

OKLAHOMA STATE UNIVERSITY

NIBLACK RESEARCH SCHOLARS

2025-26

FINAL REPORTS



**NIBLACK RESEARCH
SCHOLARS**

PREFACE

Oklahoma State University is pleased to present the enclosed reports of the 2025-26 Niblack Research Scholars.

Thanks to the continued support of OSU alumnus and retired Pfizer executive Dr. John Niblack, the Niblack Research Scholars program affords OSU students the unique opportunity to conduct real scientific research as undergraduates. Dr. Niblack himself participated in research at OSU during his undergraduate experience and believes it impacted his decision to pursue science as a career. His vision and passion to inspire a new generation of scientists lives on in this program.



Dr. John and Heidi Niblack with the 2025-26 Niblack Research Scholars.

(front row, L to R)

Nichollette DuCom, Ty Young, Keira Hine, Heidi Niblack, Dr. John Niblack, Parker Huizinga,
Brandon Lee, Rachel Davis

(back row, L to R)

Cody Moss, MaKayla Friend, Taylor Mills, OSU V.P. for Research Dr. Kenneth Sewell,
Molly McGaughy, Noor Atiyeh, Chole Tolle, Omar Ameen

SESSION ONE:

Brandon Lee

“Development of a New Treatment for Cystic Fibrosis Patients with Bacterial Lung Infections”

Research Area: Biochemistry and Molecular Biology

Faculty Sponsor: Dr. Yong Cheng

Graduate Student Mentor: Carlyn Guthrie

Parker Huizinga

“Investigating Micronutrient–Gene Interactions Influencing Serum Urate Concentrations Using a Custom Polygenic Risk Score”

Research Area: Nutritional Sciences

Faculty Sponsor: Dr. Winyoo Chohanadisai

Graduate Student Mentor: Parker Johnson

Cody Moss

“Matcha Supplementation Attenuates High-Fat, High-Sugar Diet–Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice”

Research Area: Nutritional Sciences

Faculty Sponsor: Dr. Yoo Kim

Graduate Student Mentor: Gopal Lamichhane

Development of a New Treatment for Cystic Fibrosis Patients with Bacterial Lung Infections

Brandon Lee

Biochemistry and Molecular Biology

Faculty Sponsor: Dr. Yong Cheng

Graduate Student Mentor: Carlyn Guthrie

ABSTRACT

Cystic fibrosis (CF) is a genetic disorder that impairs chloride ion transport, resulting in thick mucus accumulation that promotes chronic lung infections in humans. Among these pathogens, nontuberculous mycobacteria (NTM) are particularly difficult to treat due to multidrug therapies, often requiring 18-24 months of treatment and increasing antibiotic resistance limiting their effectiveness. This study investigated novel treatment as potential addition to existing NTM therapies while examining extracellular vesicle (EV) production in infected macrophages. EVs released from infected and uninfected macrophages were isolated using sucrose density gradient centrifugation and characterized using BCA protein assays, NanoSight nanoparticle tracking analysis, and Western blotting for EV-carried host proteins. NanoSight analysis quantified EV concentration and size distributions among experimental groups. Western blotting detected β -actin in EV samples and lysed cell controls, providing information regarding protein expression and EV characterization. Collectively, these methods establish a framework for evaluating novel antimycobacterial therapy and investigating host cell responses during NTM infection. Continued optimization of these approaches may support the development of improved therapies and future EV-based drug delivery strategies for patients with cystic fibrosis.

1. Introduction

Cystic fibrosis (CF) is caused by pathogenic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on human chromosome 7 (Chen et al., 2021). Normally, the CFTR protein allows chloride and water to accumulate, but CF patients are unable to carry out chloride ion transport (Chen et al., 2021). This leads to a lack of hydration and a buildup of extracellular mucus, allowing nontuberculous mycobacteria (NTM) to thrive.

Nontuberculous mycobacteria have been found in approximately 10% of CF patients (Skolnik et al., 2016). Globally, NTM infections have increased by approximately 4% annually, with more than 80% of epidemiologic studies reporting rising infection rates (Dahl et al., 2022). Most NTM infections are made up of either *Mycobacterium avium* complex (MAC) or

Mycobacterium abscessus (MABSC) making up 95% of all NTM infecting in CF patients (Skolnik et al., 2016). MAC accounts for 75% of NTM infections while MABSC accounts for the remaining 20% of infections (Skolnik et al., 2016). Treatment of NTM is very lengthy and often lasts between 18 to 24 months in total (Skolnik et al., 2016). Thus, developing alternative treatment or more efficient treatment of NTM within CF patients will prove great benefit.

Extracellular vesicles (EVs) are membrane-bound particles released by cells that can transport proteins, lipids, and nucleic acids between cells. They play an important role in host-pathogen interactions during NTM infection. Cheng and Schorey (2019) demonstrated that EVs released from *Mycobacterium tuberculosis*-infected macrophages can transport mycobacterial RNA to recipient cells, activating innate immune responses and promoting bacterial killing. These findings demonstrate that EVs can transport bacterial components from infected macrophages and influence surrounding cells during mycobacterial infection.

More recent studies have demonstrated important roles for both bacterial and host-derived EVs during *M. abscessus* infection. For instance, it has been found that clarithromycin treatment increased EV release from *M. abscessus* infection and altered the trafficking of mycobacterial proteins into EVs (Vermeire et al., 2024). EVs released following clarithromycin treatment also increased bacterial resistance to the antibiotic, suggesting a possible role for bacterial EVs in antimicrobial resistance. It has also been demonstrated that *M. abscessus* infection altered release and protein composition of exosomes produced by macrophages (Vermeire et al., 2025). These changes resulted in differing metabolism and promotion of intracellular *M. abscessus* growth. These findings demonstrate that EVs from both bacterium and infected host cells can influence the outcome of *M. abscessus* infection.

Host-derived EVs may also alter the intracellular environment of recipient macrophages. Ramos-Wolfley et al. (2025) found that EVs released from *M. abscessus*-infected macrophages increased iron uptake in recipient macrophages and promoted intracellular bacterial growth. Collectively, these studies demonstrate that mycobacterial infection can alter EV composition and function. These changes can then affect bacterial survival and host-cell responses. Therefore, characterizing EVs released from NTM-infected macrophages may provide insight into host responses during NTM infection as well as reveal potential therapeutic applications.

2. Materials and Methods

EV Isolation. A sucrose gradient isolation was performed to purify extracellular vesicles (EVs) from either infected or uninfected macrophages as did previously (Kowal et al., 2016). A sucrose gradient from high molar (2.1 M) to low molar (0.4 M) was created to isolate EVs based on their density. After an overnight ultra-centrifugation, the EVs are separated throughout the sucrose gradient and then multiple fractions were collected.

BCA assay. A BCA assay (Thermo Scientific Cat. No. 23277) was performed to determine the total protein concentration in each collected fraction. This allows samples to later be normalized or to identify which fractions have the highest protein concentration.

NanoSight analysis. A NanoSight NS300 (Malvern Panalytical, UK) was used to measure EV concentration, diameter, and size distribution as did previously (Vermeire et al., 2025). This can be used to directly compare how many vesicles are yielded from healthy versus NTM-infected macrophages.

Western blot analysis. Finally, a Western blot was performed to determine which proteins are present using human specific antibodies. A Western blot can confirm successful EV isolation and detect specific protein expression. A Western blot was performed to test for β -actin in each collected fraction. β -actin was detected using a β -actin primary antibody (Invitrogen, Cat. No. 15739), followed by a mouse secondary antibody (Invitrogen, Cat. No. G21040). Protein bands were visualized using a Pico chemiluminescent substrate (Thermo Scientific, Cat. No. 34580).

3. Results

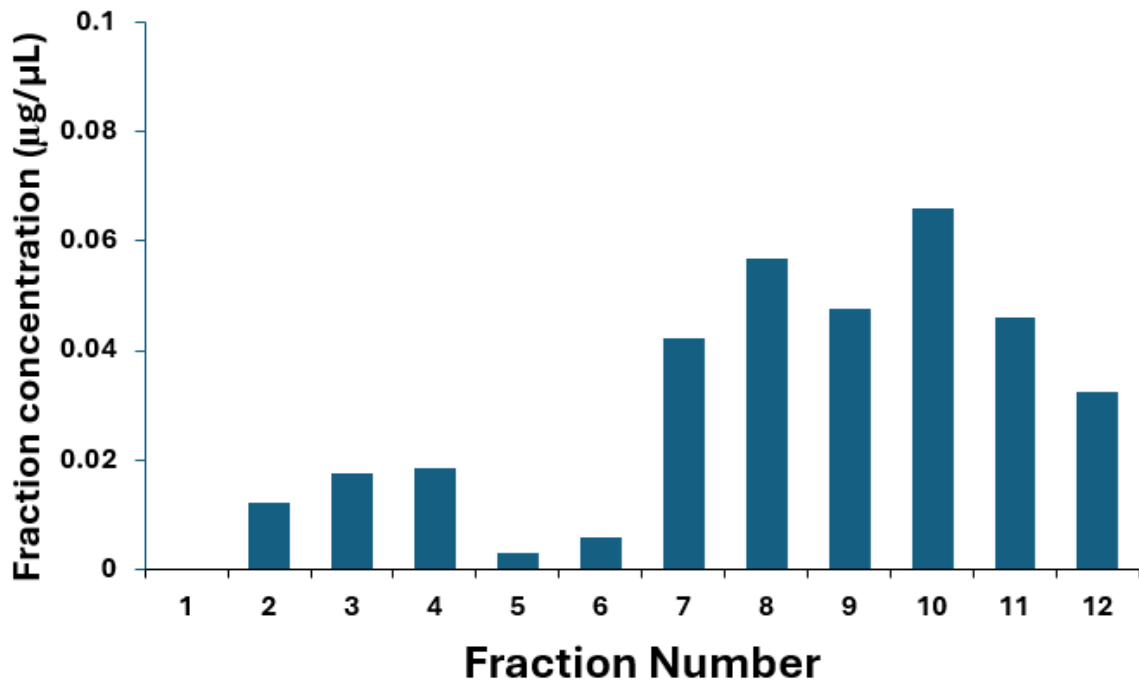


Figure 1: BCA assay results. BCA results from the 12 fractions collected from the sucrose gradient (*M.ab*-infected macrophage sample).

The BCA assay (Fig.1) demonstrated differences in total protein concentrations across the 12 fractions collected from the sucrose gradient. Protein concentrations were relatively low in fractions 1-6 while protein concentrations were higher in fractions 7-12. Fraction 10 contained the highest protein concentration followed by fractions 8, 9, and 11. These results indicate that protein-containing material was concentrated in the later fractions of the sucrose gradient. Because the BCA assay measures total protein rather than EV-specific proteins, the increase protein concentration alone cannot confirm these fractions contained the greatest abundance of extracellular vesicles.

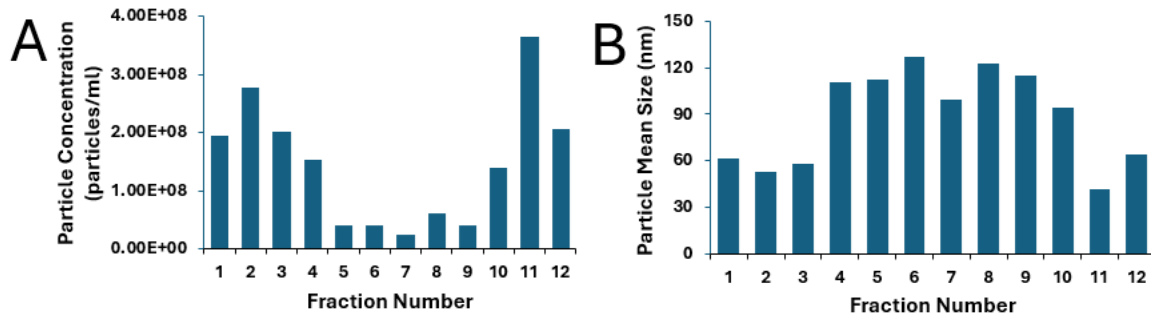


Figure 2: NanoSight results for 12 collected fractions. A) The concentration of particles per ml. B) The mean size of particles.

