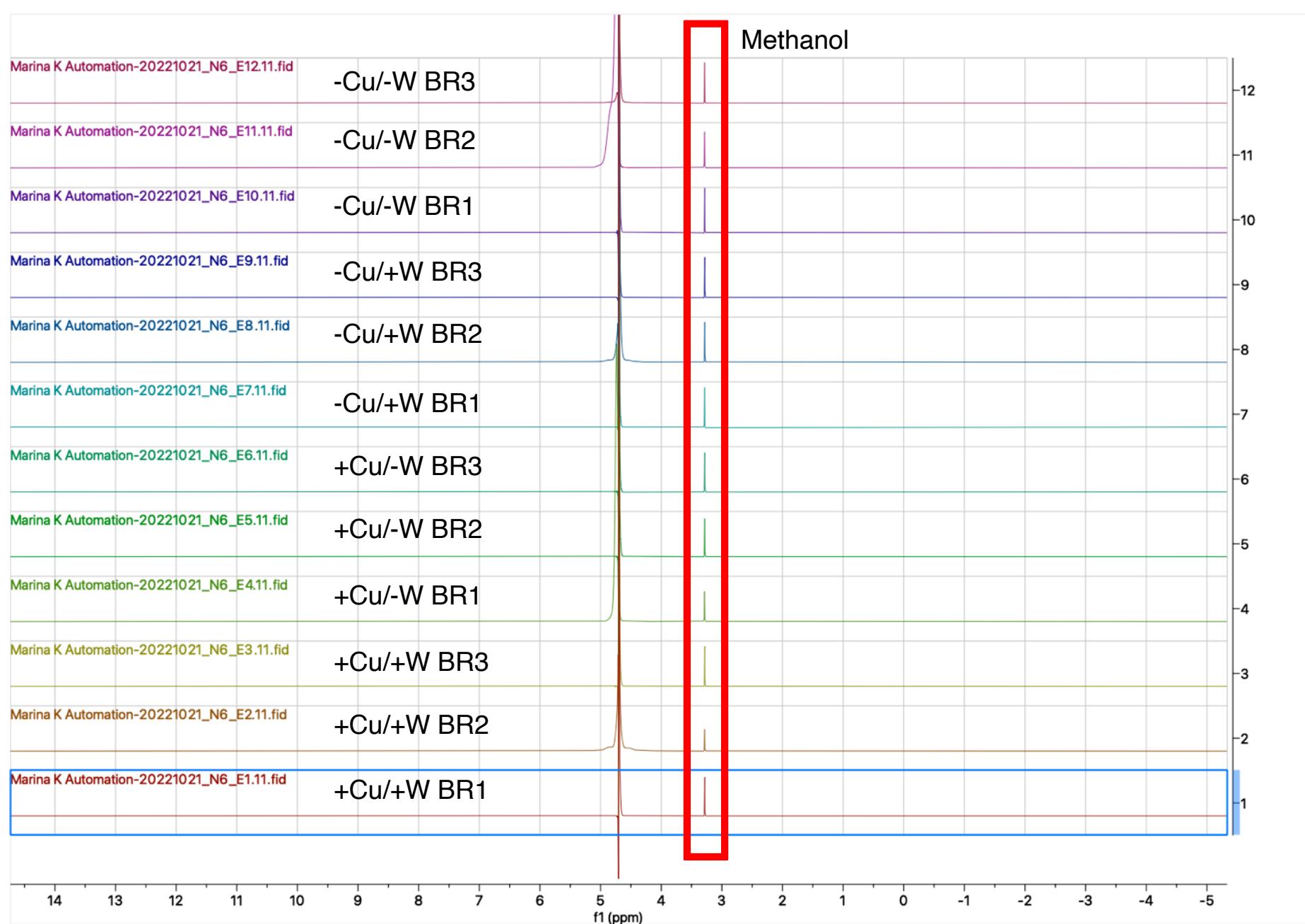
CREATING METHANOL STANDARD CURVE



CREATING SODIUM FORMATE STANDARD CURVE

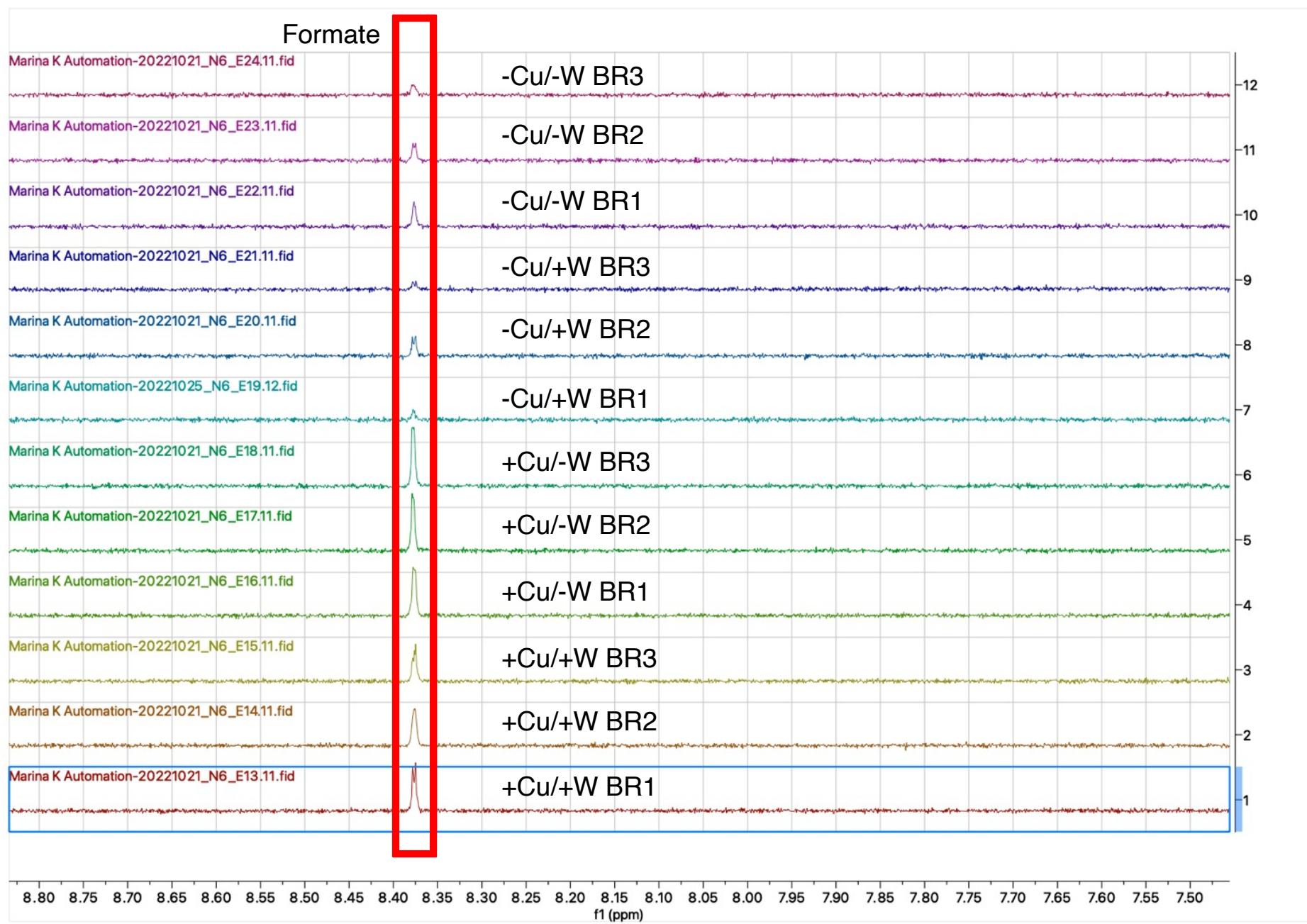


SAMPLE PROCESSING – INOCULATION

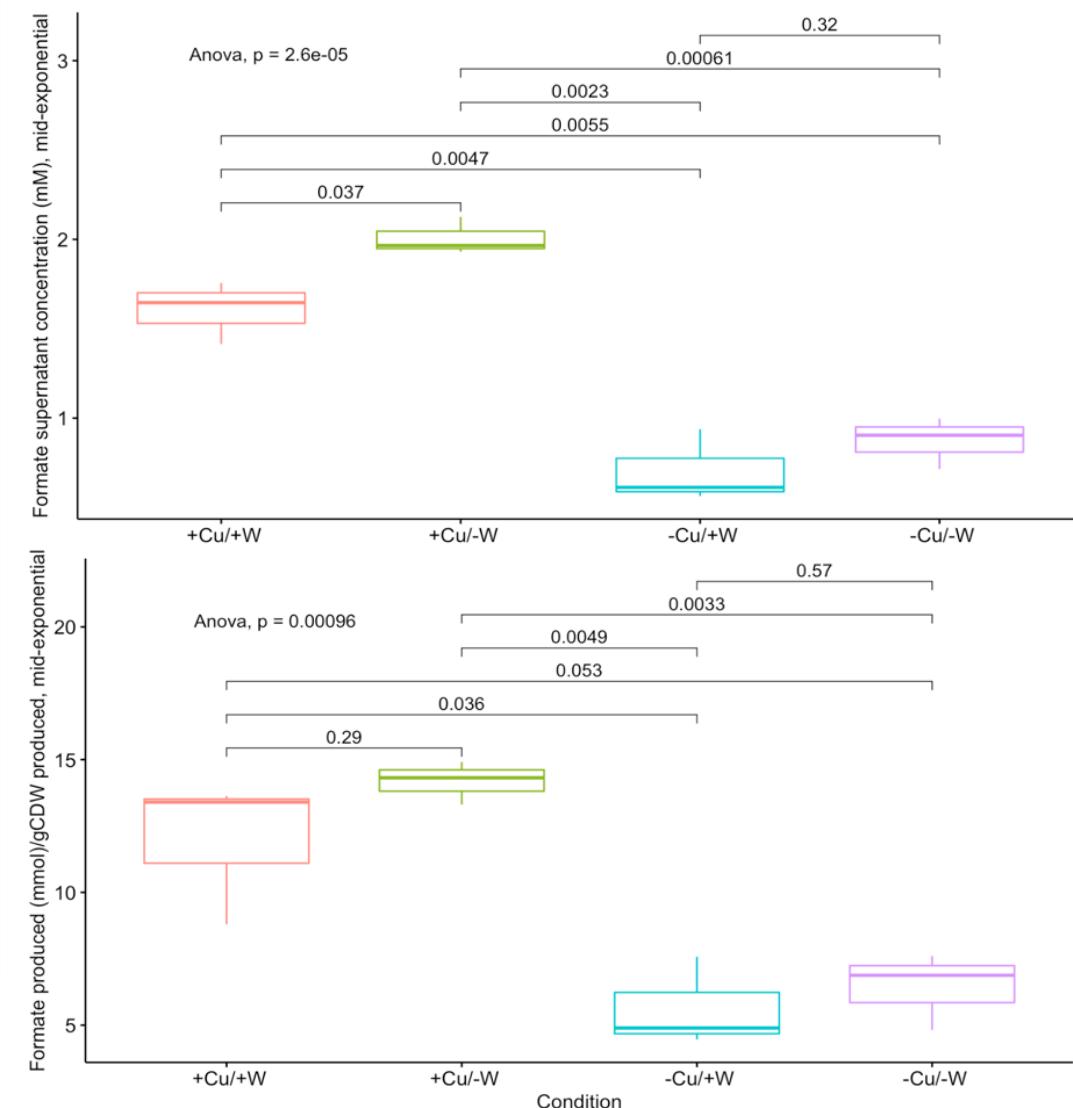
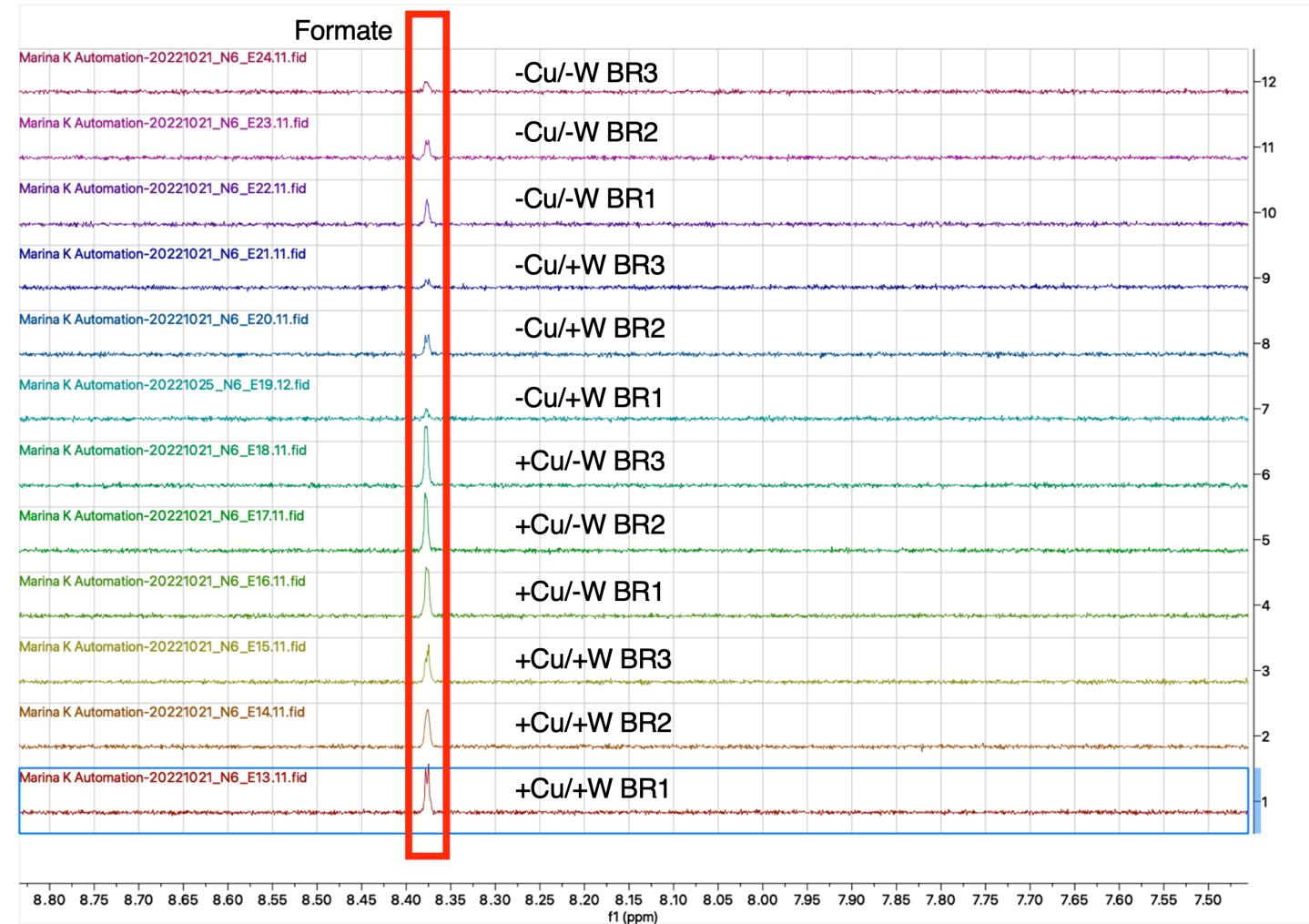