NanoSight analysis (Fig.2) demonstrated differences in both particle concentration and mean particle size among the 12 sucrose-gradient fractions. Particle concentrations (Fig.2A) were relatively high in fractions 1-4 and 10-12, with fraction 11 containing the highest particle concentration. In contrast, fractions 5-9 generally contain fewer particles. Mean particle size (Fig.2B) also varies across the gradient. Fractions 1-3 and 11-12 contained smaller particles, whereas fractions 4-10 generally contained larger particles. Fraction 11 was particularly notable because it contained the highest particle concentration while also having the smallest mean particle size. These results indicate that sucrose-gradient fractionation separated populations with distinct particle concentrations and sizes. The particle concentration measured by NanoSight did not directly correspond with total protein concentration measured by BCA, suggesting that protein and particle abundance varied independently among the collected fractions.

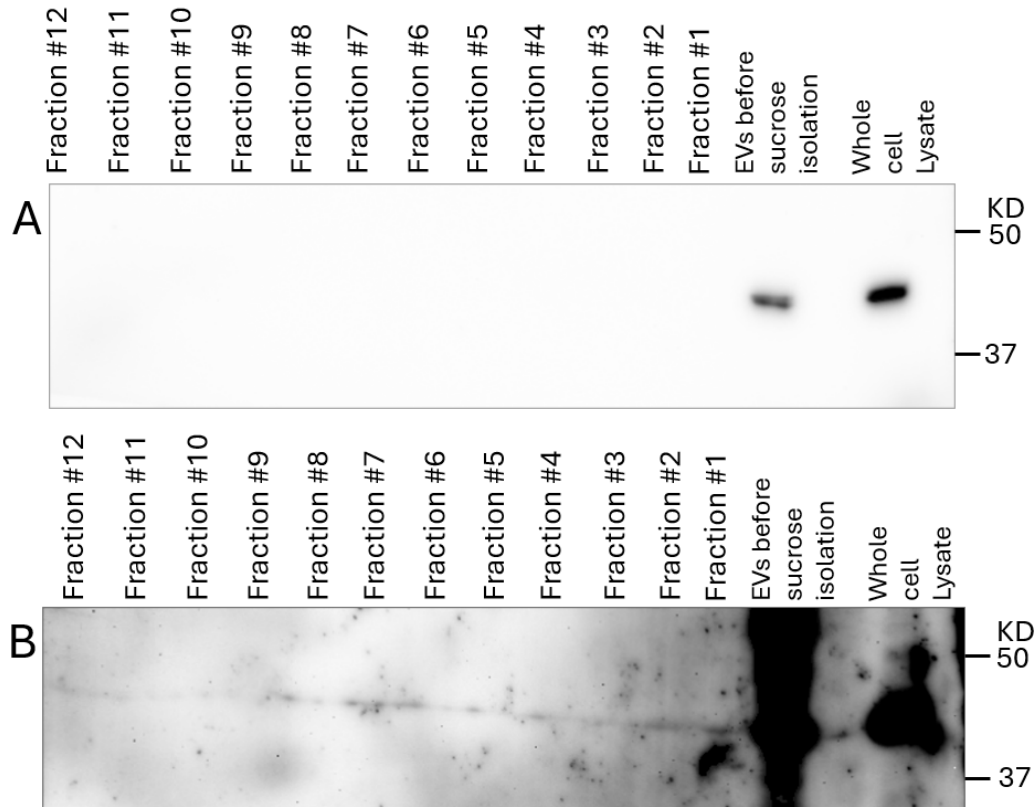


Figure 3: Western Blot results for host β -actin. A) Western blot with two minutes exposure. B) Western blot with twenty minutes exposure.

Western blot analysis detected β -actin at approximately 42 kDa in the whole cell lysate positive control and in the EV sample before sucrose-gradient isolation. Detection of β -actin in the positive control supports successful antibody detection, while its presence in the pre-fractionation EV sample indicates that β -actin was present before sucrose-gradient separation. β -actin was below the detection limit with short exposure time as shown in figure 3A. However, β -actin is detected in 12 fractions in figure 3B with extended exposure time. β -actin intensity in fraction 7 was more prominent while fraction 10 has only a faint band. Bands seem to have merged due to small loading lane size.

4. Discussion and Conclusions

Nontuberculous mycobacterial (NTM) infections remain a significant challenge in patients with cystic fibrosis because treatment often requires prolonged therapy and antibiotic resistance is becoming increasingly common (Skolnik et al., 2016). The goal of this project was to develop novel treatment as potential additions to current treatment regimens, and investigate how extracellular vesicles (EVs) may contribute to host responses in infection.

The BCA assay identified fractions with higher protein concentrations for downstream analysis. NanoSight analysis quantified both EV concentration and particle size, allowing

comparisons between infected and uninfected macrophages. Because EVs naturally transport proteins, lipids, and nucleic acids between cells, they may also serve as a promising vehicle for targeted drug delivery (Hu et al., 2025). Understanding how NTM infection change EV characteristics may contribute to the development of EV-based therapeutic strategies for intracellular pathogens such as NTM.

Western blot analysis was performed to evaluate protein expression within isolated samples. Detection of β -actin in the infected samples before sucrose isolation confirmed the presence of cellular protein. β -actin was detected in all 12 fractions but only after extended exposure. This suggests that additional optimization of the EV isolation protocol or increased sample yield may be necessary to improve recovery of EV-associated proteins for future studies.

Future work should optimize EV isolation and Western blot protocols. This will help improve confirmation of EV isolation. Furthermore, the characterization of protein cargo of EVs, and investigating the engineering of EVs as a method of drug delivery would all prove beneficial towards furthering the project.

5. Summary

Overall, this project intends to demonstrate the value of host cell-derived EVs and potential application as anti-NTM drugs. While additional experiments are needed to fully determine the therapeutic potential of EVs and to optimize EV isolation, these methods provide a foundation for identifying host-directed novel treatments for NTM infections in cystic fibrosis patients.

6. Appendices

6a. Acknowledgements

I would like to thank Dr. and Mrs. Niblack for their support. I would also like to thank Dr. Cheng for his guidance throughout the year on the project. Thank you to my graduate student, Carlyne, for her assistance in executing experiments. Lastly, thank you to Dr. Tan, Mark, Layne, and Amber for your help in teaching me specific protocols that I have used throughout the year.

6b. Papers Published

Lee, B., Brown, J. E., Wood, A., Guthrie, C., Tan, X., Matts, R., & Cheng, Y. (July 2026). Identification of Novel Therapeutic Compounds for Nontuberculous Mycobacterial Infections in Cystic Fibrosis. Poster presented at the Undergraduate Summer Research Expo, Stillwater, OK.

Lee, B., Brown, J. E., Wood, A., Guthrie, C., Tan, X., Matts, R., & Cheng, Y. (April 2026). Development of a New Drug Regimen for Cystic Fibrosis Patients with Bacterial Lung Infections. Poster presented at the Undergraduate Research Symposium, Stillwater, OK.

Jason E. Brown*, **Brandon Lee***, Audrey Kinser, Xuejuan Tan, Robert Matts and Yong Cheng. 2025. Anthraquinone Derivatives as Novel Antimycobacterial Agents against *Mycobacterium abscessus* and *Mycobacterium avium*. In preparation. *Co-first authorship

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<https://doi.org/10.1007/s40506-016-0092-6>
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<https://doi.org/10.1002/pmic.202400181>
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<https://doi.org/10.1002/pmic.202300332>

Development of a New Treatment for Mycobacterial Lung Infections

Presented by: Brandon Lee

Graduate Mentor: Carlyn Guthrie

Faculty Sponsor: Dr. Yong Cheng

Department: Biochemistry and Molecular Biology

Nontuberculous Mycobacteria (NTM)

A One Health Challenge in Rural America

Healthy People
Healthy Animals
Healthy Environments
Stronger Rural Communities

Human Health

NTM can cause lung, skin, and disseminated infections, especially in people with underlying conditions.

- Chronic lung disease
- Immunocompromised individuals
- Older adults
- Rural residents with environmental exposure

People, animals, and the environment share the same ecosystems.

RURAL USA
Shared Environments
Shared Risks
Shared Solutions

Animal Health

NTM infect a wide range of animals, including livestock, companion animals, and wildlife.

- Cattle and other livestock
- Companion animals (dogs, cats)
- Wildlife (deer, rodents, etc.)
- Potential for zoonotic transmission

Environmental Health

NTM are ubiquitous in soil, water, and biofilms, and are resilient in natural and built environments.

- Surface and groundwater
- Soil and dust
- Agricultural settings
- Biofilms (plumbing, water systems)
- Natural and farm environments

NTM
At the
Human-Animal-
Environment
Interface



Safe Water and Sanitation



Sustainable Agriculture



Animal Health Surveillance



Community Awareness



Research and Innovation



Collaborative One Health Solutions



Generated by Dr.
Cheng and AI

ONE HEALTH | SHARED ENVIRONMENTS | HEALTHY COMMUNITIES | BRIGHTER FUTURES

Nontuberculous Mycobacteria (NTM) as a Disease

- Environmental mycobacteria found worldwide
- Commonly inhabit soil, natural water, drinking-water systems, and biofilms
- Can persist in household and healthcare plumbing
- Exposure may occur through environmental sources
- (Falkinham, 2009)

- Non-specific signs and symptoms, including shortness of breath, cough, fatigue, malaise and weight loss.
- Most commonly occur in patients with underlying lung disease such as cystic fibrosis, bronchiectasis and emphysema
- (CDC, 2025)

Background: CF and NTM

- ~10% of CF Patients have NTM (Skolnik et al., 2016)
- Typical NTM treatment is 18-24 months (Skolnik et al., 2016)

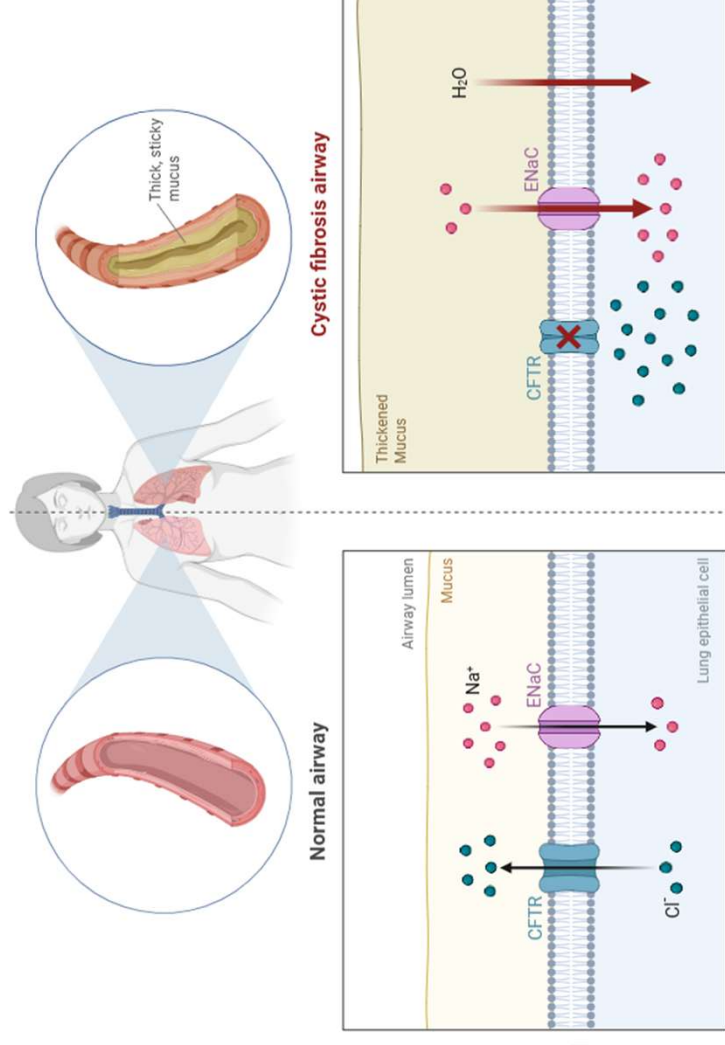
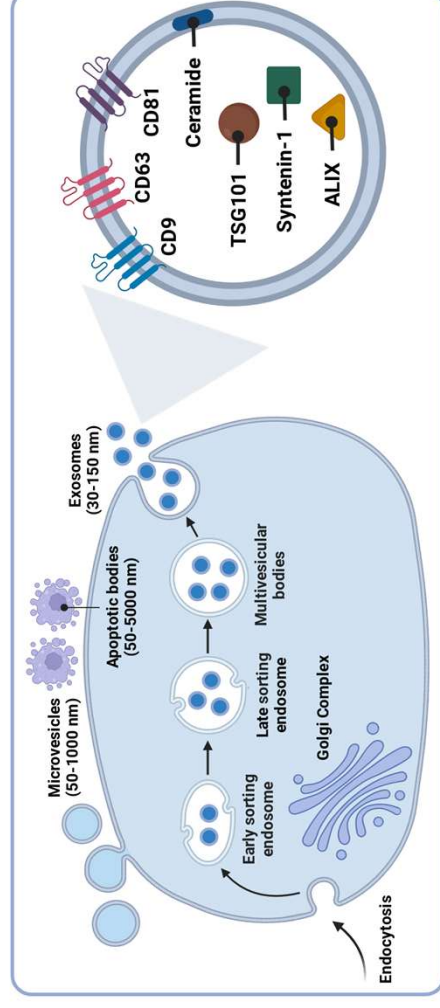


Image by Hansson (2019).

Background: Extracellular Vesicles

- Exosomes: formed within endosomes and released through multivesicular bodies
- Microvesicles: bud directly from the plasma membrane
- Apoptotic bodies: released during programmed cell death

(Yáñez-Mó et al., 2015; Welsh et al., 2024)



Depiction of an EV and its 3 major categories (Zhang, 2024).



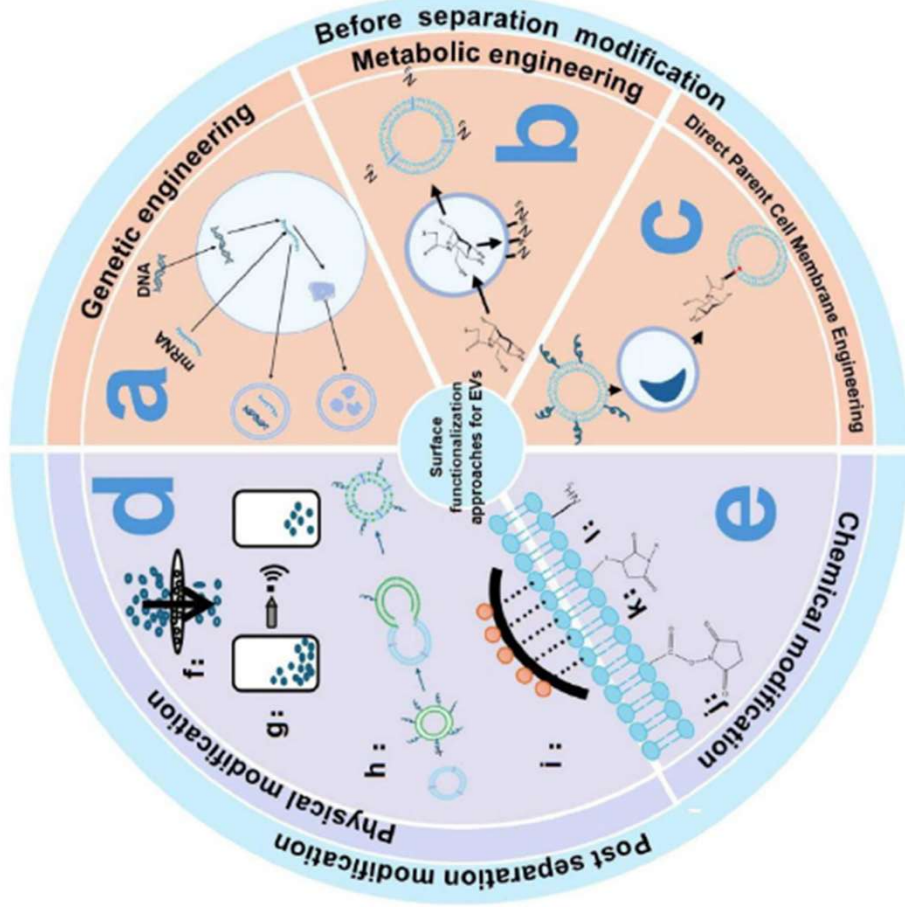
Background: EV as new anti-bacterial agent

- Mycobacterial RNA transported by EVs → innate immune activation (Cheng & Schorey, 2019)
- *M. abscessus* infection alters macrophage EV release/composition (Vermeire et al., 2025)
- Host EVs alter iron uptake and promote intracellular bacterial growth (Ramos-Wolfley et al., 2025)

Background: EV as drug-deliver system

- EVs naturally transport biological cargo such as proteins and nucleic acids between cells.
- EVs can be engineered before isolation by modifying the parent cell, allowing desired molecules or surface features to be incorporated into newly produced EVs.
- Isolated EVs can be modified or loaded with therapeutics using physical or chemical methods, creating potential targeted drug-delivery vehicles.

(Hu et al., 2025)



Potential EV modifications before and after separation (Hu et al., 2025)

Investigate extracellular vesicles produced during NTM infection and establish methods that may support future EV-based therapeutic strategies.

01

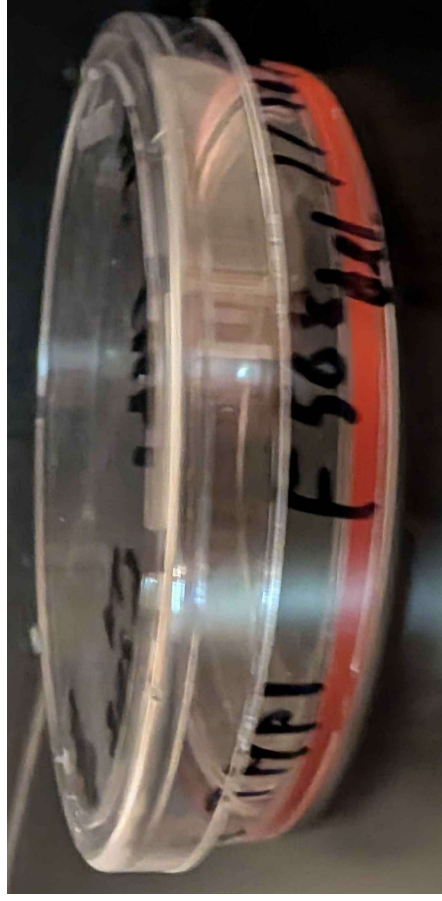
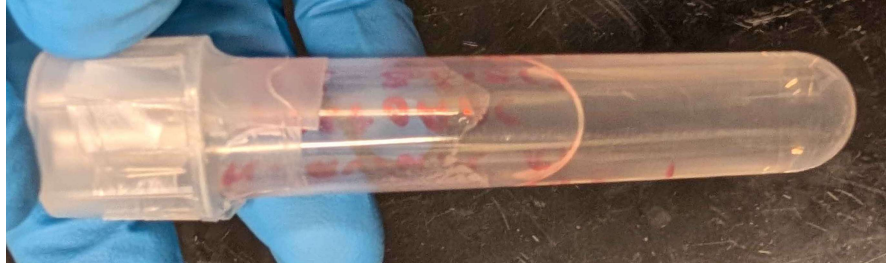
Isolate EVs

02

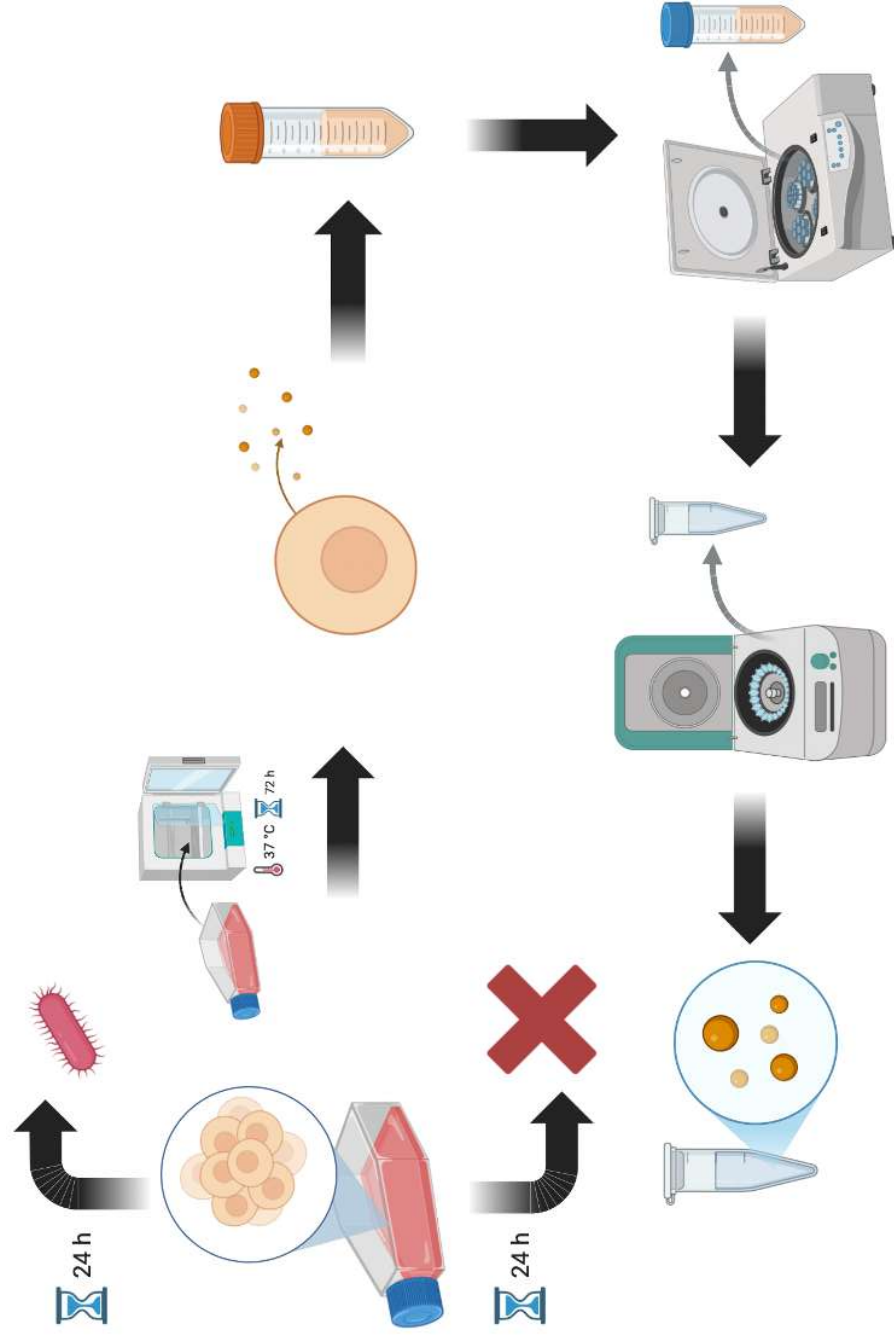
03

04

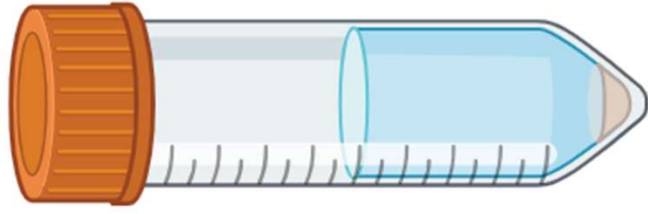
Macrophage/ Bacterial Culture



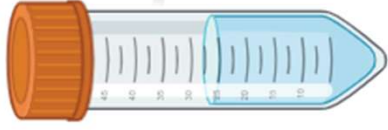
EV Isolation from Cell Culture



Workflow diagram of EV isolation created by Amber Meeker



Create a sucrose gradient
(0.4M-2.1M)