Condition	% MeoH
+Cu/+W	0.0940 +/- 0.00640
+Cu/-W	0.0919 +/- 0.00742
-Cu/+W	0.0944 +/- 0.00398
-Cu/-W	0.0913 +/- 0.00480

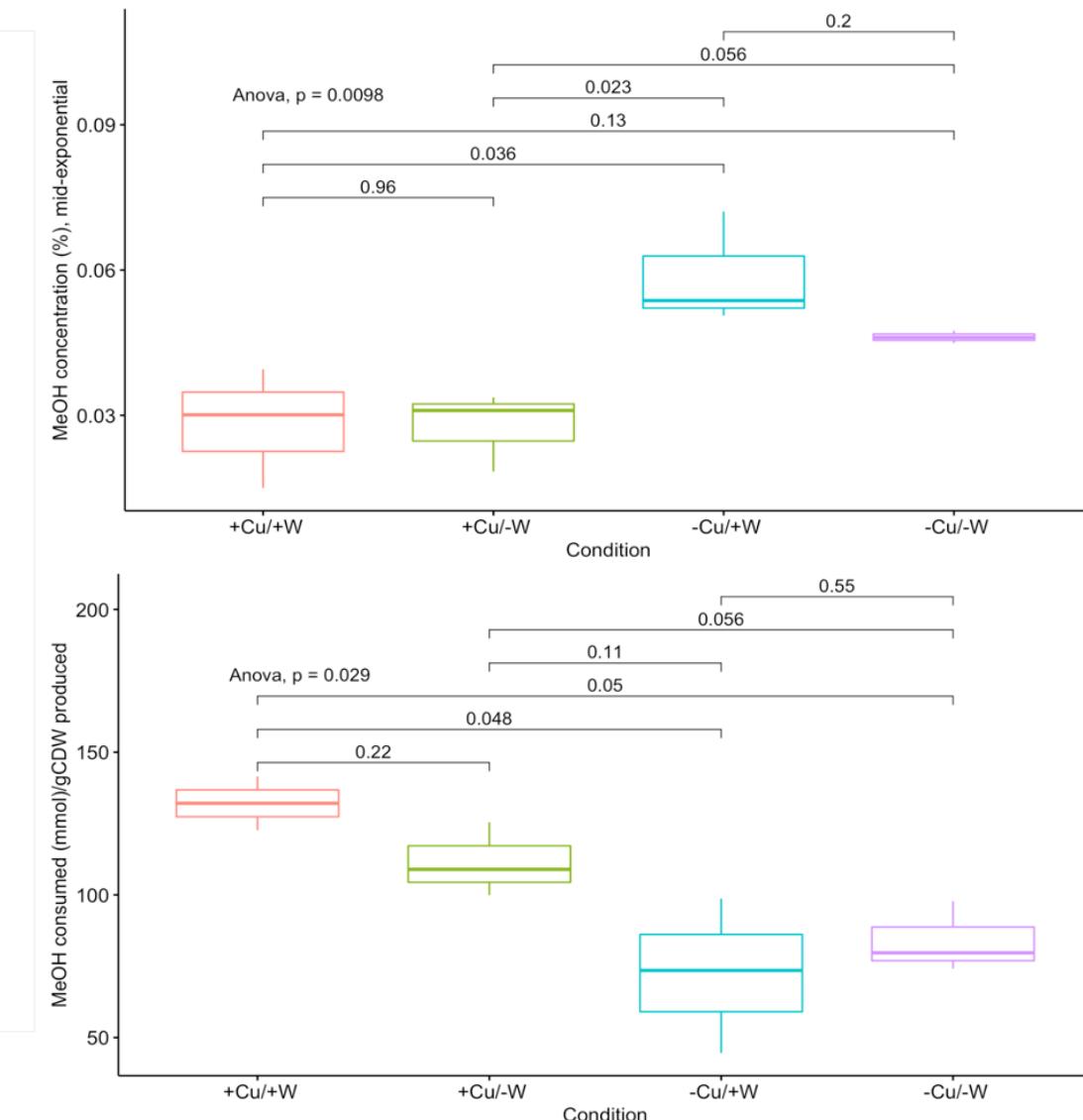
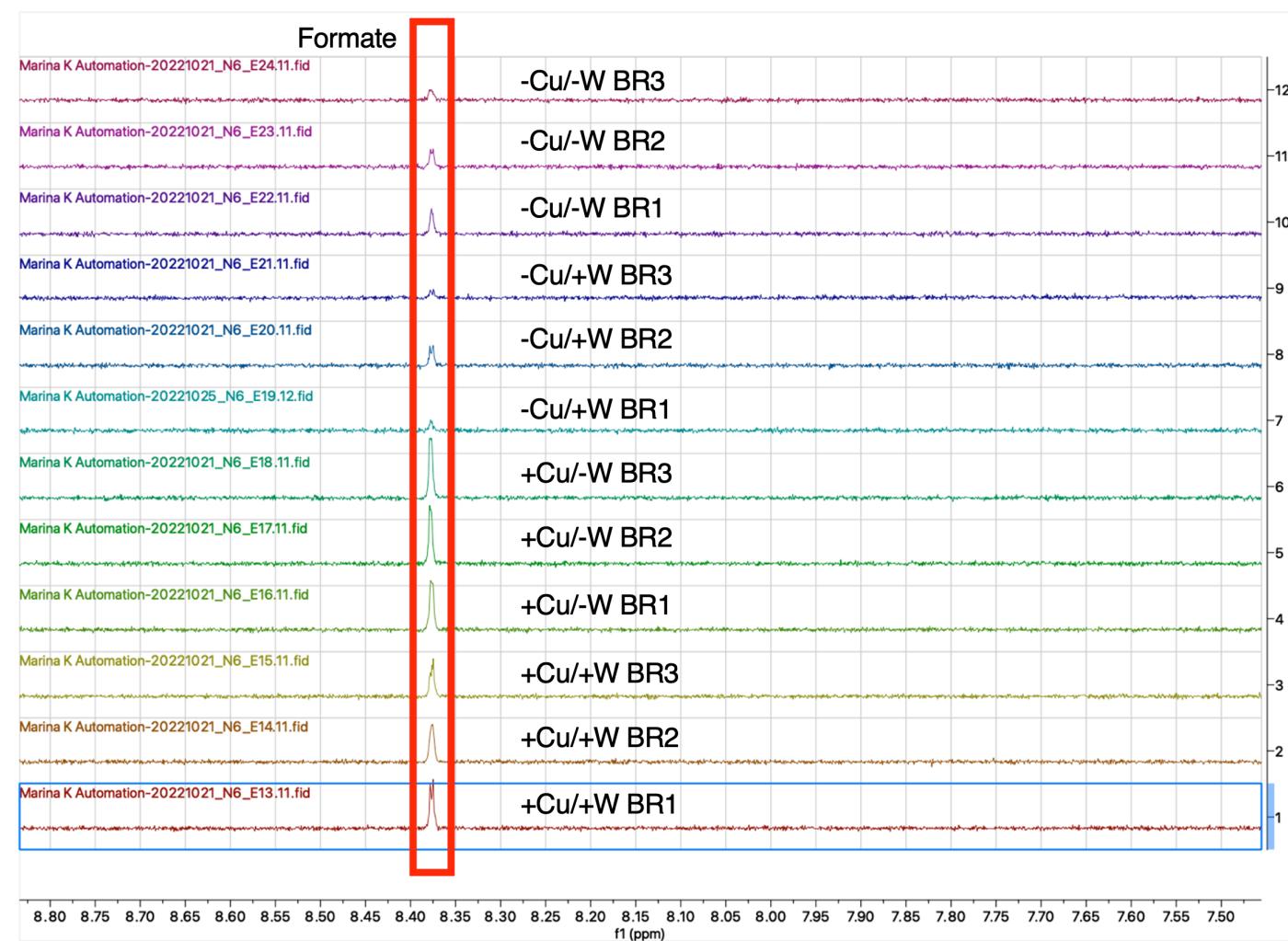
SAMPLE PROCESSING – MID-EXPONENTIAL (18.5 HRS)



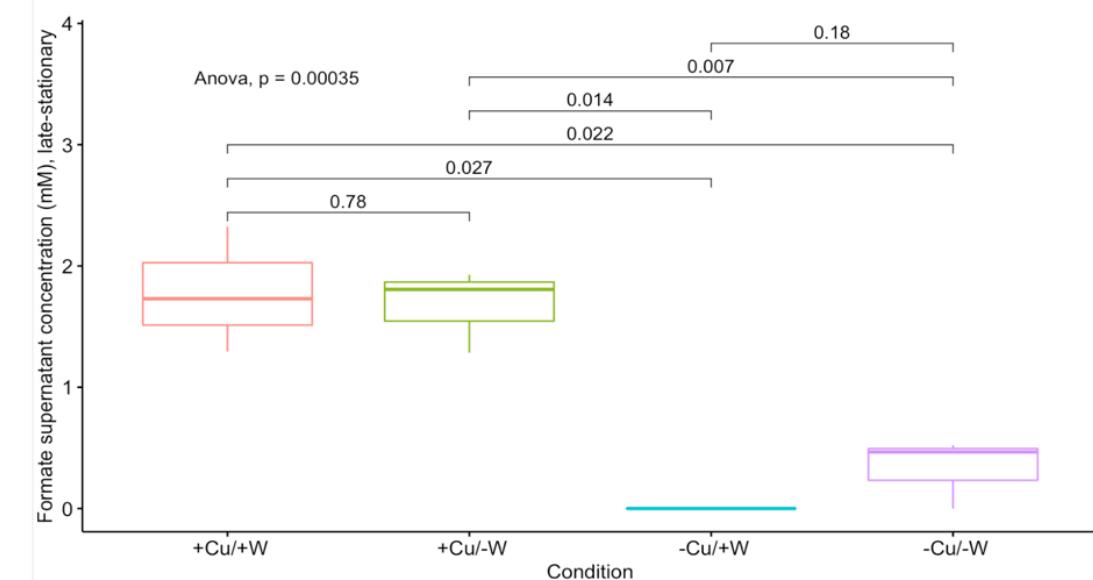
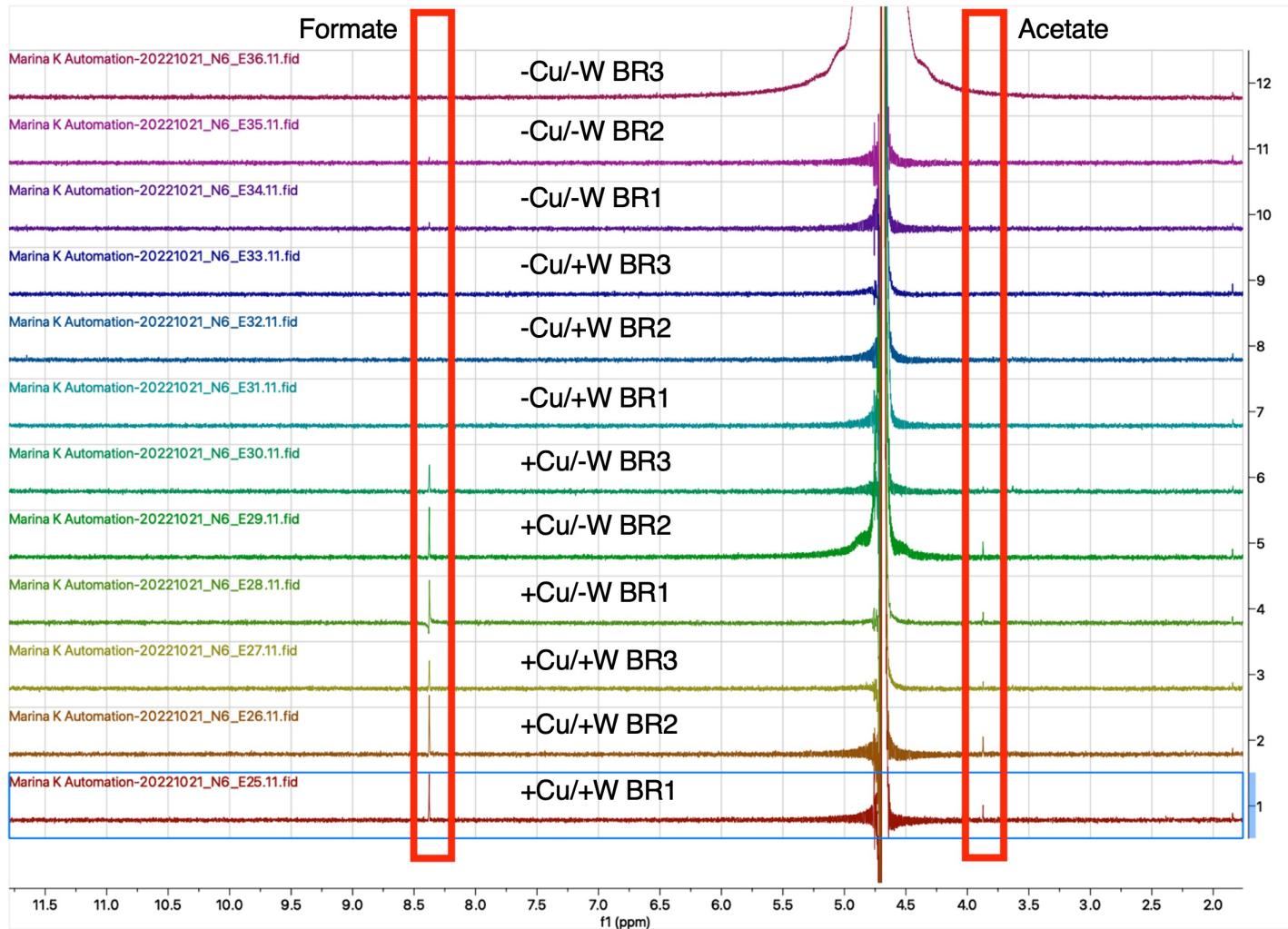
SAMPLE PROCESSING – MID-EXPONENTIAL (18.5 HRS)



SAMPLE PROCESSING – MID-EXPONENTIAL (18.5 HRS)



SAMPLE PROCESSING – LATE-STATIONARY (42 HRS)