Ultracentrifuge overnight



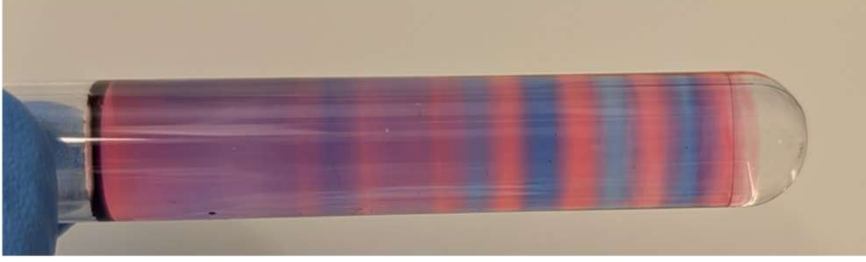
Extract 1ml fractions

Image created using Biorender

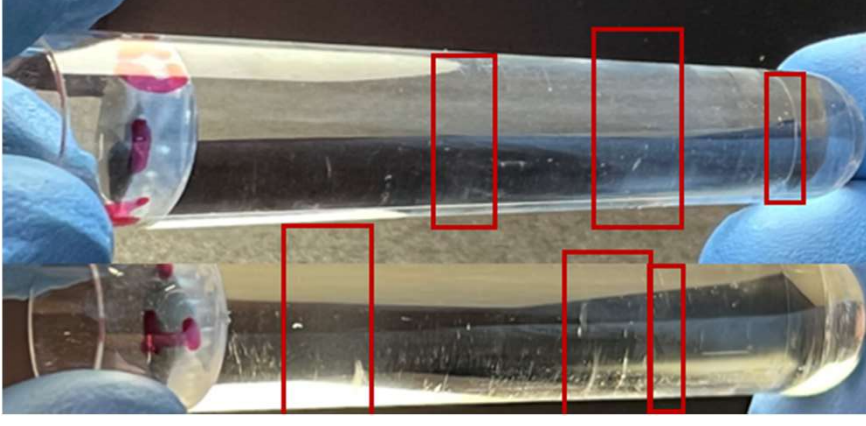
Ultracentrifuge



Sucrose Gradient separated EVs



Sucrose
gradient
visualized with
dye.



Experimental results
with sucrose gradient.

Investigate extracellular vesicles produced during NTM infection and establish methods that may support future EV-based therapeutic strategies.

01

Isolate EVs

02

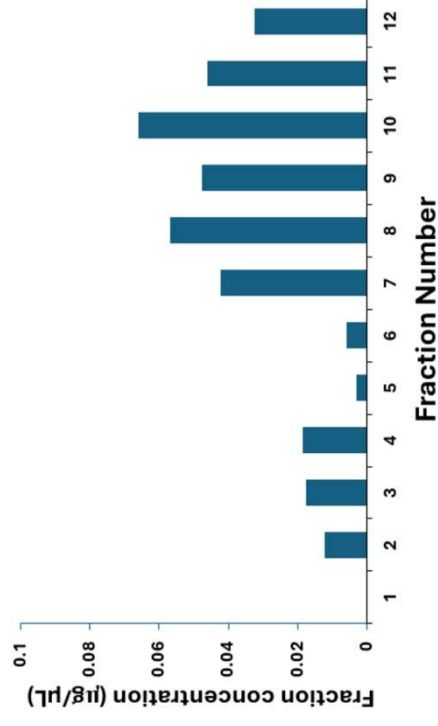
Characterize
protein
distribution

03

04

Results: BCA assay Results

- BCA results from the 12 fractions collected from the sucrose gradient (*M.ab*-infected macrophage sample).



Investigate extracellular vesicles produced during NTM infection and establish methods that may support future EV-based therapeutic strategies.

01

Isolate EVs

02

Characterize
protein
distribution

03

Characterize
Particle
concentration
and size

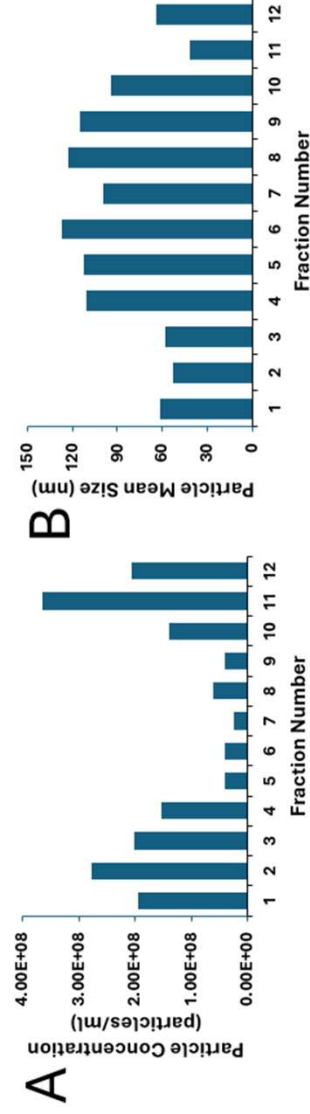
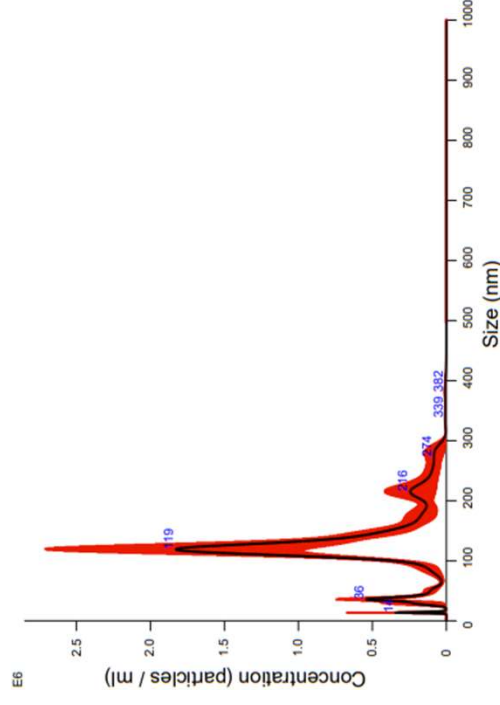
04

Results: NanoSight

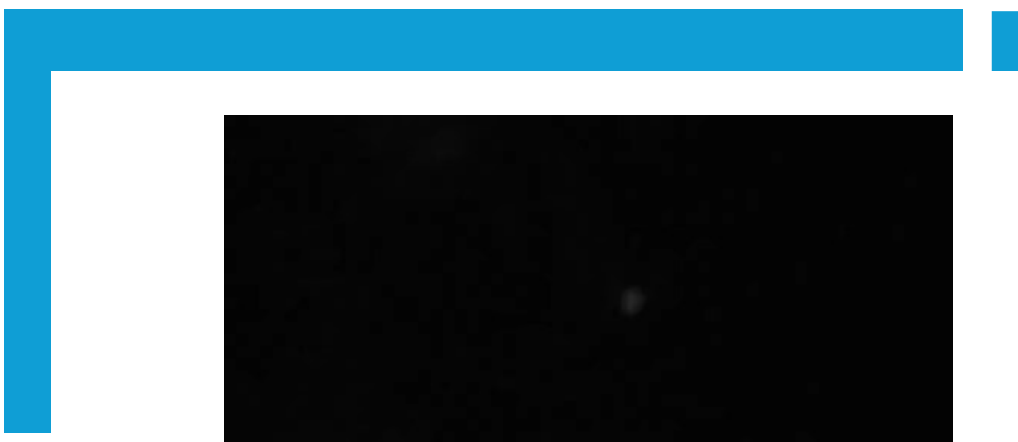
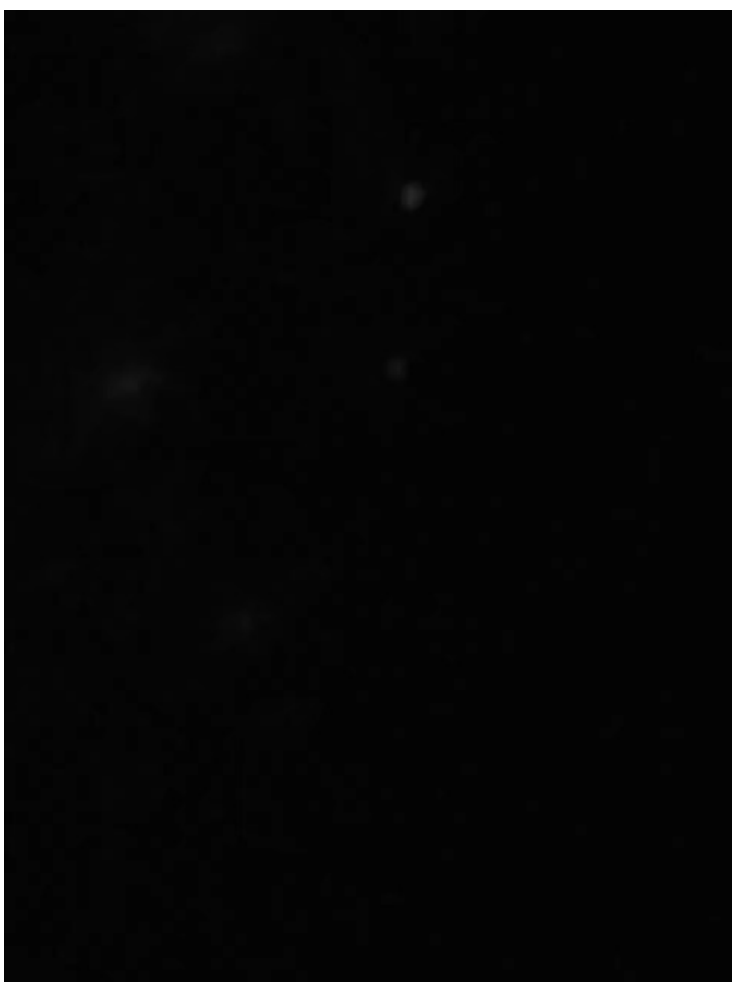
NanoSight results for 12 collected factions.

A) The concentration of particles per ml.

B) The mean size of particles.



NanoSight Video



Investigate extracellular vesicles produced during NTM infection and establish methods that may support future EV-based therapeutic strategies.

01

Isolate EVs

02

Characterize
protein
distribution

03

Characterize
Particle
concentration
and size

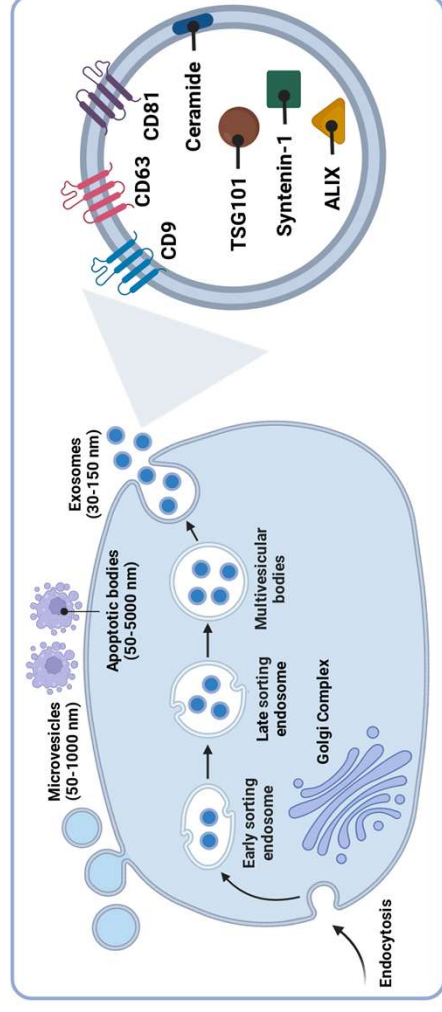
04

Evaluate host
protein
expression

Why test for EV Markers?

Biomarkers can:

- Identify EVs
- Characterize EVs
- Determine how EVs affect NTM/host cells
 - Identify therapeutic mechanisms

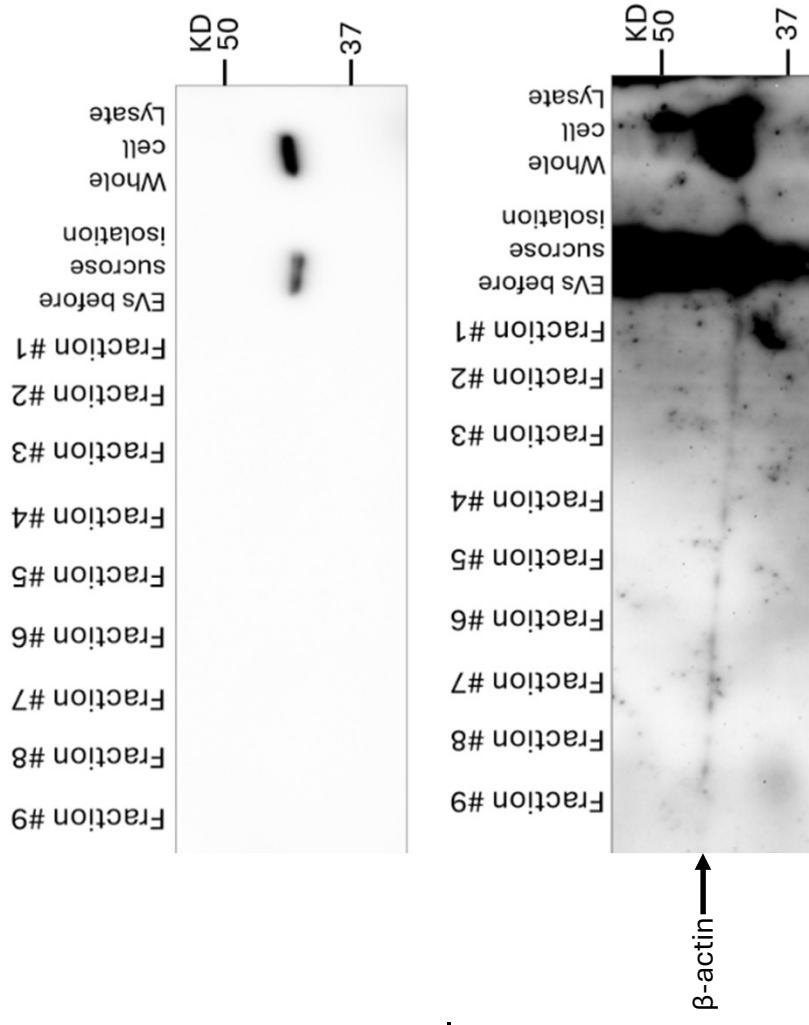


Depiction of an EV and its 3 major categories (Zhang, 2024).

Results: Western Blot

- Western Blot results for host β -actin. Human specific antibodies were used to detect β -actin.

Western blot analysis detected β -actin at approximately 42 kDa in the whole cell lysate positive control, EV sample before sucrose-gradient isolation, and multiple collected fractions.



Conclusions

Sucrose-gradient fractionation
separated distinct particle
populations

BCA and NanoSight produced
different distribution patterns

B-actin was visible after Extended
exposure.

Future Directions



Optimize EV isolation



Optimize Western blotting



Further characterize EVs



Use EVs as potential method of drug delivery

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Investigating Micronutrient–Gene Interactions Influencing Serum Urate Concentrations Using a Custom Polygenic Risk Score

Parker Huizinga

Nutritional Sciences

Faculty Sponsor: Dr. Winyoo Chohanadisai

Graduate Student Mentor: Parker Johnson

ABSTRACT

Gout is a chronic inflammatory disease strongly associated with elevated serum urate concentrations and influenced by both genetic and dietary factors. This study investigated micronutrient-gene interactions affecting serum urate levels and evaluated these interactions using a custom polygenic risk score (PRS). Data from the UK Biobank were analyzed using linear regression models to examine associations between micronutrients, candidate single nucleotide polymorphisms (SNPs), and serum urate concentrations while controlling for demographic, lifestyle, and genetic covariates. Preliminary analyses identified SNP rs12498742 within the *SLC2A9* gene as having significant interactions with multiple micronutrients. To determine whether these interactions reflected broader polygenic susceptibility or were primarily driven by *SLC2A9*, a full and custom PRS excluding the *SLC2A9* region was generated. Several micronutrients, including magnesium, zinc, calcium, manganese, and B-vitamins, were associated with lower serum urate concentrations. Individuals carrying *SLC2A9* risk alleles also demonstrated stronger urate-lowering responses to micronutrients such as folate, magnesium, biotin, and non-heme iron. However, these interaction signals disappeared after removal of the *SLC2A9* region from the PRS, suggesting that the observed nutrient-gene interactions were primarily driven by this locus rather than broader genome-wide susceptibility. These findings highlight the importance of validating nutrient-gene interactions and support the future application of precision nutrition for personalized dietary interventions and chronic disease prevention.

1. Introduction

Millions of people worldwide suffer from gout, a painful inflammatory arthritis caused by the deposition of monosodium urate crystals within joints and connective tissues (Timsans et al., 2024). Gout develops primarily due to elevated serum urate concentrations, commonly referred to as hyperuricemia, which serves as the strongest predictor of disease risk and severity. Beyond its role in gout, elevated serum urate has been associated with an increased risk of cardiovascular disease mortality, chronic kidney disease, hypertension, type 2

diabetes, and metabolic dysfunction-associated steatotic liver disease (Ali et al., 2019; Wu et al., 2021; Tian et al., 2022; Lv et al., 2024; Chang & Liu, 2025). Higher serum urate concentrations have also been linked to greater visceral and hepatic fat accumulation, highlighting its broader relevance to cardiometabolic health (Rospleszcz et al., 2020). As a result, identifying modifiable factors that influence serum urate concentrations may have important implications for preventing both gout and other chronic diseases.

However, gout is not solely determined by urate levels alone. Genetics, dietary intake, lifestyle behaviors, obesity, and other environmental factors all contribute to disease development and progression (Jeon & Yoo, 2023; Timsans et al., 2024). Recent research has increasingly demonstrated that both genetic predisposition and nutritional exposures influence how individuals regulate urate metabolism and respond to inflammatory processes associated with gout.

Dietary intake plays a particularly important role in gout pathophysiology. Foods rich in purines, especially red meats and seafood, can elevate serum urate concentrations since urate is a product of purine metabolism (Ragab et al., 2017; Zhang et al., 2022). Conversely, several micronutrients have been associated with lower urate concentrations and reduced gout risk. Vitamin C, for example, has been shown to reduce oxidative stress and inflammation while potentially enhancing renal urate excretion (Choi & Curhan, 2008). Minerals such as magnesium, zinc, manganese, and calcium have also been implicated in urate metabolism and inflammatory regulation, with calcium specifically demonstrating associations with gout risk. Despite these findings, it remains unclear how micronutrients interact with genetic variation to influence gout susceptibility and serum urate concentrations.

One major source of genetic variability arises from single nucleotide polymorphisms (SNPs), which are small variations occurring at a single nucleotide position within DNA. Although SNPs may appear minor, they can significantly alter gene function, protein expression, and metabolic regulation (Shastry, 2009). Genome-wide association studies (GWAS) have identified numerous SNPs associated with gout and serum urate concentrations, including variants within genes involved in urate transport and excretion (Köttgen et al., 2013; Jeon & Yoo, 2023). Among these genes, *SLC2A9* has consistently emerged as one of the strongest genetic determinants of serum urate concentrations and gout susceptibility (Köttgen et al., 2013).

The interaction between genes and nutrients is a central concept within the field of nutrigenomics, which examines how dietary factors influence gene expression and how genetic variation alters responses to nutrients (Zhang et al., 2022). Nutrient-gene interactions may explain why individuals respond differently to similar diets and why certain dietary interventions are more effective in some populations than others. These interactions form the

scientific foundation of precision nutrition, an emerging field focused on tailoring dietary recommendations according to an individual's genetic profile, lifestyle, and biological characteristics (Zhang et al., 2022). Precision nutrition seeks to move beyond generalized dietary guidelines toward personalized interventions capable of reducing chronic disease risk and improving health outcomes.

A major challenge in studying nutrient-gene interactions is distinguishing whether observed interactions reflect true genome-wide susceptibility or are primarily driven by a single highly influential genetic locus. Polygenic risk scores (PRS) provide one method of addressing this issue. A PRS summarizes the cumulative contribution of many genetic variants to estimate an individual's inherited predisposition to a disease or trait (Escott-Price et al., 2019; Chang et al., 2022). However, when a particularly strong gene exerts a dominant influence, it may artificially inflate or obscure nutrient-gene interaction signals within the PRS.

The primary objective of this study was to investigate micronutrient-gene interactions influencing serum urate concentrations using UK Biobank data while evaluating the validity of these interactions through the development of a custom polygenic risk score. Specifically, we aimed to determine whether nutrient-gene interactions associated with serum urate were truly polygenic or primarily driven by the *SLC2A9* gene region. By isolating and validating these interactions, this study sought to further advance the understanding of nutrigenomics and support the future application of precision nutrition strategies for chronic disease prevention.

2. Experimental Details

This study utilized data from the UK Biobank, a large longitudinal cohort consisting of over 500,000 participants between the ages of 40 and 69 years recruited between 2006 and 2010 (Sudlow et al., 2015). The UK Biobank provides extensive phenotypic, lifestyle, dietary, and genetic information collected through questionnaires, interviews, biological sampling, imaging, and long-term follow-up assessments. Genetic data were generated through large-scale genotyping and imputation methods, while additional participant information included biomarkers, socioeconomic measures, medical diagnoses, anthropometric measurements, and dietary intake data (Bycroft et al., 2018). Participants also had the option to provide blood, urine, and other biomedical samples for analysis (Sudlow et al., 2015). The breadth and depth of the UK Biobank dataset provide a unique opportunity to investigate the interaction between genetics, nutrition, and chronic disease risk.

In preliminary analyses, data from 502,154 UK Biobank participants were analyzed to examine associations between micronutrients, genetic variation, and serum urate concentrations. Linear regression analyses were conducted in R Studio to evaluate

relationships between individual micronutrients and serum urate levels. Following these analyses, associations between serum urate and 30 candidate SNPs previously identified through the Köttgen et al. genome-wide association study were examined. Finally, nutrient-gene interaction analyses were performed to determine whether interactions between these SNPs and 23 micronutrients significantly influenced serum urate concentrations.

All regression analyses included several covariates to minimize confounding and improve model accuracy. These covariates included sex, body mass index (BMI), smoking status, alcohol intake, Townsend deprivation index as a measure of socioeconomic status, income, education level, kidney disease prevalence, diuretic use, and ten genetic principal components to account for population stratification.

Preliminary analyses identified SNP rs12498742 located within the *SLC2A9* gene on chromosome 4 as having significant interactions with multiple micronutrients. Because *SLC2A9* is strongly associated with urate transport and gout susceptibility, we hypothesized that this gene region may disproportionately influence nutrient-gene interaction signals. To test this hypothesis, a custom polygenic risk score (PRS) was developed to estimate genetic susceptibility to elevated serum urate concentrations while excluding the *SLC2A9* gene region.

To construct the PRS, GWAS summary statistics from the Köttgen et al. serum urate study was obtained from the European Bioinformatics Institute GWAS catalog (Köttgen et al., 2013). These summary statistics served the basis to estimate genetic predisposition toward elevated serum urate concentrations in our cohort. Genetic target data for UK Biobank participants were extracted using the PLINK2 software platform. Quality control procedures included removal of ambiguous SNPs, multi-allelic variants, and SNPs with minor allele frequencies below 0.01 (Chang et al., 2022).