METAL LIMITATION EXPERIMENTS SUMMARY

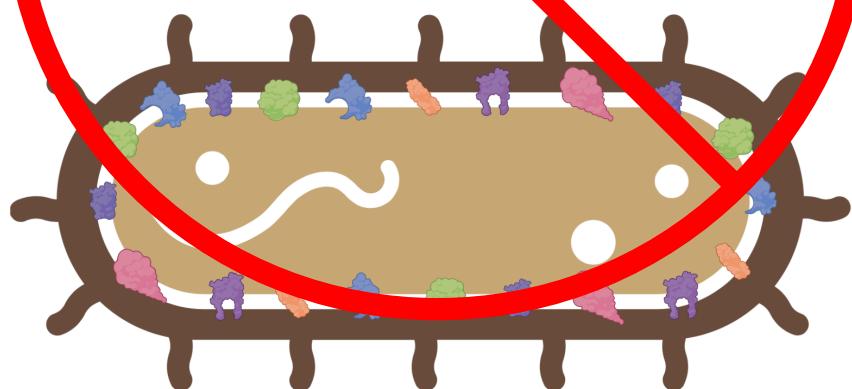
Condition	Growth Phase	Time (hrs)	O.D.	g CDW	% MeOH	mM Formate
+Cu/+W	Inoculation	0	0.05	0.001725	0.0940 +/- 0.00064	0
+Cu/-W					0.0919 +/- 0.00742	
-Cu/+W					0.0944 +/- 0.00398	
-Cu/-W					0.0913 +/- 0.00480	
+Cu/+W	Mid-exponential	18.5	0.449 +/- 0.060	0.0155 +/- 0.0021	0.0282 +/- 0.0124	1.608 +/- 0.172
+Cu/-W			0.462 +/- 0.044	0.0159 +/- 0.0015	0.0277 +/- 0.0082	2.008 +/- 0.104
-Cu/+W			0.414 +/- 0.032	0.0143 +/- 0.0012	0.0588 +/- 0.0116	0.705 +/- 0.204
-Cu/-W			0.447 +/- 0.029	0.0154 +/- 0.0010	0.0462 +/- 0.0013	0.872 +/- 0.144
+Cu/+W	Late-stationary	42	0.554 +/- 0.020	0.0191 +/- 0.00066	0	1.783 +/- 0.517
+Cu/-W			0.567 +/- 0.010	0.0196 +/- 0.00036		1.673 +/- 0.342
-Cu/+W			0.823 +/- 0.047	0.0284 +/- 0.00161		0
-Cu/-W			0.769 +/- 0.028	0.0266 +/- 0.00095		0.329 +/- 0.287

METAL LIMITATION EXPERIMENTS – CONCLUSIONS

- Formate overflow begins after inoculation during early exponential growth
- By mid-exponential growth, all 4 conditions had overflow of formate out of the cell and into the growth media
- By late-stationary growth, cells cultivated in -Cu/+W condition consumed all formate which was produced during mid-exponential growth
 - Cells cultivated in -Cu/-W conditions also consumed formate
- By late-stationary growth, cells cultivated in +Cu conditions have prolonged formate accumulation in the supernatant
 - Overflow of acetate was also noted, which was not observed during mid-exponential growth
 - Acetate overflow was not observed in -Cu conditions
- When a high percentage of cell volume is occupied by ICMs/low free cell volume (+Cu), high formate overflow is observed
- When a low percentage of cell volume occupied by ICMs/high free cell volume (-Cu), formate is consumed

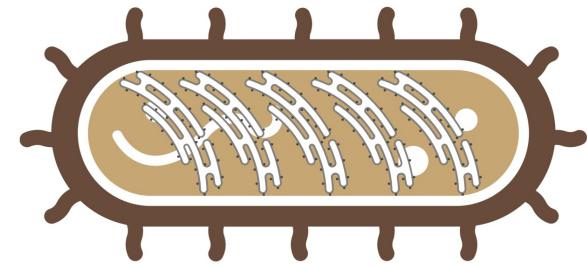
Real Estate Hypothesis

Fast-growing cells are saturated with membrane-bound machinery and lack the free space required to produce additional respiratory enzymes

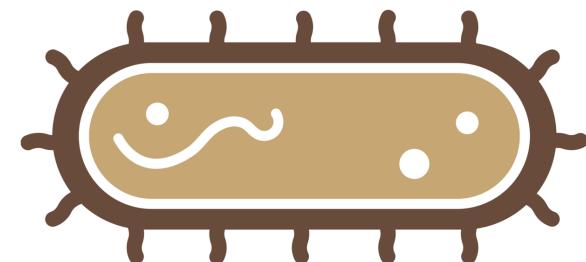


Anabolism Hypothesis

Fast-growing cells are saturated with membrane-bound machinery and lack the free volume required for efficient anabolic metabolism



+Cu = High ICM = High membrane enzyme saturation/low free volume



-Cu = Low ICM = Low membrane enzyme saturation/high free volume

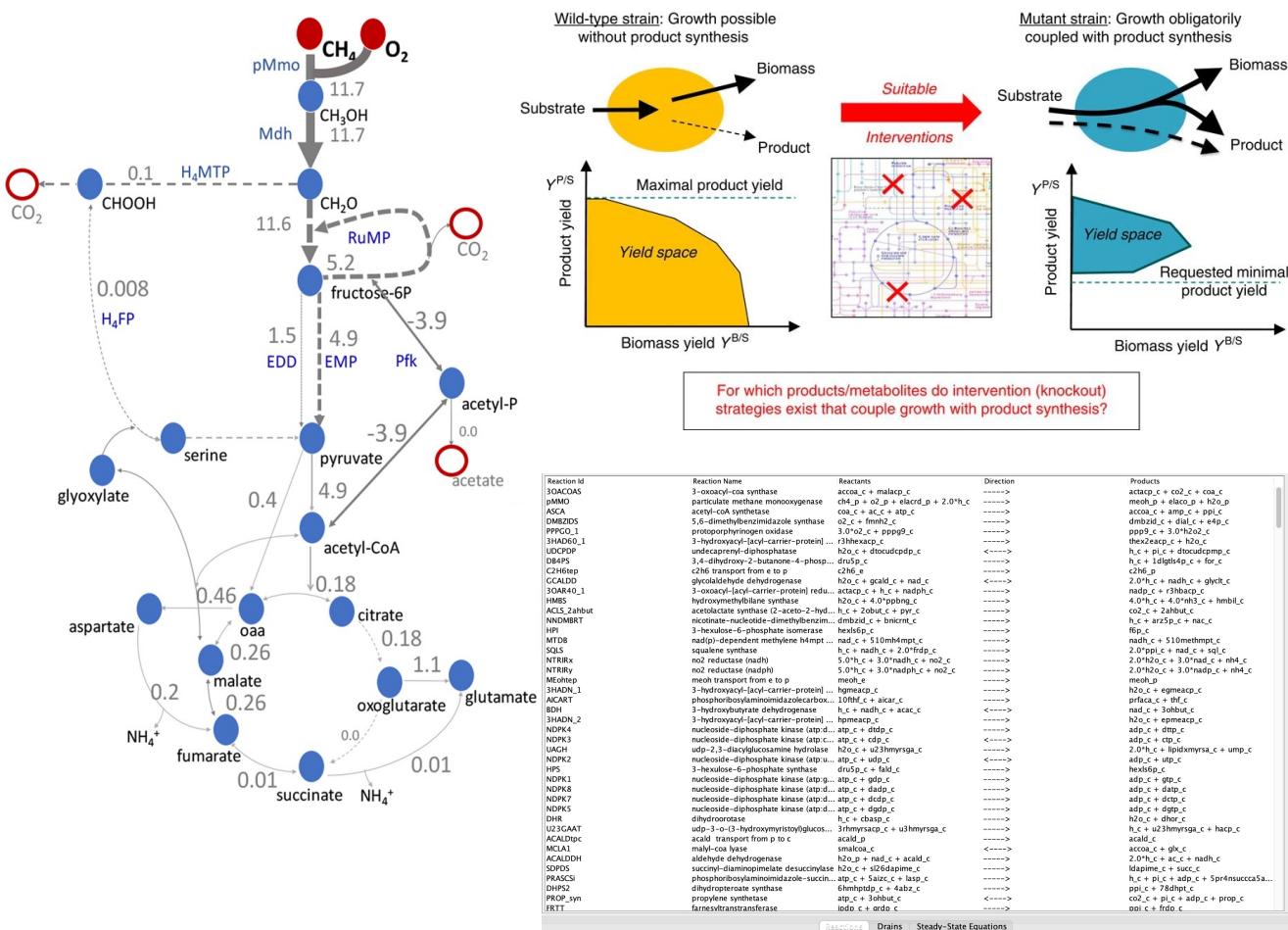
COMPUTATIONAL MODEL TO CAPTURE OVERFLOW METABOLISM IN *M. ALCALIPHILUM*

System of differential equations representing key components of *Methylomicrobium alcaliphilum*'s metabolism

Master's Thesis: Aim 2

Metabolic Modeling

Flux Balance Analysis

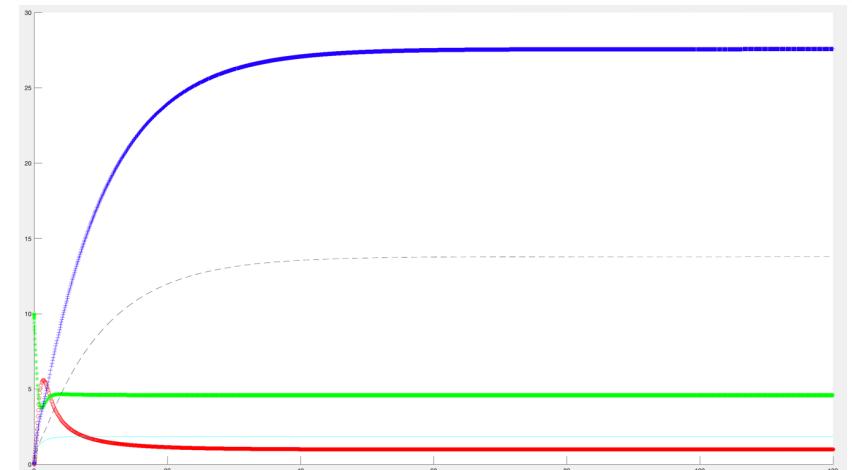
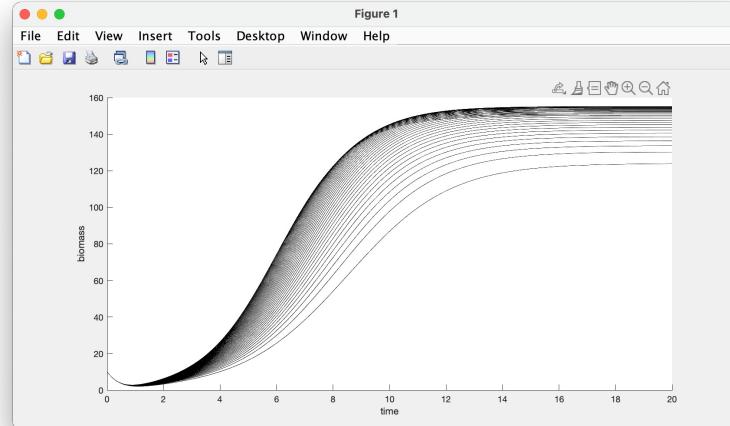


Differential Equations

$$\frac{dy}{dx} = f(x)$$

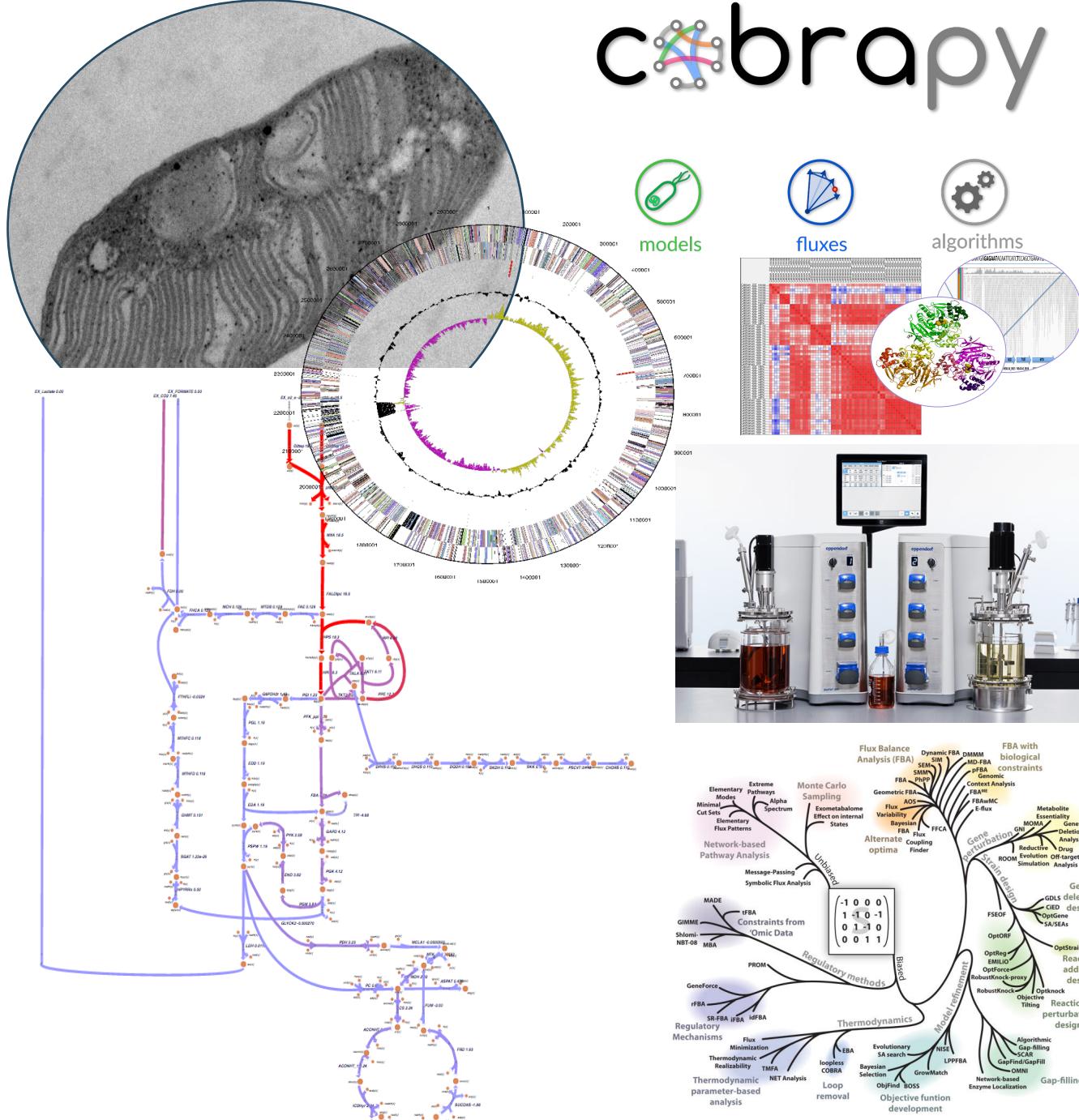
$$\frac{dy}{dx} = f(x, y)$$

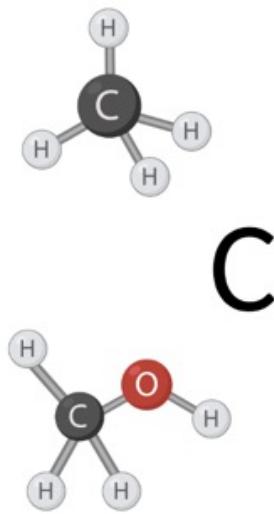
$$x_1 \frac{\partial y}{\partial x_1} + x_2 \frac{\partial y}{\partial x_2} = y$$



Limitations of Flux Balance Modeling

- FBA does not consider critical information about cell geometry
 - Cell volume
 - Cell shape
 - ICM fraction/composition
 - Enzyme turnover rates
- Does not predict formate to be an excreted waste product (like CO₂ and H₂O)
- The metabolic model can be manually updated by incorporating new enzymatic reactions/pathways for non-native metabolites





C

E

ICMs

C_{in}

K_R

ICMs

ICMs

B

K_F

W
(formate)

System of differential equations

$$\frac{dC_{in}}{dt} = K_{in} \times \frac{C}{N} \times E - K_f \times C_{in} \times F - K_r \times f_2 \times C_{in} \times R$$

- Carbon-in

$$\frac{dB}{dt} = (e \times K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) \times \left(1 - \frac{B}{B_{max}}\right) - (K_{BF} + K_{BR} + K_{BE}) \times B - d_B \times B$$