Polygenic risk scores were generated using PRSice-2 software within the UK Biobank cloud computing environment for 108,992 participants (Choi et al., 2019). Both the base PRS and the custom PRS were adjusted for age, sex, and forty genetic principal components. To create the custom PRS, the *SLC2A9* gene region was systematically excluded using progressively larger exclusion windows. Exclusion began at one million base pairs on each side of the gene and expanded in 500,000 base pair increments up to ten million base pairs. A 5.5 million base pair exclusion window was ultimately identified as the optimal threshold for minimizing the association between the custom PRS and SNP rs12498742 while preserving overall genome-wide urate risk prediction.

Following PRS generation, linear regression analyses were performed to validate associations between the PRSs and serum urate concentrations. Additional analyses examined relationships between rs12498742 and both the base and custom PRSs to confirm

successful attenuation of the *SLC2A9* contribution within the custom score. Finally, regression models were conducted to evaluate interactions between micronutrients and both PRSs on serum urate concentrations.

3. Results

Initial analyses examining associations between micronutrients and serum urate concentrations demonstrated that several vitamins and minerals were significantly associated with lower serum urate levels. These included riboflavin, pantothenic acid, biotin, vitamin B12, calcium, magnesium, total iron, non-heme iron, zinc, manganese, copper, and iodine, among others. These findings supported previous evidence suggesting that dietary micronutrients may influence urate metabolism and inflammatory processes associated with gout risk.

Subsequent analyses evaluating nutrient-gene interactions identified a particularly strong interaction signal involving SNP rs12498742 within the *SLC2A9* gene region. Compared to the other candidate SNPs analyzed, rs12498742 demonstrated a substantially greater number of significant interactions with micronutrients, suggesting that this locus may play a dominant role in mediating nutrient effects on serum urate concentrations.

Several micronutrients displayed altered effects on serum urate concentrations depending on interactions with their genotype at rs12498742. Specifically, riboflavin, pantothenic acid, biotin, calcium, and magnesium demonstrated stronger associations with lower serum urate concentrations among individuals carrying one or two rs12498742 risk alleles compared to individuals without risk alleles. These findings indicate that genetic variation within *SLC2A9* modifies the physiological response to certain micronutrients and may influence the effectiveness of dietary interventions targeting urate reduction.

When a genome-wide polygenic risk score incorporating the *SLC2A9* region was analyzed, similar nutrient interaction patterns were observed. Individuals with higher genetic susceptibility demonstrated stronger micronutrient interactions influencing serum urate concentrations. However, after removing the *SLC2A9* gene region from the PRS to generate the custom score, these nutrient interaction signals disappeared. This finding suggests that the previously observed nutrient-gene interactions were not broadly polygenic in nature but were instead largely driven by the *SLC2A9* locus itself.

Together, these findings demonstrate that *SLC2A9* rs12498742 serves as a key driver of micronutrient-gene interactions affecting serum urate concentrations and highlight the importance of validating nutrient-gene interaction findings through targeted genetic modeling approaches such as custom polygenic risk scores.

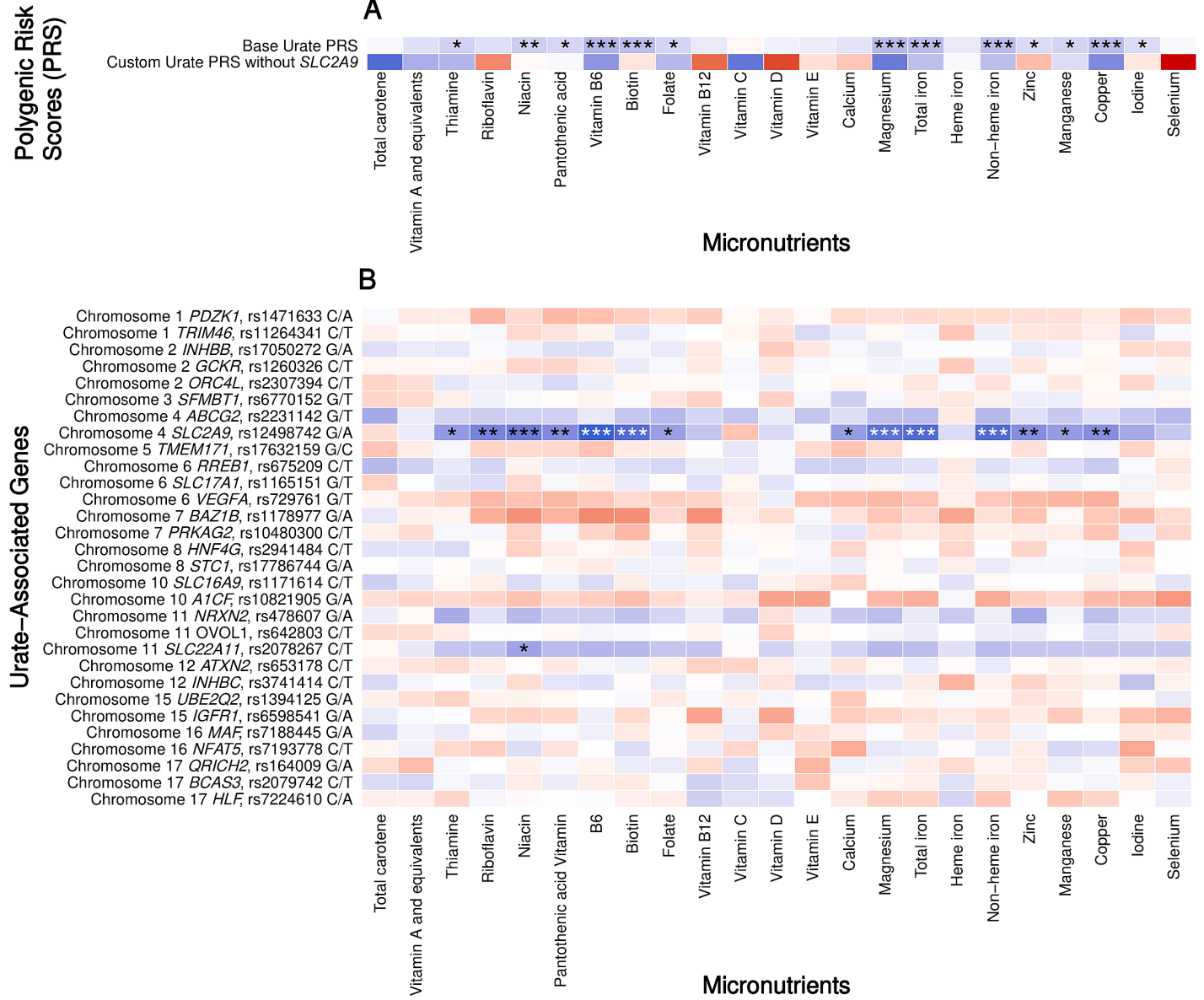


Figure 1: Associations between micronutrient–genetic interactions and serum urate concentrations. Results were analyzed using linear regression, with blue and red indicating negative and positive beta coefficients, respectively. Sample sizes ranged from 107,028 to 108,992 participants. Asterisks denote statistically significant interactions after Benjamini–Hochberg false discovery rate (FDR) correction: *FDR < 0.05, **FDR < 0.01, and ***FDR < 0.001.

4. Discussion and Conclusions

The findings of this study demonstrate that nutrient-gene interactions influencing serum urate concentrations are strongly mediated by genetic variation within the *SLC2A9* gene region. While several micronutrients were initially associated with reduced serum urate concentrations, the interaction analyses revealed that these effects differed substantially depending on genotype. In particular, individuals carrying risk alleles at rs12498742 demonstrated stronger associations between micronutrient intake and lower serum urate concentrations, especially for riboflavin, pantothenic acid, biotin, calcium, and magnesium. These findings suggest that genetic variation within *SLC2A9* may alter nutrient responsiveness and contribute to interindividual differences in gout susceptibility.

The *SLC2A9* gene is biologically relevant because it encodes a urate transporter involved in renal uric acid handling. Variants within this gene have consistently demonstrated strong associations with serum urate concentrations and gout risk across multiple genome-wide association studies (Köttgen et al., 2013). Therefore, the identification of nutrient interactions at this locus is biologically plausible and further supports the role of *SLC2A9* as a central regulator of urate metabolism.

One of the most significant contributions of this study was the development and application of a custom polygenic risk score excluding the *SLC2A9* region. Traditional PRSs are designed to summarize cumulative genome-wide genetic risk; however, highly influential loci can dominate these scores and potentially create misleading interaction signals. By systematically removing the *SLC2A9* region and reevaluating nutrient interactions, this study demonstrated that the observed interactions were largely driven by a single dominant locus rather than broader genome-wide susceptibility (Escott-Price et al., 2019; Chang et al., 2022). This approach highlights the importance of validating nutrient-gene interactions and improving the methodological rigor of nutrigenomic research.

These findings have important implications for the future of precision nutrition. Traditional dietary recommendations are typically generalized across populations despite significant biological variation between individuals (Zhang et al., 2022). However, this study demonstrates that individuals with different genetic profiles may respond differently to the same nutrients. As a result, dietary interventions designed to reduce gout risk or lower serum urate concentrations may need to be personalized according to genotype rather than universally applied.

The long-term potential of precision nutrition is substantial. In the future, clinicians may be able to use genetic information to identify individuals at elevated risk for gout and provide personalized nutritional recommendations aimed at preventing disease onset before symptoms occur. For example, individuals carrying specific *SLC2A9* variants may benefit from targeted micronutrient interventions that optimize urate metabolism and reduce inflammation. More broadly, the framework established in this study may extend beyond

gout to other chronic diseases influenced by nutrient-gene interactions, including obesity, cardiovascular disease, diabetes, and inflammatory disorders.

Future research should focus on replicating these findings in independent and more diverse populations to improve generalizability. Additionally, mechanistic studies are needed to better understand how micronutrients biologically interact with *SLC2A9* and related metabolic pathways. Expanding this work through multi-omics approaches, including metabolomics, transcriptomics, and proteomics, may further clarify the complex biological networks underlying nutrient-gene interactions.

Emerging technologies such as artificial intelligence and machine learning may also accelerate the development of precision nutrition. Integrating genomic information with dietary intake, biomarker data, and lifestyle factors could allow predictive models to generate highly individualized dietary recommendations tailored to a person's unique biological profile. As precision nutrition continues to evolve, studies such as this one provide important foundational evidence supporting the transition from generalized nutrition guidelines toward personalized strategies capable of improving chronic disease prevention and long-term health outcomes.

5. Summary

This study investigated micronutrient-gene interactions influencing serum urate concentrations using UK Biobank data and a custom polygenic risk score approach. Several micronutrients, including riboflavin, pantothenic acid, biotin, calcium, magnesium, iron, zinc, manganese, copper, and iodine, were associated with lower serum urate concentrations. Interaction analyses identified SNP rs12498742 within the *SLC2A9* gene as the primary driver of nutrient-gene interactions affecting serum urate concentrations. Individuals carrying rs12498742 risk alleles demonstrated stronger associations between micronutrient intake and lower serum urate concentrations than non-carriers. Although these interaction patterns persisted when using a standard genome-wide polygenic risk score, they disappeared after removing the *SLC2A9* region from the score, indicating that the observed effects were primarily driven by this locus rather than broader polygenic susceptibility. These findings highlight the importance of validating nutrient-gene interactions and support the growing role of precision nutrition in developing personalized dietary interventions for chronic disease prevention and gout risk reduction.

6. Appendices

6a. Acknowledgements

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6b. Papers Published

Published Papers:

1. Johnson PR, Huizinga P, Emerson W, Chohanadisai W. **Associations between dietary micronutrient intake and serum urate concentrations are dependent on SLC2A9 polymorphism rs12498742: A UK Biobank cohort study.** J Nutr. 2026;156(7):2164-2176. doi:10.1016/j.tjn.2026.04.032

Peer-Reviewed Publications (Accepted / Under Review)

1. Johnson PR, Keirns BH, Meade GE, Huizinga PA, Kim AY, Sigdel R, Cardona CJ, Montgomery MR, Emerson SR, Chowanadisai W.
Metabolic risk factors contribute to cardiovascular disease and MASLD incidence in people with normal weight obesity: A UK Biobank study.
International Journal of Obesity. Accepted, awaiting publication (2026).