- Biomass production

$$\frac{dN}{dt} = d_B \times B$$

- Cell division rate

$$\frac{dR}{dt} = K_{BR} \times B - d_R \times R$$

- Cellular respiration

$$\frac{dF}{dt} = K_{BF} \times B - d_F \times F$$

- Cellular fermentation

$$\frac{dE}{dt} = K_{BE} \times B \times \left(1 - \frac{E}{E_{max}}\right) - d_E \times E$$

- Enzymatic carbon membrane transporters (C to C_{in})

$$\frac{dW}{dt} = f_2 \left[(K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) - (e \times K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) \left(1 - \frac{B}{B_{max}}\right) \right]$$

- Waste production/formate excretion (regulated by ICM proportion)

Where:

$$\text{cell_volume} = 10 \times B$$

$$\text{cell_area} = 4\pi \times \left(\frac{3 \times \text{cell_volume}}{4\pi}\right)^{\frac{2}{3}}$$

$$\text{ICM_volume} = P_f \times \text{cell_volume}$$

$$\text{ICM_area} = 4\pi \times \left(\frac{3 \times \text{ICM_volume}}{4\pi}\right)^{\frac{2}{3}}$$

$$f_2 = P_f \times \frac{\text{ICM_area}}{\text{cell_area}}$$

$$B_{max} = 1 \times (\text{cell_volume} - \text{ICM_volume})$$

$$E_{max} = 1 \times \text{cell_area}$$

- C = extracellular carbon source available,
- C_{in} = intracellular carbon source available,
- K_F = intracellular carbon utilization rate via fermentation enzymes,
- K_R = intracellular carbon utilization rate via respiratory enzymes,
- K_{BE} = biomass to membrane transporter (i.e., methane monooxygenase, methanol dehydrogenase) production rate to bring carbon into the cell (C to C_{in}),
- K_{BF} = biomass to fermentation enzymes production rate,
- K_{BR} = biomass to respiratory enzymes production rate,
- K_{in} = rate of carbon conversion/transport into intracellular space
- d_F = decay rate of fermentation enzymes,
- d_R = decay rate of respiratory enzymes,
- d_E = decay rate of membrane transporter enzymes,
- d_B = biomass turnover rate,
- f₂ = ICM proportion,
- e = efficiency of biomass production through fermentation,
- B_{max} = maximum biomass able to be produced,
- E_{max} = maximum membrane transporters capable of being produced on cell membrane



Dr. Parag Katira



Software: MATLAB

R2022a

Package: ode45

differential equation solver

SYSTEM OF DIFFERENTIAL EQUATIONS

$$\frac{dC_{in}}{dt} = K_{in} \times \frac{C}{N} \times E - K_f \times C_{in} \times F - K_r \times f_2 \times C_{in} \times R$$

$$\begin{aligned} \frac{dB}{dt} \\ = & (e \times K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) \times \left(1 - \frac{B}{B_{max}}\right) \\ - & (K_{BF} + K_{BR} + K_{BE}) \times B - d_B \times B \end{aligned}$$

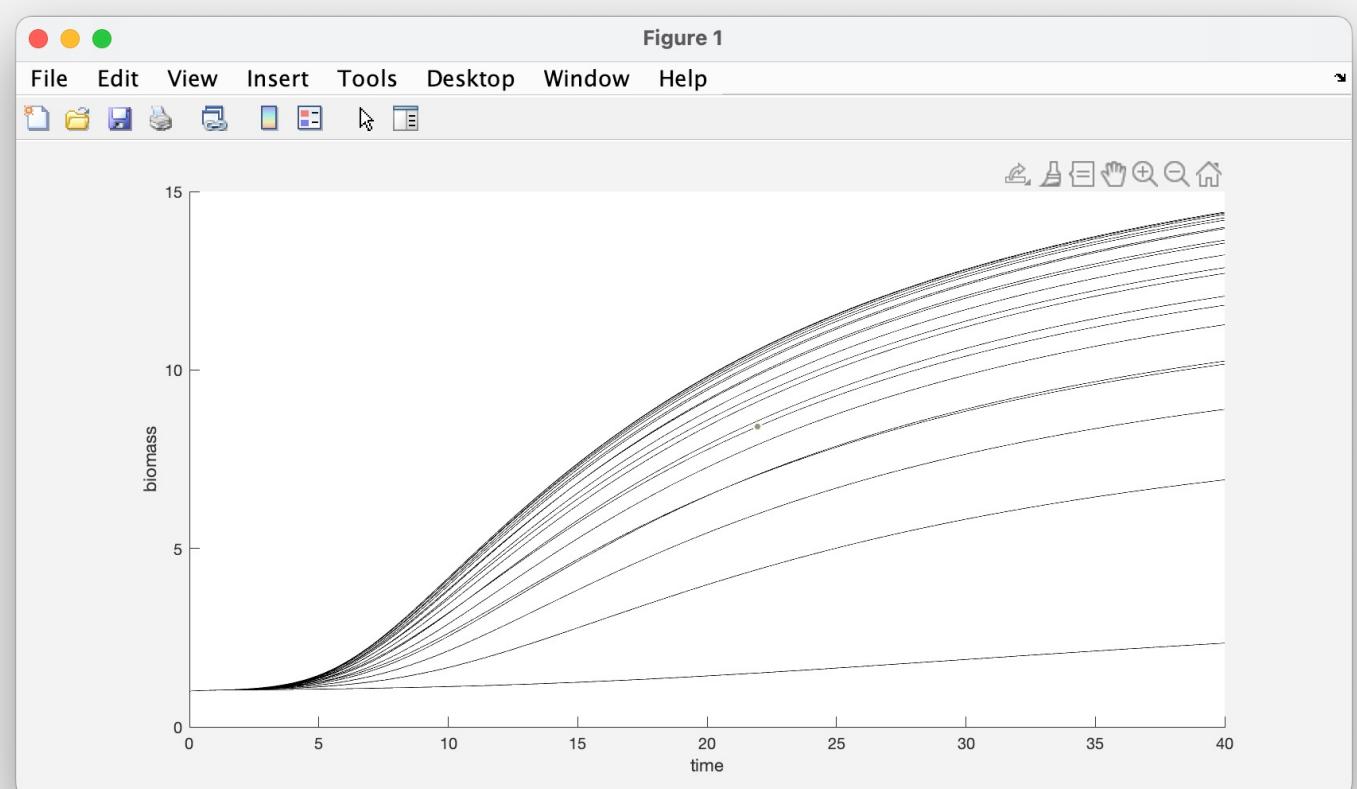
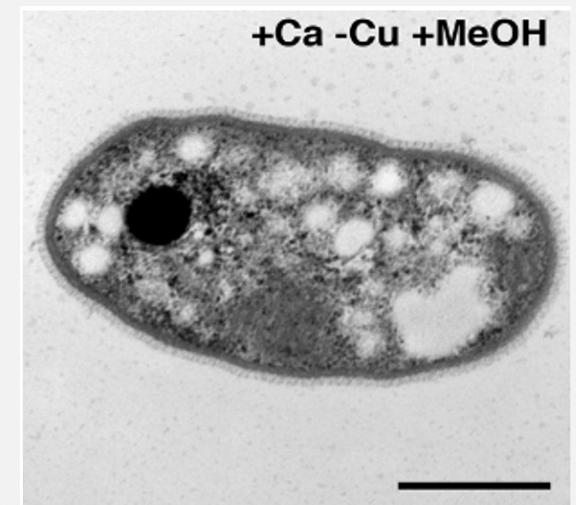
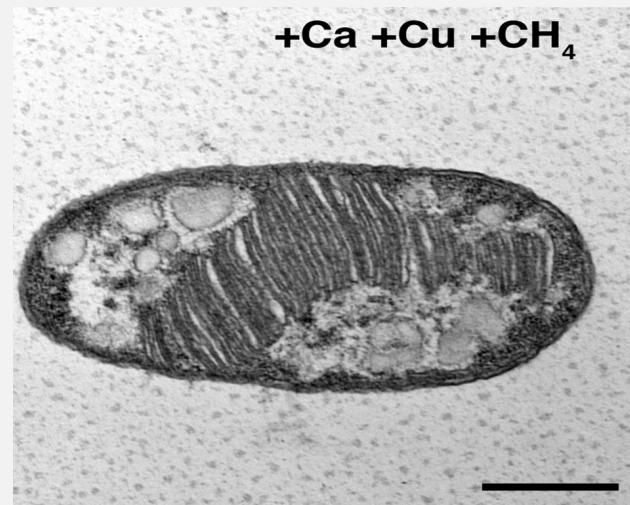
$$\frac{dN}{dt} = d_B \times B$$

$$\frac{dR}{dt} = K_{BR} \times B - d_R \times R$$

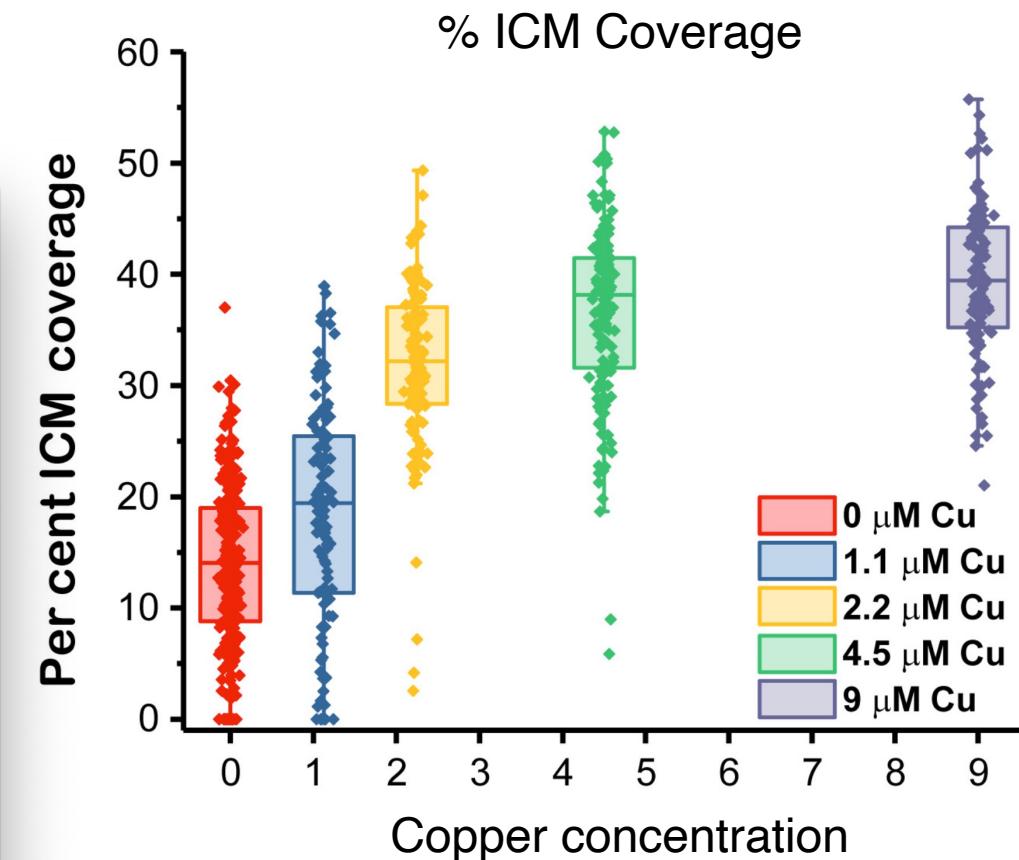
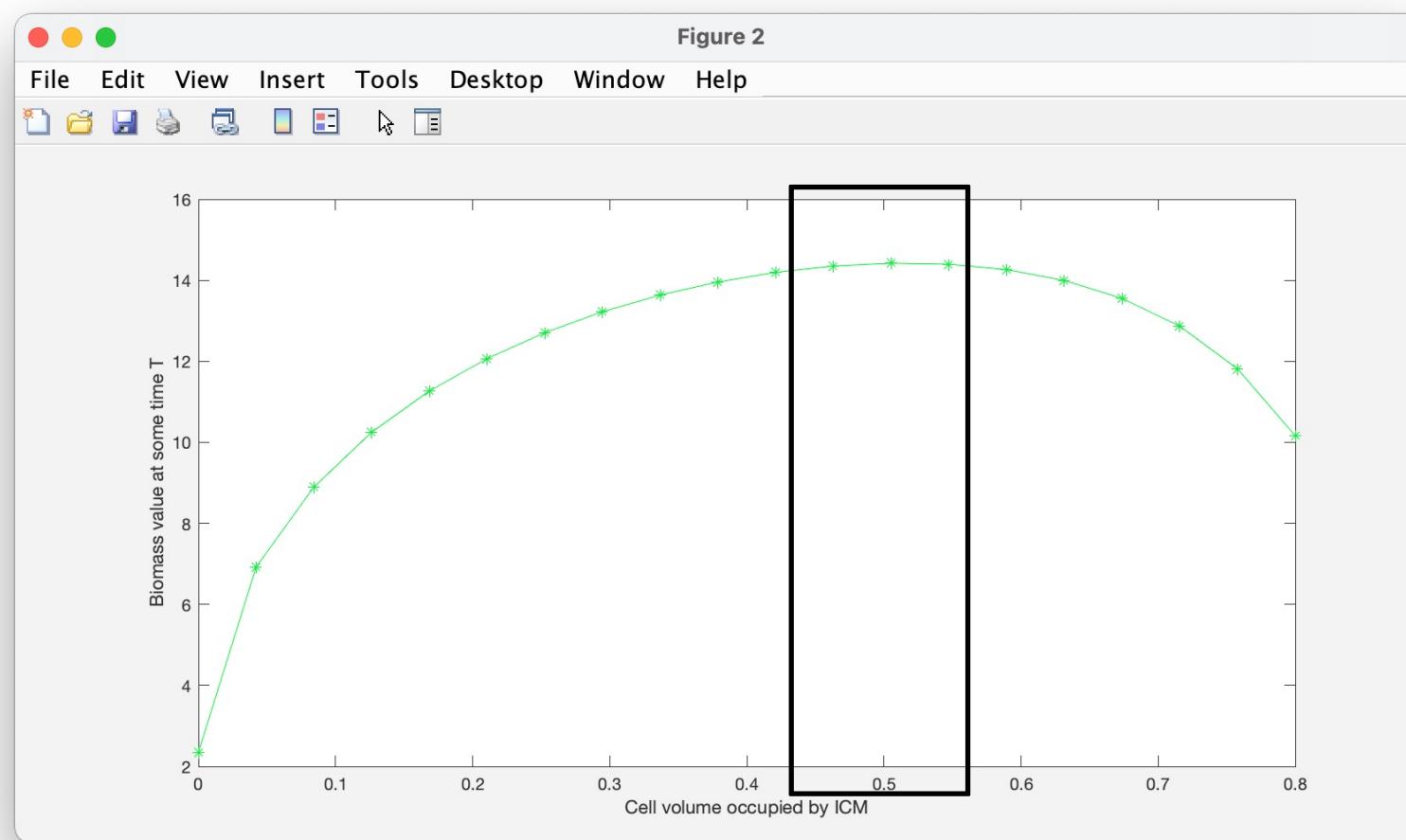
$$\frac{dF}{dt} = K_{BF} \times B - d_F \times F$$

$$\frac{dE}{dt} = K_{BE} \times B \times \left(1 - \frac{E}{E_{max}}\right) - d_E \times E$$

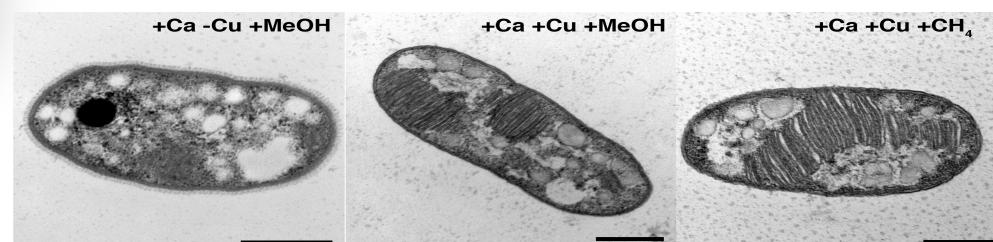
$$\begin{aligned} \frac{dW}{dt} \\ = & f_2 \left[(K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) \right. \\ - & \left. (e \times K_f \times C_{in} \times F + K_r \times f_2 \times C_{in} \times R) \left(1 - \frac{B}{B_{max}}\right) \right] \end{aligned}$$



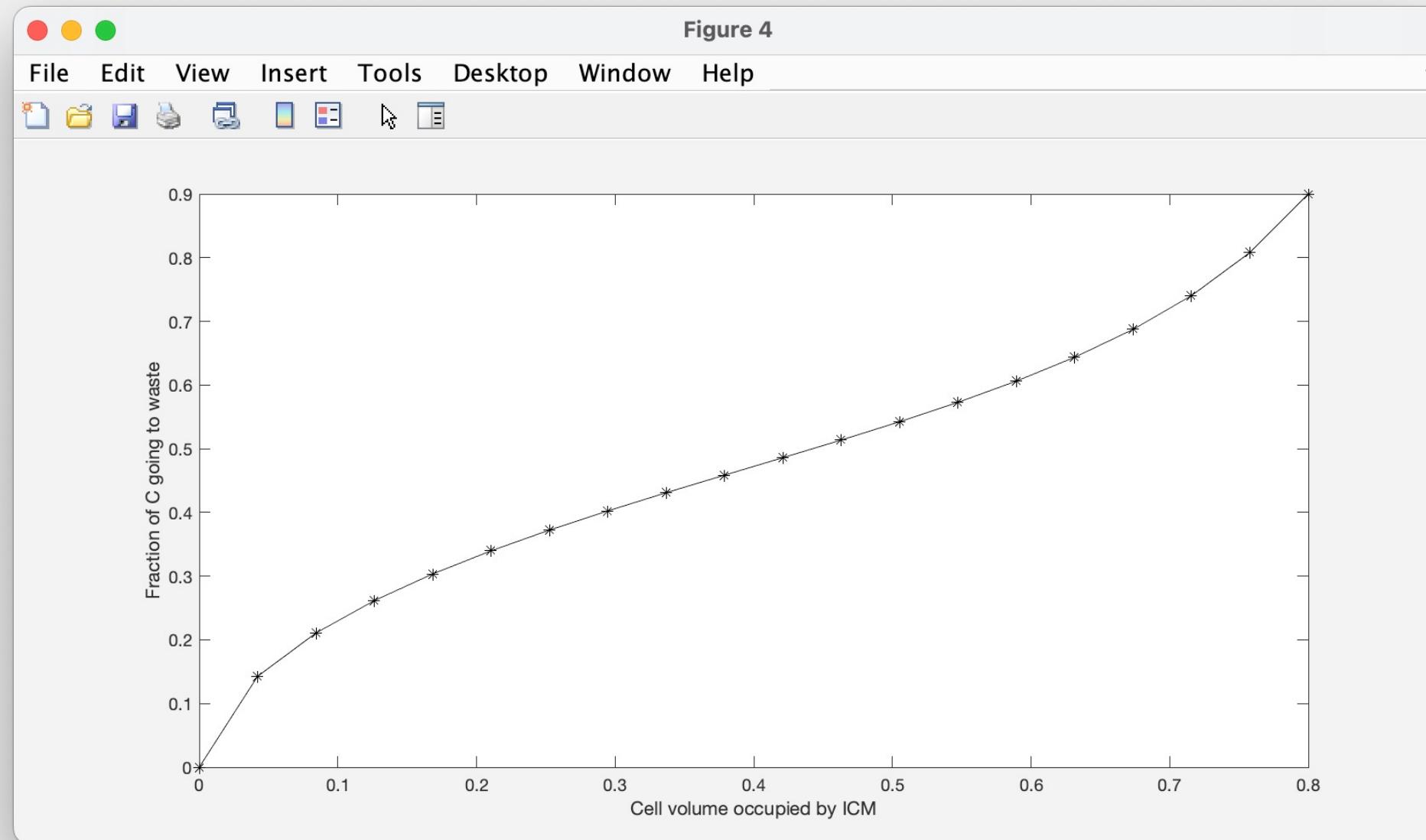
ICM INFLUENCE ON BIOMASS PRODUCTION



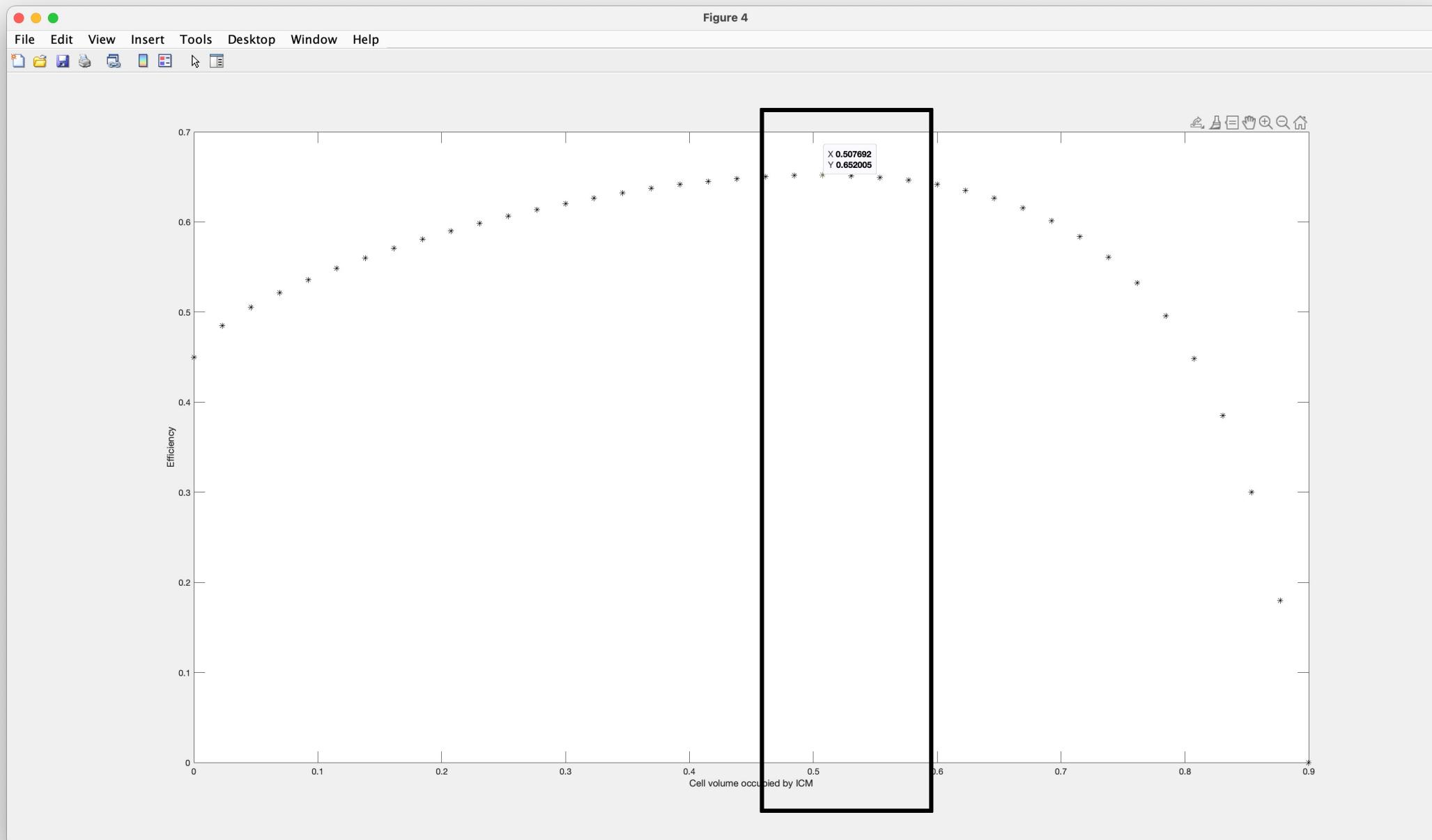
Whiddon, K. T., et al. (2019). Fluorescence-based analysis of the intracytoplasmic membranes of type I methanotrophs. *Microbial biotechnology*, 12(5), 1024-1033.



ICM INFLUENCE ON CARBON CONVERSION EFFICIENCY



EFFICIENCY (CARBON UTILIZED/WASTE PRODUCED)



OVERFLOW MODEL: SUMMARY AND NEXT DIRECTIONS

- When ICM fraction is high, waste is being produced (formate) which cannot be effectively utilized
 - Excreted as outflow
- More free cytosol = more formaldehyde assimilation
- Higher ICM fraction = less free cytosol = limited formaldehyde utilization → Increased formate production
- Lower ICM fraction = more free cytosol = increased formaldehyde utilization → Decreased formate production
- Conversion efficiency peaks with ICM fraction 0.45-0.55; correlates well with prior studies
 - After reaching a critical ICM fraction threshold, efficiency begins to rapidly decline with continually increasing ICM proportion
- The current model captures the critical features of *M. alcaliphilum*'s C₁ metabolism.
 - Formate secretion needs to be adjusted (i.e., Overflow begins immediately)
 - Formate was not intended to be re-used as a carbon source during initial equation design
- Additional model complexity can be added, as needed

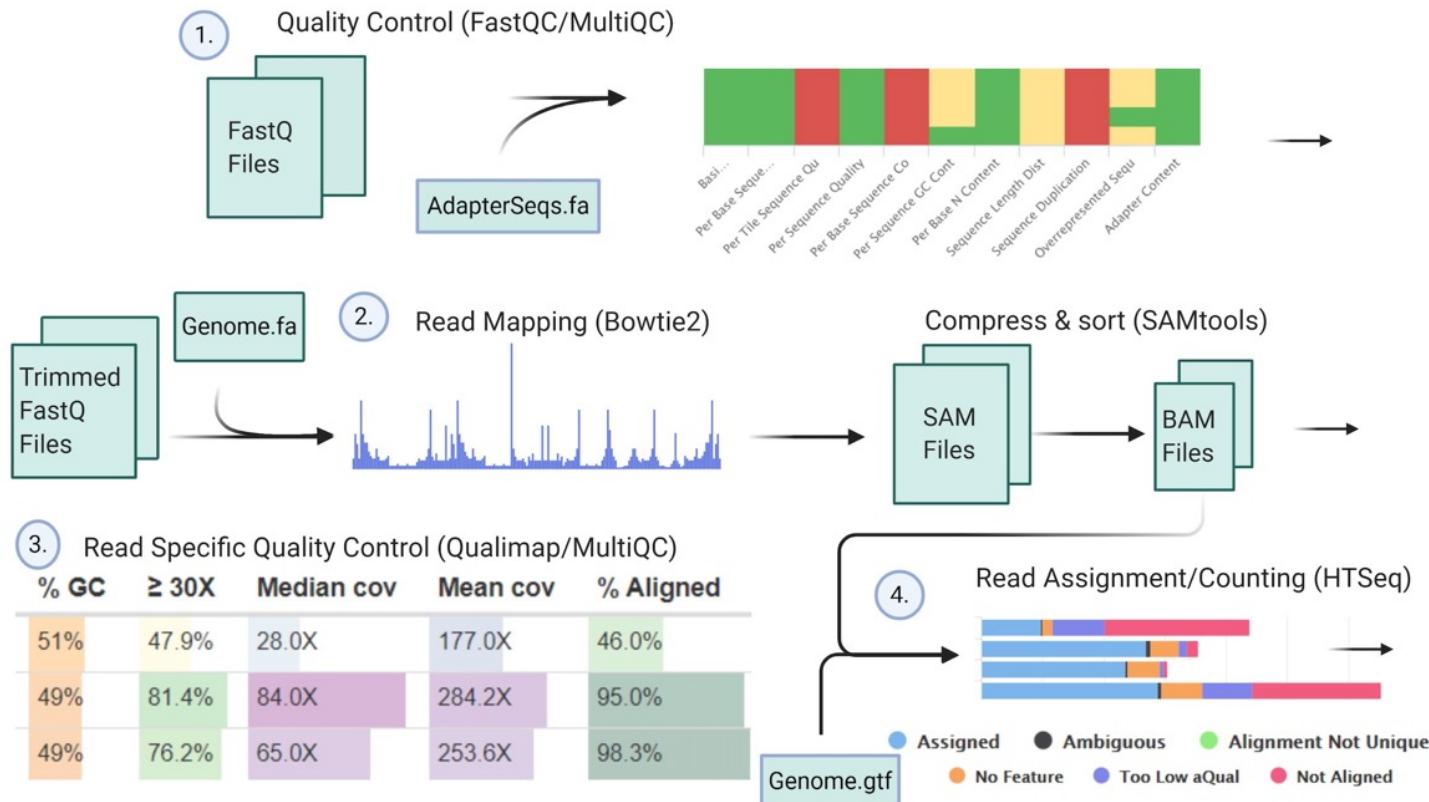
RNA-SEQ ANALYSES TO ASSESS GENE ACTIVITY OF CORE C1 PATHWAYS IN METAL LIMITED CONDITIONS