Presentations/Posters:

1. Huizinga PA, Johnson PR, Kim AY, Cardona CJ, Sigdel R, Montgomery MR, Emerson SR, Chowanadisai W.
Genes, Diet, and Urate: Investigating Micronutrient Interactions for Personalized Risk Prediction Using a Custom Polygenic Risk Score.
Poster Presentation, OSU Undergraduate Research Expo.
2. Huizinga PA, Johnson PR, Kim AY, Cardona CJ, Sigdel R, Montgomery MR, Emerson SR, Chowanadisai W.
Diet, DNA, and Macular Degeneration: Investigating Micronutrient–SNP Interactions on Photoreceptor Segment Thickness.
Poster Presentation, OSU Research Symposium.

Conference Abstracts / Accepted Presentations

1. Johnson PR†, Huizinga PH, Cardona CJ, Sigdel R, Joyce JM, Montgomery MR, Lin D, Kim Y, Chowanadisai W.
Associations Between ARMS2 and CFH Polymorphisms, Micronutrients, and Eye Measures: Photoreceptor and Macular Thickness.
Accepted, American Society for Nutrition Conference (2026).

6c. Literature Cited

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NIBLACK RESEARCH SCHOLARS
PROGRAM

Investigating Micronutrient–Gene Interactions Influencing Serum Urate Concentrations

*Using a custom polygenic risk score to evaluate whether
nutrient–gene interactions are truly polygenic or driven
by SLC2A9*

Parker Huizinga
Nutritional Sciences
Faculty Sponsor: Dr. Winyoo Chohanadisai
Graduate Student Mentor: Parker Johnson

Key terminology before we start

A quick vocabulary map for the genetics and precision nutrition pieces of the project.

SNP

Single nucleotide polymorphism; a common one-letter DNA difference that can influence biological traits.

PRS

Polygenic risk score; one score that adds small effects from many SNPs to estimate inherited tendency for a trait.

Micronutrient-gene interaction

When the relationship between a nutrient and serum urate changes depending on genotype.

UK Biobank

A large online health “library” containing data for genetics, clinical information, biomarkers, diet/lifestyle information, and health outcomes for over 500,000 participants.

PRS as a golf scorecard

Each SNP is like one hole: it contributes a small amount to the final score.

SNP	SNP	SNP
1	2	3
+1	+0	+2

The PRS adds many variants into one total genetic risk estimate.

Removing the SLC2A9 “hole” tests whether the nutrient-gene signal still holds without the dominant genetic contributor.

Background: why serum urate matters

Serum urate is a clinically meaningful biomarker shaped by both biology and behavior.

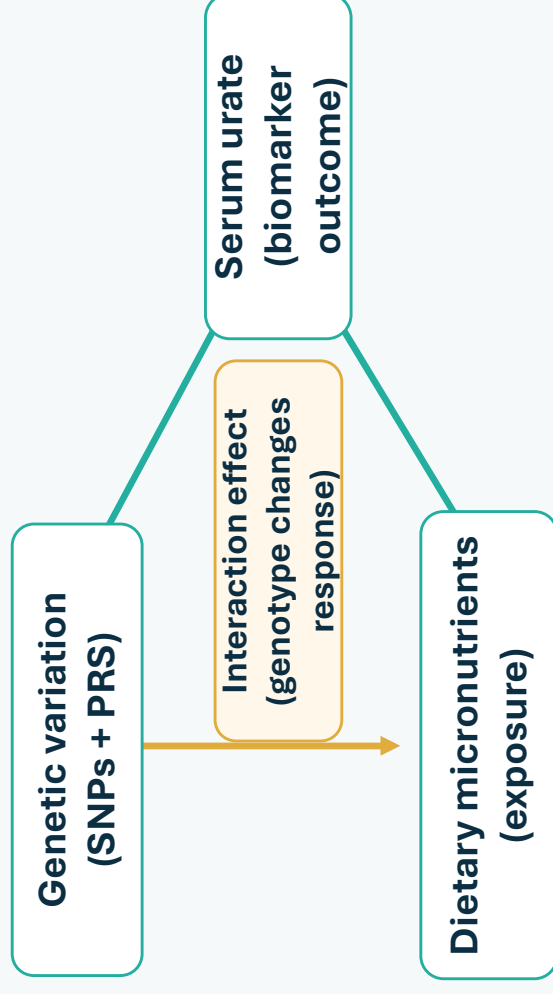
Clinical relevance

- Final byproduct of purine metabolism and strongest biomarker underlying gout risk.
- Higher urate is linked with kidney, cardiovascular, type 2 diabetes, and metabolic disease risk.
- Useful precision-nutrition model because genetic variation and diet both influence urate.

Why this project matters

The key question is whether genetic background changes the nutrient-urate relationship.

Citations: Johnson et al., J Nutr, 2026; Dalbeth et al., Nat Rev Dis Primers, 2019.



Direct effects: genetics and diet each relate to urate.
Interaction effect: the nutrient-urate association changes by genotype.

Background: why validate the PRS?

A dominant gene region can make an interaction signal look more polygenic than it really is.

My project logic



The validation problem

- A PRS should summarize broad inherited tendency across many variants.
- If one locus dominates, PRS × nutrient signals may reflect that locus—not genome-wide risk.
- For serum urate, *SLC2A9* is biologically important because it affects urate transport.

If a signal is truly polygenic, it should persist after *SLC2A9* is removed.
If it disappears, the dominant *SLC2A9* locus was likely driving the result.

Citations: Sudlow et al., PLoS Med, 2015; UK Biobank; Johnson et al., J Nutr, 2026.

Research question and project goals

A validation problem in nutrigenomics.

Are micronutrient–gene interactions affecting serum urate truly polygenic, or are they mainly driven by *SLC2A9*?

Phase 1 Identify micronutrients associated with serum urate concentrations.

Phase 2 Test interactions between 23 micronutrients and candidate urate-associated SNPs.

Phase 3 Build a custom PRS excluding *SLC2A9* to evaluate whether interaction signals remain.

MY PRIMARY ROLE:
custom PRS strategy

Hypothesis *SLC2A9* may disproportionately influence nutrient–gene interaction signals for serum urate.

Dataset and variables

UK Biobank provided linked dietary, biomarker, lifestyle, and genetic data.

Cohort context

The UK Biobank includes questionnaires, biomarkers, biological samples, genotyping/imputation, and long-term follow-up for more than 500,000 adults.

502,154
participants in
preliminary analyses

108,992
participants in
PRS analyses

Outcome Serum urate concentrations

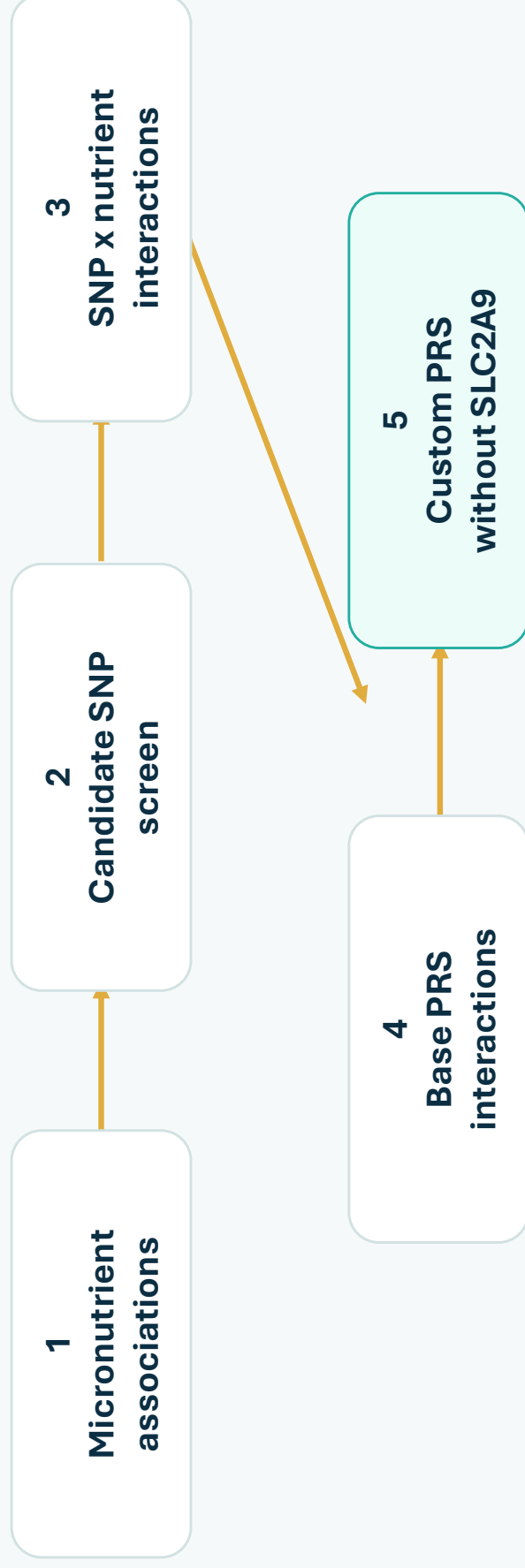
Nutrition 23 dietary micronutrients

Genetics 30 candidate SNPs + base/custom PRS

Covariates Age, genetic sex, BMI, smoking, alcohol, socioeconomic status, income, education, kidney disease, diuretic use, 10 genetic PCs, seafood, dairy, meat, fruits/vegetables, total energy, and fructose intake.

Methods: analytical workflow

Regression modeling moved the project from association testing to validation.



Validation logic

If interactions disappear after *SLC2A9* is removed, the dominant locus was likely driving the signal.

Tools: R Studio, PLINK2, PRSice-2, UK Biobank RAP, and Benjamini-Hochberg FDR correction.

Custom PRS strategy

Testing whether one dominant locus was creating the interaction signal.

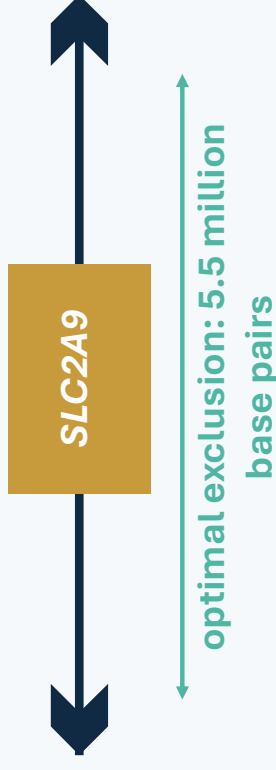
The problem

Traditional PRSs summarize genome-wide genetic risk, but a highly influential gene can dominate the score and make interaction signals look more polygenic than they really are.

The solution

We systematically excluded the SLC2A9 region and retested associations with the custom urate PRS.