Master's Thesis: Aim 3

RNA-SEQ ANALYSIS PIPELINE



ANACONDA®



Johnson, Z. J., Krutkin, D. D., Bohutskyi, P., & Kalyuzhnaya, M. G. (2021). Metals and methylotrophy: Via global gene expression studies. *Methods in Enzymology*, 650, 185-213.

```

paired_end_whole_pipeline.sh x
#!/bin/bash

#Directory from where the script is executed
parent_directory=$(dirname $0)

project_name=$1 #name of project (plain input)
organism_name=$2 #short name of organism, needed to build reference when mapping with bowtie2 (plain input)
fastq_gz_dir=$3 #directory with all of the fastq files (path to directory)
fasta_reference=$4 #reference fasta (path to .fna file)
seq_adapter=$5 #Sequencing adapter sequence (path to .fa file)
gtf_ref=$6 #GTF reference file (path to .gtf file)

#Check to see if the user input is a directory
if [[ ! -d $fastq_gz_dir ]]; then
    echo "Enter valid directory with fastq files. Exiting..."
    exit
fi

if [[ ! -d $parent_directory/$project_name ]]; then
    echo "The directory $parent_directory/$project_name exists, skipping creation of directory..."
else
    echo "Directory does not exist... creating $parent_directory/$project_name"
    mkdir -p $parent_directory/$project_name/{01_fastq,02_trimmed_fastq,03_mapped_reads/{SAM,sBAM},04_gene_counts}
fi

#Pathnames stored as variables to be called on
QC1=$parent_directory/$project_name/Quality_Control/01_fastq
tfastq=$parent_directory/$project_name/02_trimmed_fastq
QC2=$parent_directory/$project_name/Quality_Control/02_trimmed_fastq
indexed=$parent_directory/$project_name/ref_seqs/$organism_name
mappedSAM=$parent_directory/$project_name/03_mapped_reads/SAM
sortedBAM=$parent_directory/$project_name/03_mapped_reads/sBAM
QC3=$parent_directory/$project_name/Quality_Control/03_mapped_reads
counts=$parent_directory/$project_name/04_gene_counts
ref_seqs=$parent_directory/$project_name/ref_seqs

#number of sample files
fil_num=$(find $fastq_gz_dir -name "*.fastq.gz" | wc -l)
echo "Processing $fil_num files in $fastq_gz_dir"

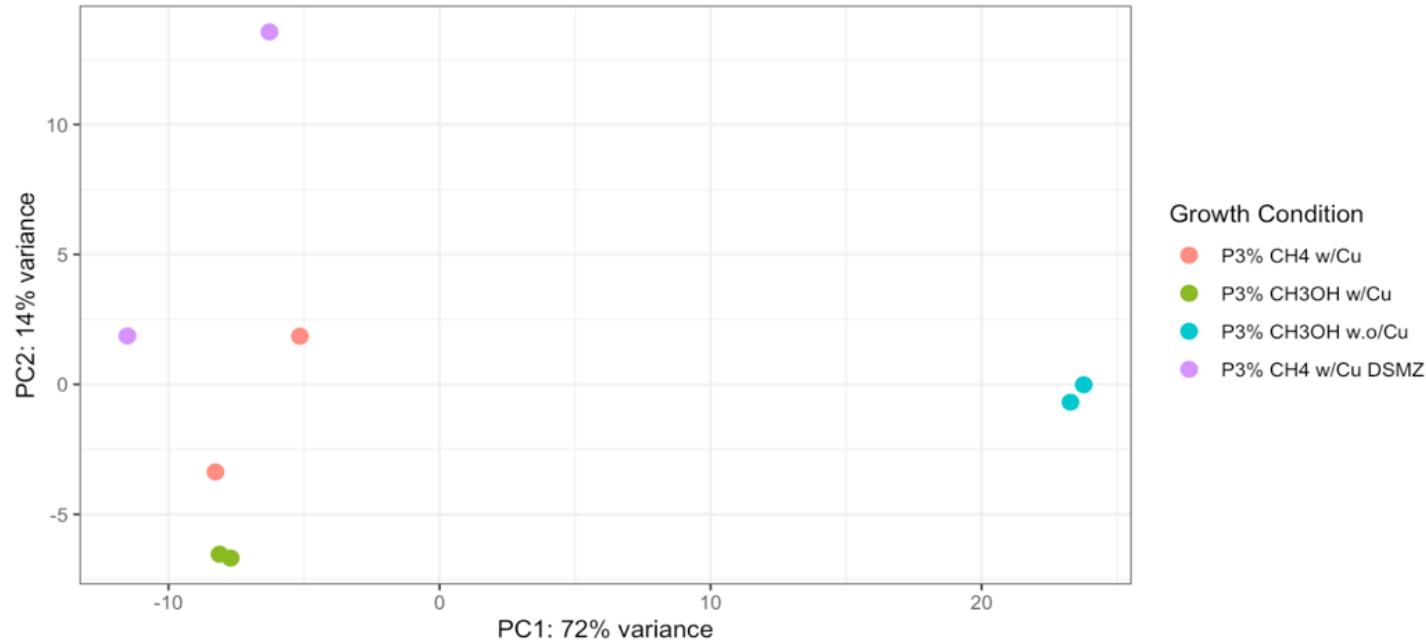
#####
> $tfastq/trimmomatic_stdout.txt
> $tfastq/trimmomatic_stderr.txt

mate1=""
mate2=""

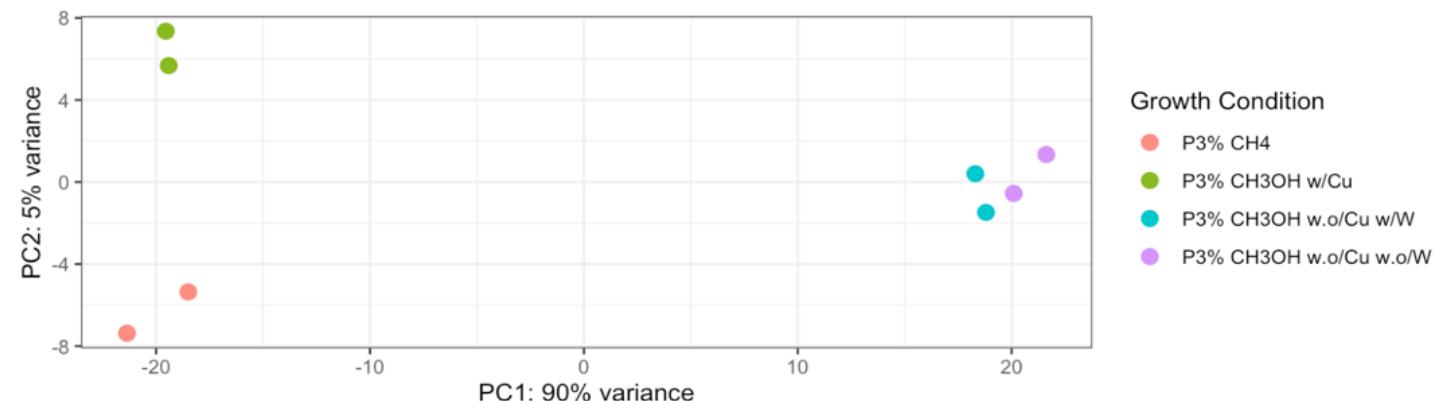
```

SAMPLES USED FOR ANALYSES

Total RNA-seq samples

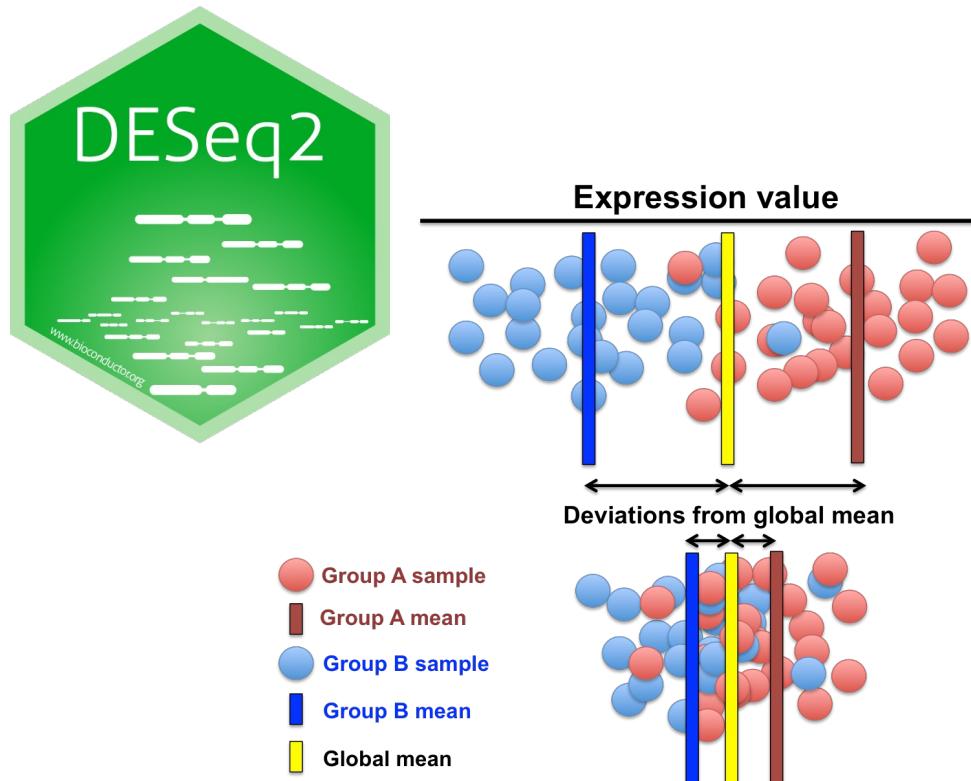


mRNA-seq samples



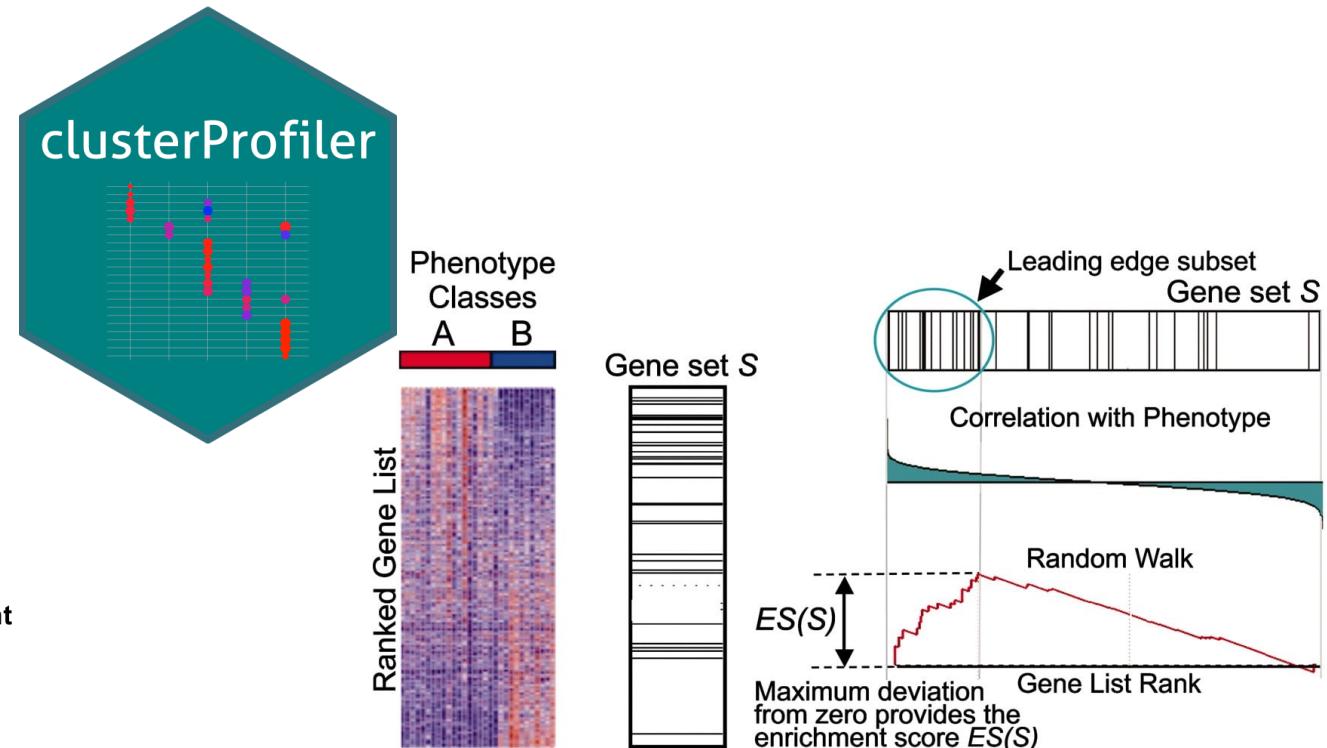
POST-PROCESSING ANALYSES

Differential Gene Expression Analysis



Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, 15(12), 1-21.

Gene Set Enrichment Analysis



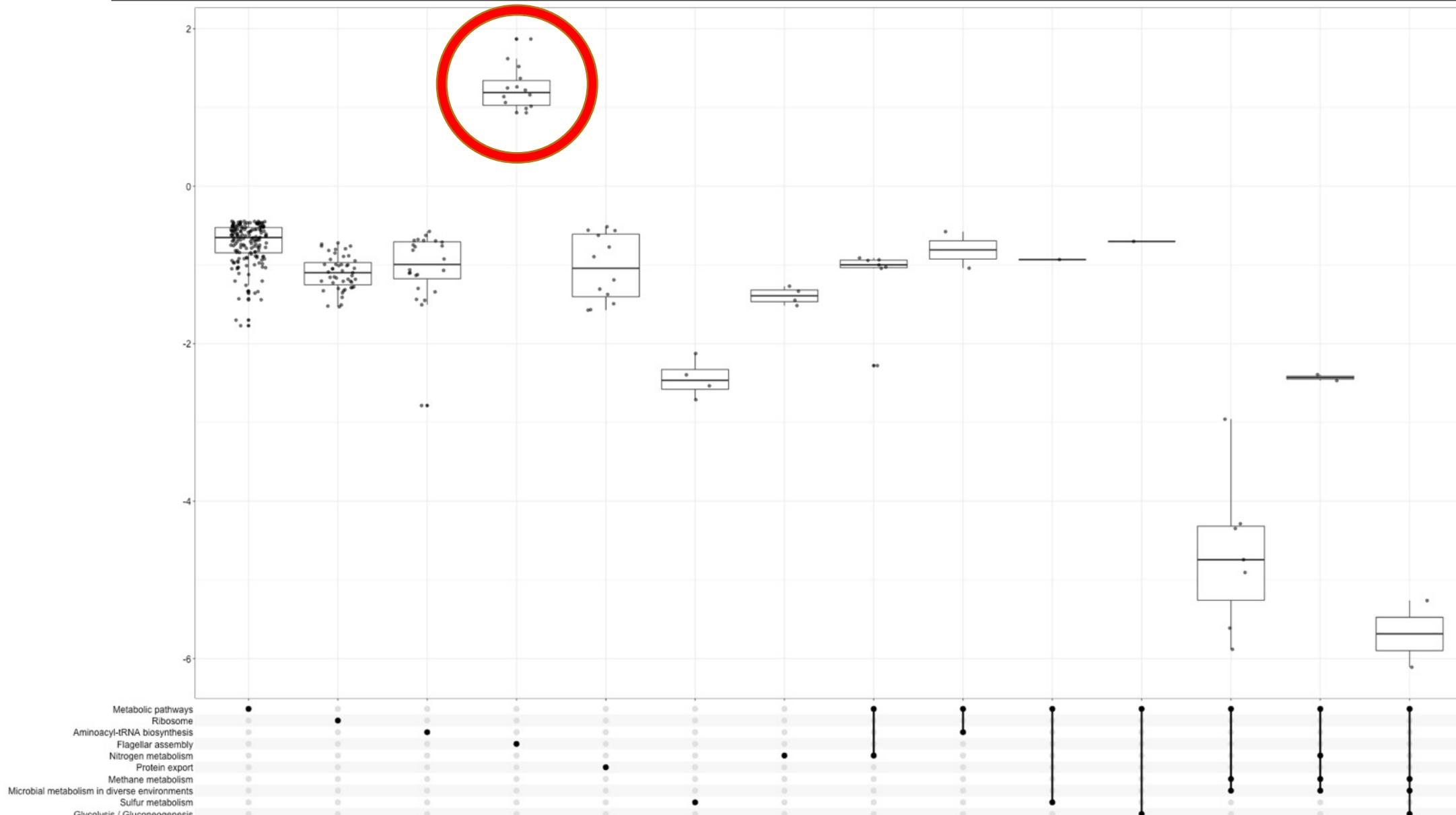
Subramanian, A. et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, 102(43), 15545-15550.

Wu, T. et al. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovation*, 2(3), 100141.

Enzyme/Pathway	Function	Gene ID (Mage)	NCBI ID	Protein ID	P3% MeOH +Cu vs P3% MeOH -Cu	
					Log2foldChange	padj
Methane Oxidation (pMMO)	Particulate methane monooxygenase subunit C	MALCv4_0514	MEALZ_0514	CCE22212	-0.50	2.57E-08
	Particulate methane monooxygenase subunit A	MALCv4_0515	MEALZ_0515	CCE22213	-1.93	3.12E-101
	Particulate methane monooxygenase subunit B	MALCv4_0516	MEALZ_0516	CCE22214	-1.82	5.23E-101
Methanol Oxidation (MxaF-MDH)	MxaL protein	MALCv4_3438	MEALZ_3438	CCE25101	-3.09	3.32E-47
	MxaK protein	MALCv4_3439	MEALZ_3439	CCE25102	-4.88	8.70E-32
	MxaC protein	MALCv4_3440	MEALZ_3440	CCE25103	-5.49	3.21E-43
	MxaA protein	MALCv4_3441	MEALZ_3441	CCE25104	-5.23	2.01E-85
	MxaS protein	MALCv4_3442	MEALZ_3442	CCE25105	-5.71	1.32E-68
	MxaP protein	MALCv4_3443	MEALZ_3443	CCE25106	-6.05	7.75E-61
	MxaR protein	MALCv4_3444	MEALZ_3444	CCE25107	-6.06	1.25E-150
	MxaP protein (Cytochrome cl)	MALCv4_3446	MEALZ_3446	CCE25109	-5.63	1.23E-132
	MxaF methanol dehydrogenase, small subunit	MALCv4_3445	MEALZ_3445	CCE25108	-6.00	9.57E-97
	MxaF methanol dehydrogenase, large subunit	MALCv4_3448	MEALZ_3448	CCE25111	-6.15	0.00E+00
Methanol Oxidation (XoxF-MDH)	MxaB DNA binding response regulator	MALCv4_3449	MEALZ_3449	CCE25112	-4.04	7.40E-28
	XoxF methanol dehydrogenase	MALCv4_3497	MEALZ_3497	CCE25159	3.16	1.03E-155
	MxaJ-like protein	MALCv4_3498	MEALZ_3498	CCE25160	-0.33	3.32E-01
Formaldehyde Oxidation	Cytochrome X (putative XoxG4)	MALCv4_2642	MEALZ_2642	CCE24317	1.97	8.24E-77
	Formaldehyde-activating enzyme	MALCv4_2428	MEALZ_2428	CCE24109	-0.88	5.24E-15
	Formaldehyde-activating enzyme 4	MALCv4_1456	MEALZ_1456	CCE23144	0.04	8.95E-01
	Formaldehyde-activating enzyme 2	MALCv4_0850	MEALZ_0850	CCE22544	1.40	6.59E-30
Formate Oxidation	Sulfide:quinone oxidoreductase/aldehyde dehydrogenase	MALCv4_0272	MEALZ_0272	CCE1972	0.42	3.54E-01
	Tungsten-containing formate dehydrogenase, beta subunit	MALCv4_1883	MEALZ_1883	CCE23569	-0.58	6.77E-07
	Tungsten-containing formate dehydrogenase, alpha subunit	MALCv4_1882	MEALZ_1882	CCE23568	-0.72	1.27E-10
	Molybdenum containing formate dehydrogenase, delta	MALCv4_0215	MEALZ_0215	CCE21915	0.58	4.35E-01
	Molybdenum containing formate dehydrogenase, accessory	MALCv4_0216	MEALZ_0216	CCE21916	-0.06	9.14E-01
	Molybdenum containing formate dehydrogenase, alpha	MALCv4_0217	MEALZ_0217	CCE21917	0.05	9.84E-01
	Na(+)-translocating NADH-quinone reductase subunit F	MALCv4_2228	MEALZ_2228	CCE23914	-0.33	7.73E-02
	Na(+)-translocating NADH-quinone reductase subunit E	MALCv4_2229	MEALZ_2229	CCE23915	-0.39	1.23E-01
	Na(+)-translocating NADH-quinone reductase subunit D	MALCv4_2230	MEALZ_2230	CCE23916	-0.51	2.07E-02
	Na(+)-translocating NADH-quinone reductase subunit C	MALCv4_2231	MEALZ_2231	CCE23917	-0.71	7.37E-05
ETS Complex I	Na(+)-translocating NADH-quinone reductase subunit B	MALCv4_2232	MEALZ_2232	CCE23918	-0.60	1.00E-04
	Na(+)-translocating NADH-quinone reductase subunit A	MALCv4_2233	MEALZ_2233	CCE23919	-0.41	4.81E-03
	Quinolinate synthase A	MALCv4_2234	MEALZ_2234	CCE23920	0.14	5.92E-01
	NAD-reducing hydrogenase hox5, beta subunit	MALCv4_1304	MEALZ_1304	CCE22993	0.51	3.77E-03
	NAD-reducing hydrogenase hox5, delta subunit	MALCv4_1305	MEALZ_1305	CCE22994	0.38	3.23E-01
	NADH:ubiquinone oxidoreductase, gamma subunit	MALCv4_1306	MEALZ_1306	CCE22995	0.54	6.77E-02
	NAD-reducing hydrogenase hox5, alpha subunit	MALCv4_1307	MEALZ_1307	CCE22996	0.51	1.24E-03
	NADH dehydrogenase	MALCv4_1287	MEALZ_1287	CCE22976	-0.40	2.97E-01
	NADH ubiquinone oxidoreductase 2	MALCv4_3726	MEALZ_3726	CCE25382	-0.08	9.27E-01
	sdhX, hypothetical protein	MALCv4_2678	MEALZ_2678	CCE24353	0.75	1.34E-01
ETS Complex II	sdhB, succinate dehydrogenase	MALCv4_2679	MEALZ_2679	CCE24354	0.78	8.87E-03
	sdhA, succinate dehydrogenase	MALCv4_2680	MEALZ_2680	CCE24355	0.96	1.47E-09
	sdhE, succinate dehydrogenase, hydrophobic membrane	MALCv4_2681	MEALZ_2681	CCE24356	1.08	4.89E-02
	Succinate dehydrogenase cytochrome b556 subunit	MALCv4_2682	MEALZ_2682	CCE24357	0.63	1.95E-01
ETS Complex III	Cytochrome c1	MALCv4_0632	MEALZ_0632	CCE22327	-0.24	4.94E-01
	Cytochrome b	MALCv4_0633	MEALZ_0633	CCE22328	-0.40	6.56E-02
	Ubiquinol-cytochrome c reductase	MALCv4_0634	MEALZ_0634	CCE22329	-0.39	1.72E-01
	Cytochrome B557	MALCv4_1724	MEALZ_1724	CCE23411	0.51	1.44E-01
Cytochromes	Bacterioferritin-associated ferredoxin Bfd	MALCv4_1725	MEALZ_1725	CCE23412	0.52	5.61E-01
	Cytochrome c6	MALCv4_0938	MEALZ_0938	CCE22632	0.40	2.20E-01
	Cytochrome c class I	MALCv4_0390	MEALZ_0390	CCE22090	-0.59	6.28E-03
	Cytochrome B561	MALCv4_0602	MEALZ_0602	CCE22297	0.15	8.52E-01
	Cytochrome P460	MALCv4_0918	MEALZ_0918	CCE22612	0.07	8.85E-01
	Cytochrome c'-beta	MALCv4_0702	MEALZ_0702	CCE22397	0.67	2.87E-03
	Cytochrome c class I	MALCv4_1120	MEALZ_1120	CCE22811	0.51	8.28E-02
	Cytochrome c family protein	MALCv4_1295	MEALZ_1295	CCE22984	-0.69	1.54E-01
	Cytochrome c peroxidase	MALCv4_3827	MEALZ_3827	CCE25482	0.36	3.91E-01
	Cytochrome C oxidase polypeptide III	MALCv4_2312	MEALZ_2312	CCE23993	-1.51	5.01E-18
ETS Complex IV (cytochrome ca3 oxidase)	Cytochrome C oxidase assembly protein	MALCv4_2313	MEALZ_2313	CCE23994	-1.24	4.93E-05
	Cytochrome aa3 oxidase, subunit I	MALCv4_2314	MEALZ_2314	CCE23995	-1.23	5.01E-19
	Cytochrome C oxidase, subunit II	MALCv4_2315	MEALZ_2315	CCE23996	-1.23	4.66E-19
	Bacteriohemerythrin	MALCv4_2316	MEALZ_2316	CCE23997	-0.34	7.99E-01
ETS Complex IV (cytochrome ba3 oxidase)	Cytochrome C oxidase, CbaD subunit	MALCv4_1292	MEALZ_1292	CCE22981	-1.68	1.71E-01
	Cytochrome C oxidase, subunit II	MALCv4_1293	MEALZ_1293	CCE22982	0.14	8.84E-01
	Cytochrome C oxidase, subunit I	MALCv4_1294	MEALZ_1294	CCE22983	-1.13	9.14E-05
ATP Biosynthesis	ATP synthase, subunit beta 2	MALCv4_3735	MEALZ_3735	CCE25391	0.63	1.84E-02
	ATP synthase, subunit b2	MALCv4_3741	MEALZ_3741	CCE25397	0.29	6.17E-01
RuMP & PPP	3-hexulose-6-phosphate isomerase	MALCv4_3952	MEALZ_3952	CCE25608	-0.07	7.25E-01
	3-hexulose-6-phosphate synthase	MALCv4_3953	MEALZ_3953	CCE25609	-0.01	9.71E-01
	Hexulose-6-phosphate synthase and isomerase	MALCv4_1912	MEALZ_1912	CCE23598	0.23	2.37E-01
	Transaldolase	MALCv4_3948	MEALZ_3948	CCE25604	-0.13	4.18E-01
	Transketolase	MALCv4_3951	MEALZ_3951	CCE25607	-0.42	3.83E-05
	Fructose-bisphosphate aldolase	MALCv4_3947	MEALZ_3947	CCE25603	-0.30	1.36E-02
EMP	Glyeraldehyde 3-phosphate dehydrogenase	MALCv4_3079	MEALZ_3079	CCE24745	-0.04	8.62E-01
	Pyruvate kinase II	MALCv4_3080	MEALZ_3080	CCE24746	-0.27	5.07E-02
	Phosphoglycerate kinase	MALCv4_3549	MEALZ_3549	CCE25207	-0.52	2.34E-03
EDD/oxPPP	Glucose-6-phosphate isomerase	MALCv4_0104	MEALZ_0104	CCE21808	-0.57	3.77E-03
	Glucose-1-dehydrogenase I	MALCv4_1699	MEALZ_1699	CCE23386	0.67	1.84E-01
	2-dehydro-3-deoxyphosphoconitate aldolase	MALCv4_1362	MEALZ_1362	CCE23051	-0.79	1.28E-02
	6-phosphogluconate dehydratase	MALCv4_1363	MEALZ_1363	CCE23052	-0.92	1.10E-07
TCA	Aconitase hydratase, acnA	MALCv4_0310	MEALZ_0310	CCE22010	0.87	4.80E-06
	Citrate synthase, gltA2	MALCv4_1360	MEALZ_1360	CCE23049	0.03	9.35E-01
	Succinate semialdehyde dehydrogenase, gabD	MALCv4_1576	MEALZ_1576	CCE22363	-0.15	7.34E-01
	Dihydrolipoyl dehydrogenase, odhL	MALCv4_1578	MEALZ_1578	CCE22365	0.38	2.21E-01
	2-oxoglutarate dehydrogenase E2, sucB	MALCv4_1579	MEALZ_1579	CCE23266	0.42	1.80E-01
	2-oxoglutarate dehydrogenase E1, sucA	MALCv4_1580	MEALZ_1580	CCE23267	0.53	2.81E-03
	Citrate synthase, gltA	MALCv4_3024	MEALZ_3024	CCE24690	-0.24	3.46E-01
	Aconitase hydratase 2, acnB	MALCv4_3025	MEALZ_3025	CCE24691	-0.16	3.56E-01
	Iso citrate dehydrogenase, NAD-dependent, icd	MALCv4_3026	MEALZ_3026	CCE24692	-0.18	3.72E-01
	Succinyl-CoA ligase, subunit alpha	MALCv4_3290	MEALZ_3290	CCE24955	0.37	2.37E-01
Serine Cycle & H4-folate pathway	Iso citrate dehydrogenase, NADH-dependent, icdh	MALCv4_3844	MEALZ_3844	CCE25499	0.23	5.35E-01
	Malate thiokinase, small subunit	MALCv4_3215	MEALZ_3215	CCE24880	-1.03	6.42E-08
	Malate thiokinase, large subunit	MALCv4_3216	MEALZ_3216	CCE24881	-1.21	3.16E-04
	Malyl-CoA lyase	MALCv4_3217	MEALZ_3217	CCE24882	-0.93	2.25E-08
	Serine glyoxylate aminotransferase	MALCv4_3218	MEALZ_3218	CCE24883	-0.95	2.94E-13
	2-hydroxyacid dehydrogenase NAD-binding	MALCv4_3219	MEALZ_3219	CCE24884	-0.07	8.73E-01
	Malate dehydrogenase	MALCv4_3220	MEALZ_3220	CCE24885	0.20	3.36E-01
	NADP-methylenetetrahydrofolate dehydrogenase	MALCv4_3221	MEALZ_3221	CCE24886	-0.90	2.88E-03
Fatty Acid Metabolism	Glycerate 2-kinase	MALCv4_3222	MEALZ_3222	CCE24887	-0.76	4.42E-02
	Serin hydroxymethyltransferase	MALCv4_3223	MEALZ_3223	CCE24888	-0.72	2.91E-04
	Formate tetrahydrofolate ligase	MALCv4_3224	MEALZ_3224	CCE24889	-0.83	5.40E-06
	Acyl-coenzyme A dehydrogenase	MALCv4_0452	MEALZ_0452	CCE22151	1.04	1.18E-01
	3-hydroxyacyl-CoA dehydrogenase	MALCv4_0453	MEALZ_0453	CCE22152	1.15	5.77E-02
	3-ketoacyl-CoA thiolase	MALCv4_0454	MEALZ_0454	CCE22153	1.14	2.60E-01
	Squalene-hopene cyclase	MALCv4_2523	MEALZ_2523	CCE24201	0.11	9.18E-01

Enzyme/Pathway	Function	Gene ID (Mage)	NCBI ID	Protein ID	P3% MeOH +Cu+/W vs P3% MeOH -Cu-/W			=	N.S.
					Log2FoldChange	padj			
Methane Oxidation (pMMO)	Particulate methane monooxygenase subunit C	MALCV4_0514	MEALZ_0514	CCE22212	-1.08	5.89E-13			
	Particulate methane monooxygenase subunit A	MALCV4_0515	MEALZ_0515	CCE22213	-2.25	2.36E-46			= padj < 0.05
	Particulate methane monooxygenase subunit B	MALCV4_0516	MEALZ_0516	CCE22214	-2.29	7.04E-43			= padj < 0.01
	MxaL protein	MALCV4_3438	MEALZ_3438	CCE25101	-3.12	4.20E-98			= padj < 0.001
	MxaK protein	MALCV4_3439	MEALZ_3439	CCE25102	-4.68	2.14E-150			
	MxaC protein	MALCV4_3440	MEALZ_3440	CCE25103	-5.89	1.86E-274			
Methanol Oxidation (MxaF-MDH)	MxaA protein	MALCV4_3441	MEALZ_3441	CCE25104	-6.34	7.02E-301			
	MxaS protein	MALCV4_3442	MEALZ_3442	CCE25105	-6.42	1.10E-252			
	MxaP protein	MALCV4_3443	MEALZ_3443	CCE25106	-5.57	0.00E+00			
	MxaBt protein	MALCV4_3444	MEALZ_3444	CCE25107	-6.75	0.00E+00			= -1:-2
	MxaG protein (Cytochrome c1)	MALCV4_3446	MEALZ_3446	CCE25109	-7.39	0.00E+00			= -2:-3
	MxaF methanol dehydrogenase, small subunit	MALCV4_3445	MEALZ_3445	CCE25108	-6.79	3.72E-282			= -3:-4
Methanol Oxidation (XoxF-MDH)	MxaF methanol dehydrogenase, large subunit	MALCV4_3448	MEALZ_3448	CCE25111	-8.01	1.18E-45			
	MxaB DNA binding response regulator	MALCV4_3449	MEALZ_3449	CCE25112	-2.67	4.15E-04			
	XoxF methanol dehydrogenase	MALCV4_3497	MEALZ_3497	CCE25159	2.68	7.35E-76			
	MxaJ-like protein	MALCV4_3498	MEALZ_3498	CCE25160	-0.22	1.90E-01			= -4:-5
Formaldehyde Oxidation	Cytochrome X (putative XoxG4)	MALCV4_2642	MEALZ_2642	CCE24317	2.59	4.93E-79			= <-5
	Formaldehyde-activating enzyme	MALCV4_2428	MEALZ_2428	CCE24109	-0.78	1.89E-08			
	Formaldehyde-activating enzyme 4	MALCV4_1456	MEALZ_1456	CCE23144	-0.09	6.30E-01			
	Formaldehyde-activating enzyme 2	MALCV4_0850	MEALZ_0850	CCE22544	2.61	1.93E-45			
	Sulfide:quinone oxidoreductase/aldehyde dehydrogenase	MALCV4_0272	MEALZ_0272	CCE21972	0.34	5.93E-02			= 0:1
	Tungsten-containing formate dehydrogenase, beta subunit	MALCV4_1883	MEALZ_1883	CCE23569	-0.31	6.22E-02			= 1:2
	Tungsten-containing formate dehydrogenase, alpha subunit	MALCV4_1882	MEALZ_1882	CCE23568	-0.74	2.38E-08			= 2:3
	Molybdenum containing formate dehydrogenase, delta	MALCV4_0215	MEALZ_0215	CCE21915	0.45	5.70E-02			
	Molybdenum containing formate dehydrogenase, accessory	MALCV4_0216	MEALZ_0216	CCE21916	-1.02	9.52E-03			
	Molybdenum containing formate dehydrogenase, alpha	MALCV4_0217	MEALZ_0217	CCE21917	-1.43	4.46E-04			= >3
ETS Complex I	Na(+)-translocating NADH-quinone reductase subunit F	MALCV4_2228	MEALZ_2228	CCE23914	-0.90	9.58E-09			
	Na(+)-translocating NADH-quinone reductase subunit E	MALCV4_2229	MEALZ_2229	CCE23915	-1.05	7.48E-10			
	Na(+)-translocating NADH-quinone reductase subunit D	MALCV4_2230	MEALZ_2230	CCE23916	-0.59	1.18E-04			
	Na(+)-translocating NADH-quinone reductase subunit C	MALCV4_2231	MEALZ_2231	CCE23917	-0.88	4.85E-09			
	Na(+)-translocating NADH-quinone reductase subunit B	MALCV4_2232	MEALZ_2232	CCE23918	-0.97	5.88E-11			
	Na(+)-translocating NADH-quinone reductase subunit A	MALCV4_2233	MEALZ_2233	CCE23919	-0.57	4.94E-05			
	Quinolinate synthase A	MALCV4_2234	MEALZ_2234	CCE23920	0.25	1.31E-01			
	NAD-reducing hydrogenase hox5, beta subunit	MALCV4_1304	MEALZ_1304	CCE22993	0.47	9.93E-04			
	NAD-reducing hydrogenase hox5, delta subunit	MALCV4_1305	MEALZ_1305	CCE22994	0.54	4.13E-04			
	NADH:ubiquinone oxidoreductase, gamma subunit	MALCV4_1306	MEALZ_1306	CCE22995	0.80	5.75E-08			
ETS Complex II	NAD-reducing hydrogenase hox5, alpha subunit	MALCV4_1307	MEALZ_1307	CCE22996	0.69	1.95E-06			
	NADH dehydrogenase	MALCV4_1287	MEALZ_1287	CCE22976	-0.54	1.15E-03			
	NADH ubiquinone oxidoreductase 2	MALCV4_3726	MEALZ_3726	CCE25382	0.14	5.17E-01			
	sdhX, hypothetical protein	MALCV4_2678	MEALZ_2678	CCE24353	0.43	4.01E-02			
	sdhB, succinate dehydrogenase	MALCV4_2679	MEALZ_2679	CCE24354	0.80	4.80E-07			
	sdhA, succinate dehydrogenase	MALCV4_2680	MEALZ_2680	CCE24355	0.79	5.28E-08			
ETS Complex III	sdhE, succinate dehydrogenase, hydrophobic membrane	MALCV4_2681	MEALZ_2681	CCE24356	0.66	5.33E-03			
	Succinate dehydrogenase cytochrome b556 subunit	MALCV4_2682	MEALZ_2682	CCE24357	1.08	2.01E-10			
	Cytochrome c1	MALCV4_0632	MEALZ_0632	CCE22327	-0.30	7.85E-02			
	Cytochrome b	MALCV4_0633	MEALZ_0633	CCE22328	-0.31	5.24E-02			
Cytochromes	Ubiquinol-cytochrome c reductase	MALCV4_0634	MEALZ_0634	CCE22329	-0.09	6.14E-01			
	Cytochrome B557.5	MALCV4_1724	MEALZ_1724	CCE23411	0.48	1.68E-02			
	Bacterioferritin-associated ferredoxin Bfd	MALCV4_1725	MEALZ_1725	CCE23412	-0.61	4.01E-02			
	Cytochrome c6	MALCV4_0938	MEALZ_0938	CCE22632	-0.03	8.99E-01			
	Cytochrome c class I	MALCV4_0390	MEALZ_0390	CCE22090	-0.49	2.54E-03			
	Cytochrome B561	MALCV4_0602	MEALZ_0602	CCE22297	0.50	1.28E-02			
	Cytochrome P460	MALCV4_0918	MEALZ_0918	CCE22612	-0.10	6.15E-01			
	Cytochrome c'-beta	MALCV4_0702	MEALZ_0702	CCE22397	0.65	1.13E-05			
	Cytochrome c class I	MALCV4_1120	MEALZ_1120	CCE22811	0.48	2.62E-03			
	Cytochrome c family protein	MALCV4_1295	MEALZ_1295	CCE22984	-0.35	7.56E-02			
ETS Complex IV (cytochrome aa3 oxidase)	Cytochrome c peroxidase	MALCV4_3827	MEALZ_3827	CCE25482	1.17	7.28E-12			
	Cytochrome C oxidase polypeptide III	MALCV4_2312	MEALZ_2312	CCE23993	-1.79	2.07E-29			
	Cytochrome C oxidase assembly protein	MALCV4_2313	MEALZ_2313	CCE23994	-1.36	7.36E-18			
	Cytochrome aa3 oxidase, subunit I	MALCV4_2314	MEALZ_2314	CCE23995	-1.11	3.52E-13			
	Cytochrome C oxidase, subunit II	MALCV4_2315	MEALZ_2315	CCE23996	-1.10	4.81E-09			
	Bacteriohemerythrin	MALCV4_2316	MEALZ_2316	CCE23997	-0.12	7.70E-01			
ETS Complex IV (cytochrome ba3 oxidase)	Cytochrome C oxidase, CbaD subunit	MALCV4_1292	MEALZ_1292	CCE22981	-0.29	7.00E-01			
	Cytochrome C oxidase, subunit II	MALCV4_1293	MEALZ_1293	CCE22982	-0.69	1.78E-03			
	Cytochrome C oxidase, subunit I	MALCV4_1294	MEALZ_1294	CCE22983	-0.71	1.65E-04			
ATP Biosynthesis	ATP synthase, subunit beta 2	MALCV4_3735	MEALZ_3735	CCE25391	0.56	4.46E-04			
	ATP synthase, subunit b2	MALCV4_3741	MEALZ_3741	CCE25397	0.12	5.82E-01			
RuMP & PPP	3-hexulose-6-phosphate isomerase	MALCV4_3952	MEALZ_3952	CCE25608	-0.28	5.54E-02			
	3-hexulose-6-phosphate synthase	MALCV4_3953	MEALZ_3953	CCE25609	-0.09	6.02E-01			
	Hexulose-6-phosphate synthase and isomerase	MALCV4_1912	MEALZ_1912	CCE23598	-0.08	6.78E-01			
	Transaldolase	MALCV4_3948	MEALZ_3948	CCE25604	-0.33	3.32E-02			
	Transketolase	MALCV4_3951	MEALZ_3951	CCE25607	-0.53	1.17E-04			
	Fructose-bisphosphate aldolase	MALCV4_3947	MEALZ_3947	CCE25603	-0.27	7.05E-02			
EMP	Glyceraldehyde 3-phosphate dehydrogenase	MALCV4_3079	MEALZ_3079	CCE24745	0.17	2.67E-01			
	Pyruvate kinase II	MALCV4_3080	MEALZ_3080	CCE24746	-0.39	9.55E-03			
	Phosphoglycerate kinase	MALCV4_3549	MEALZ_3549	CCE25207	-0.59	8.44E-05			
	Glucose-6-phosphate isomerase	MALCV4_0104	MEALZ_0104	CCE21808	-0.41	4.69E-03			
EDD/oxPPP	Glucose-1-dehydrogenase I	MALCV4_1699	MEALZ_1699	CCE23386	0.08	7.91E-01			
	2-dehydro-3-deoxyphosphoacetone aldolase	MALCV4_1362	MEALZ_1362	CCE23051	-0.45	1.01E-02			
	6-phosphogluconate dehydrogenase	MALCV4_1363	MEALZ_1363	CCE23052	-0.51	1.02E-03			
	Aconitate hydratase, acnA	MALCV4_0310	MEALZ_0310	CCE22010	0.59	2.45E-04			
	Citrate synthase, gltA2	MALCV4_1360	MEALZ_1360	CCE23049	0.24	1.24E-01			
	Succinate semialdehyde dehydrogenase, gabD	MALCV4_1576	MEALZ_1576	CCE23263	-0.32	6.46E-02			
TCA	Dihydrolipoyl dehydrogenase, odlh	MALCV4_1578	MEALZ_1578	CCE23265	0.40	1.39E-02			
	2-oxoglutarate dehydrogenase E2, sucB	MALCV4_1579	MEALZ_1579	CCE23266	0.50	1.15E-03			
	2-oxoglutarate dehydrogenase E1, sucA	MALCV4_1580	MEALZ_1580	CCE23267	0.60	2.99E-05			
	Citrate synthase, gltA	MALCV4_3024	MEALZ_3024	CCE24690	-0.06	7.41E-01			
	Aconitate hydratase 2, acnB	MALCV4_3025	MEALZ_3025	CCE24691	-0.34	2.88E-02			
	Isocitrate dehydrogenase, NAD-dependent, icd	MALCV4_3026	MEALZ_3026	CCE24692	-0.25	1.34E-01			
Serine Cycle & H4-folate pathway	Succinyl-CoA ligase, subunit alpha	MALCV4_3290	MEALZ_3290	CCE24955	0.36	2.72E-02			
	Isocitrate dehydrogenase, NADH-dependent, icdh	MALCV4_3844	MEALZ_3844	CCE25499	0.29	7.47E-02			
	Malate thiokinase, small subunit	MALCV4_3215	MEALZ_3215	CCE24880	-1.12	5.88E-16			
	Malate thiokinase, large subunit	MALCV4_3216	MEALZ_3216	CCE24881	-1.34	3.14E-20			
	Malyl-CoA lyase	MALCV4_3217	MEALZ_3217	CCE24882	-1.14	8.05E-17			
	Serine glyoxylate aminotransferase	MALCV4_3218	MEALZ_3218	CCE24883	-0.93	1.07E-11			
	2-hydroxyacid dehydrogenase NAD-binding	MALCV4_3219	MEALZ_3219	CCE24884	0.07	7.33E-01			
	Malate dehydrogenase	MALCV4_3220	MEALZ_3220	CCE24885	0.44	6.10E-03			
Fatty Acid Metabolism	NADP-methenylenetetrahydrofolate dehydrogenase	MALCV4_3221	MEALZ_3221	CCE24886	-0.59	4.23E-04			
	Glycerate 2-kinase	MALCV4_3222	MEALZ_3222	CCE24887	-0.88	2.53E-08			
	Serine hydroxymethyltransferase	MALCV4_3223	MEALZ_3223	CCE24888	-0.63	9.94E-06			
	Formate tetrahydrofolate ligase	MALCV4_3224	MEALZ_3224	CCE24889	-0.78	1.21E-08			
	Acyl-coenzyme A dehydrogenase	MALCV4_0452	MEALZ_0452	CCE22151	0.63	3.21E-03			
	3-hydroxyacyl-CoA thiolase	MALCV4_0453	MEALZ_0453	CCE22152	0.90	1.73E-05			
	Squalene-hopene cyclase	MALCV4_0454	MEALZ_0454	CCE22153	0.32	1.67E-01			
	Dihydroxyacetone kinase	MALCV4_2523	MEALZ_2523	CCE24201	0.59	8.02E-03			
	Dihydroxyacetone phosphatase	MALCV4_3956	MEALZ_3956	CCE25612	1.60	1.16E-22			

GENE SET ENRICHMENT ANALYSIS (KEGG FUNCTIONS)



P3% MeOH +Cu/+W (control) vs P3% MeOH -Cu/-W (experimental/treatment)

RNA-SEQ ANALYSES: SUMMARY AND NEXT DIRECTIONS

- Compared to cells grown with copper, cells grown without copper have profoundly downregulated expression of genes involved with initial substrate oxidation
 - pMMO and MxaF-MDH genes are the most heavily downregulated
- XoxF-MDH genes are significantly upregulated
- Almost all KEGG functions downregulated in –Cu conditions
 - Genes involved in flagellar assembly significantly upregulated
- Total RNA-seq samples must be re-quantified with alternative package
 - HTSeq may be too conservative for rRNA genes
- Analyze normalized total RNA-seq samples after re-quantification
 - Compare normalized abundances for genes involved in **central cytosolic metabolic functions** (i.e., RuMP, PPP, EMP/ED)
 - Compare normalized abundances for genes involved in **central membrane bound functions** (i.e., pMMO, MDH, cytochromes, NADH dehydrogenase)
 - Compare normalized abundances for genes involved in **ribosomal activity** (rRNAs, ribosome binding proteins, tRNAs)

Thank you!

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Dr. Arun Sethuraman (SDSU Biology Department)



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THE METHANOTROPH TEAM