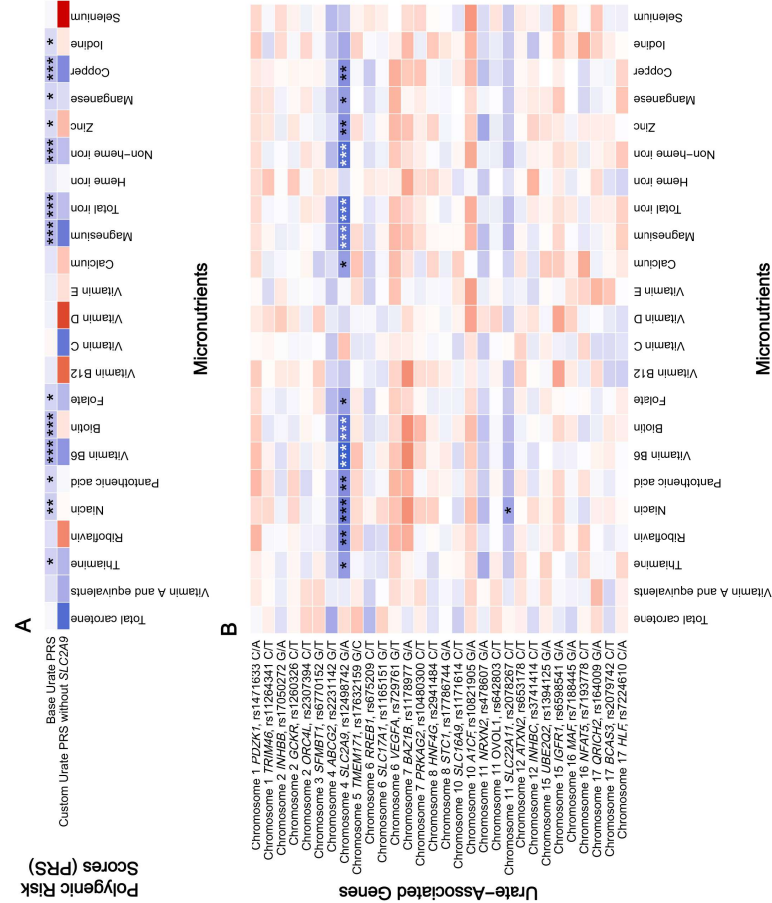
SLC2A9 exclusion window



Goal: reduce the association between the custom PRS and rs12498742 while preserving overall urate risk prediction.

Results I: interaction signals clustered at SLC2A9

The strongest pattern involved rs12498742 and multiple micronutrients.



Published Figure 3: heatmap of micronutrient-genetic interactions. Blue indicates negative beta coefficients; asterisks indicate FDR-significant interactions.

Key result

rs12498742 within *SLC2A9* showed the densest cluster of significant nutrient interactions.

- Risk allele carriers showed stronger urate-lowering associations for several nutrients.
- Signals were especially notable for riboflavin, pantothenic acid, biotin, calcium, and magnesium.
- This supported *SLC2A9* as a biologically plausible driver of nutrient responsiveness.

Results II: what disappeared after removing SLC2A9

The significant Base PRS x nutrient interactions no longer remained in the custom PRS.

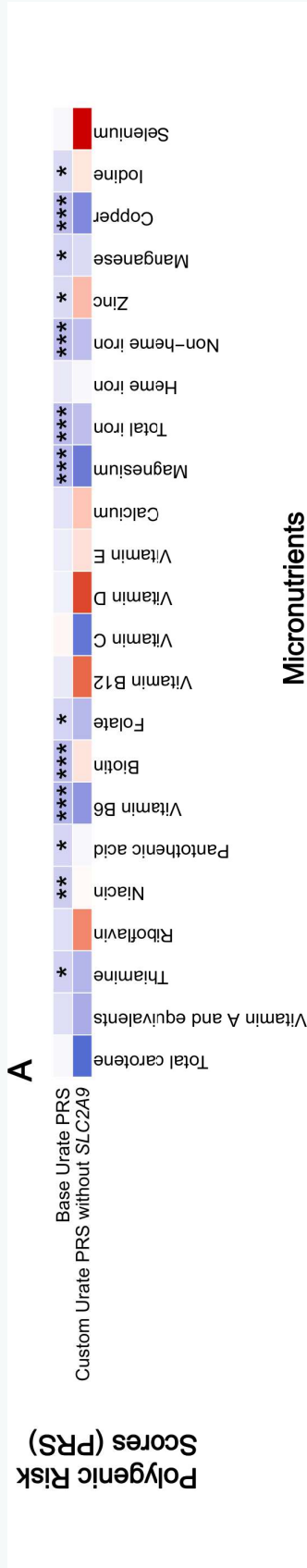


Figure 3A from the published paper: Base PRS interactions appeared before SLC2A9 removal, but disappeared in the custom PRS.

Base PRS interactions that lost significance

- B-vitamins: thiamine, niacin, pantothenic acid, vitamin B6, biotin, and folate
- Minerals / trace elements: magnesium, total iron, non-heme iron, zinc, manganese, copper, and iodine

Interpretation

The signal looked polygenic when SLC2A9 was included, but disappeared when that region was removed.

This suggests the apparent PRS x nutrient pattern was driven mainly by the SLC2A9 locus rather than genome-wide urate risk.

SCHOLARLY OUTPUT

This project resulted in a peer-reviewed publication

Published in *The Journal of Nutrition*

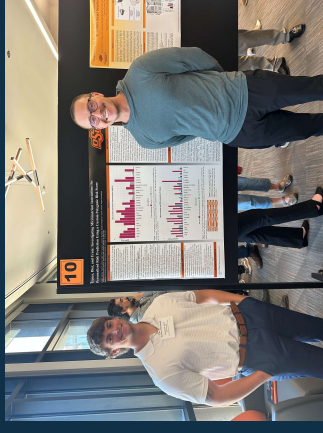
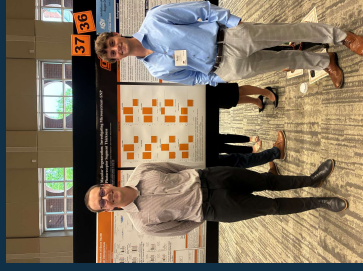
Co-author on a 2026 peer-reviewed article connecting dietary micronutrient intake, serum urate concentrations, and *SLC2A9* rs12498742.

J Nutr. 2026;156:101627.

doi:10.1016/j.tjnut.2026.101627

Additional outputs

- Poster presentation at the OSU Undergraduate Research Expo
- Additional accepted / under-review manuscripts and conference abstracts from related lab work



Conclusions and future directions

What this project contributes to precision nutrition.

Main conclusion

SLC2A9 rs12498742 appears to be a key driver of micronutrient–gene interactions affecting serum urate concentrations.

- Micronutrients were associated with lower serum urate concentrations.
- Risk allele carriers showed stronger urate-lowering responses for several nutrients.
- The disappearance of signals in the custom PRS emphasized the need to validate nutrigenomic findings.

Next steps

Replicate

Test independent and more diverse populations

Mechanism

Clarify how micronutrients interact with SLC2A9 pathways

Integrate

Combine genomics with metabolomics, biomarkers, diet, and lifestyle

Predict

Use AI / machine learning to support individualized dietary recommendations

Precision nutrition is promising only when interaction signals are carefully validated before translation into personalized recommendations.

Acknowledgements

This project was made possible by mentorship, collaboration, and support for undergraduate research.

Thank you

Dr. and Mrs. Niblack

For their generous support of undergraduate research through the Niblack Research Scholars Program

Dr. Winyoo Chwanadisai

Faculty sponsor, mentor, and guide in scientific reasoning, statistical analysis, and research design

Parker Johnson

Graduate student mentor for his guidance, troubleshooting, collaboration, and encouragement

Nutritional Sciences Research
Laboratory
Department of Nutritional Sciences
Oklahoma State University

Questions?

Matcha Supplementation Attenuates High-Fat, High-Sugar Diet–Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice

Cody Moss

Department of Nutritional Sciences

Faculty Sponsor: Dr. Yoo Kim

Graduate Student Mentor: Gopal Lamichhane

ABSTRACT

Matcha, a powdered form of green tea, is rich in bioactive compounds, including L-theanine and polyphenols, that have been associated with beneficial effects on cardiometabolic and gut health. This study aimed to evaluate whether dietary matcha supplementation attenuates high-fat, high-sugar diet (HFHSD)–induced cardiac hypertrophy and whether these effects are related to alterations in the gut microbiota along the gut-heart axis. Five-week-old male C57BL/6 mice were randomly assigned to either a normal chow diet (NCD) group or one of three HFHSD groups (HFHSD alone, HFHSD + 0.2% matcha, or HFHSD + 1% matcha). The dietary intervention lasted 10 weeks, during which body weight was continuously monitored. Results showed that after the 10-week intervention, heart weight was significantly reduced in the 1% matcha-supplemented mice compared with HFHSD controls. Wheat germ agglutinin (WGA) staining showed that both 0.2% and 1% matcha supplementation significantly reduced cardiomyocyte cross-sectional area relative to HFHSD controls. Furthermore, matcha supplementation altered gut microbiome composition, characterized by a marked increase in the abundance of beneficial taxa (*Ligilactobacillus*, *Lactobacillus*, *Oscillospiraceae*) while reducing potentially pathogenic taxa (*Enterobacteriaceae*, *Klebsiella*). In conclusion, dietary matcha supplementation attenuated HFHSD–induced cardiac hypertrophy in mice, potentially via modulation of the gut microbiota.

1. Introduction

High-fat, high-sugar diets (HFHSD) are major contributors to cardiometabolic dysfunction, which includes the development of cardiac hypertrophy. Emerging evidence highlights the critical role of the gut microbiome in systemic health. Diet–induced alterations in gut microbiota can influence cardiovascular outcomes, suggesting the presence of a vital “gut-heart axis” that may serve as a target for therapeutic intervention.

Matcha, a powdered green tea, is rich in bioactive compounds like L-theanine and polyphenols, which are associated with beneficial effects on cardiometabolic and gut health.

However, its specific ability to attenuate HFHSD-induced cardiac hypertrophy via gut microbiome modulation requires further evaluation. Therefore, this study was designed to evaluate whether dietary matcha supplementation attenuates HFHSD-induced cardiac hypertrophy and if these effects relate to alterations in the gut microbiota along the gut-heart axis.

2. Experimental Details

Five-week-old male C57BL/6 mice were utilized for the study and randomly assigned to four dietary groups: a normal chow diet (NCD), a high-fat, high-sugar diet (HFHSD), HFHSD + 0.2% ceremonial-grade matcha, or HFHSD + 1% ceremonial-grade matcha. The dietary intervention lasted for 10 weeks, with continuous monitoring of body weight throughout the study. During the 10th week, fecal samples were collected for gut microbiota analysis using 16S rRNA sequencing of the extracted genomic DNA. At the end of the experiment, blood samples were collected to assess serum lipid profiles. Additionally, metabolic organs including the liver, epididymal white adipose tissue (WAT), and heart were harvested, weighed, and collected for further analysis.

For histological analysis, the collected hearts were processed and evaluated for cardiomyocyte hypertrophy and fibrosis. This evaluation was conducted using Wheat Germ Agglutinin (WGA) staining and Picro-Sirius Red staining. ImageJ software was used to quantify the cross-sectional area, collagen area fraction, and a semi-quantitative collagen score.

Statistical analyses were performed using ordinary one-way or two-way repeated measures analysis of variance (ANOVA). This was followed by Tukey's multiple comparison tests. Data were presented as the mean $\pm$ standard error of the mean (SEM), with statistical significance indicated as $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$.

3. Results

After the 10-week dietary intervention, measurements were recorded for final body weight, final heart weight, and the ratio of heart weight to body weight. Heart weight was significantly reduced in the 1% matcha-supplemented mice compared to the HFHSD control group (**Figure 1A-C**). Wheat Germ Agglutinin (WGA) and Picro-Sirius Red staining revealed a significant increase in cardiomyocyte size and collagen deposition in HFHSD-fed mice compared with NCD animals. However, supplementation with both 0.2% and 1% matcha significantly reduced the cardiomyocyte cross-sectional area and fibrosis parameters relative to HFHSD controls (**Figure 1E, G-I**). All statistical data are presented as mean $\pm$ standard error of the mean (SEM), with significance evaluated via ANOVA.

Regarding the gut microbiome, matcha supplementation improved the gut dysbiosis induced by the HFHSD under obesity. Alpha diversity was evaluated using Observed ASVs and the Shannon Index (**Figure 2A-B**), while Beta diversity was evaluated by Principal

Coordinate Analysis (PCoA) using Weighted and Unweighted UniFrac distances (**Figure 2C-D**). The relative abundance of taxa was assessed at the Phylum, Family, and Genus levels (**Figure 2E-G**). Matcha supplementation successfully altered the microbiome composition by markedly increasing the abundance of beneficial taxa (*Ligilactobacillus*, *Lactobacillus*, *Oscillospiraceae*). Conversely, it reduced the abundance of potentially pathogenic taxa such as Enterobacteriaceae and *Klebsiella*.

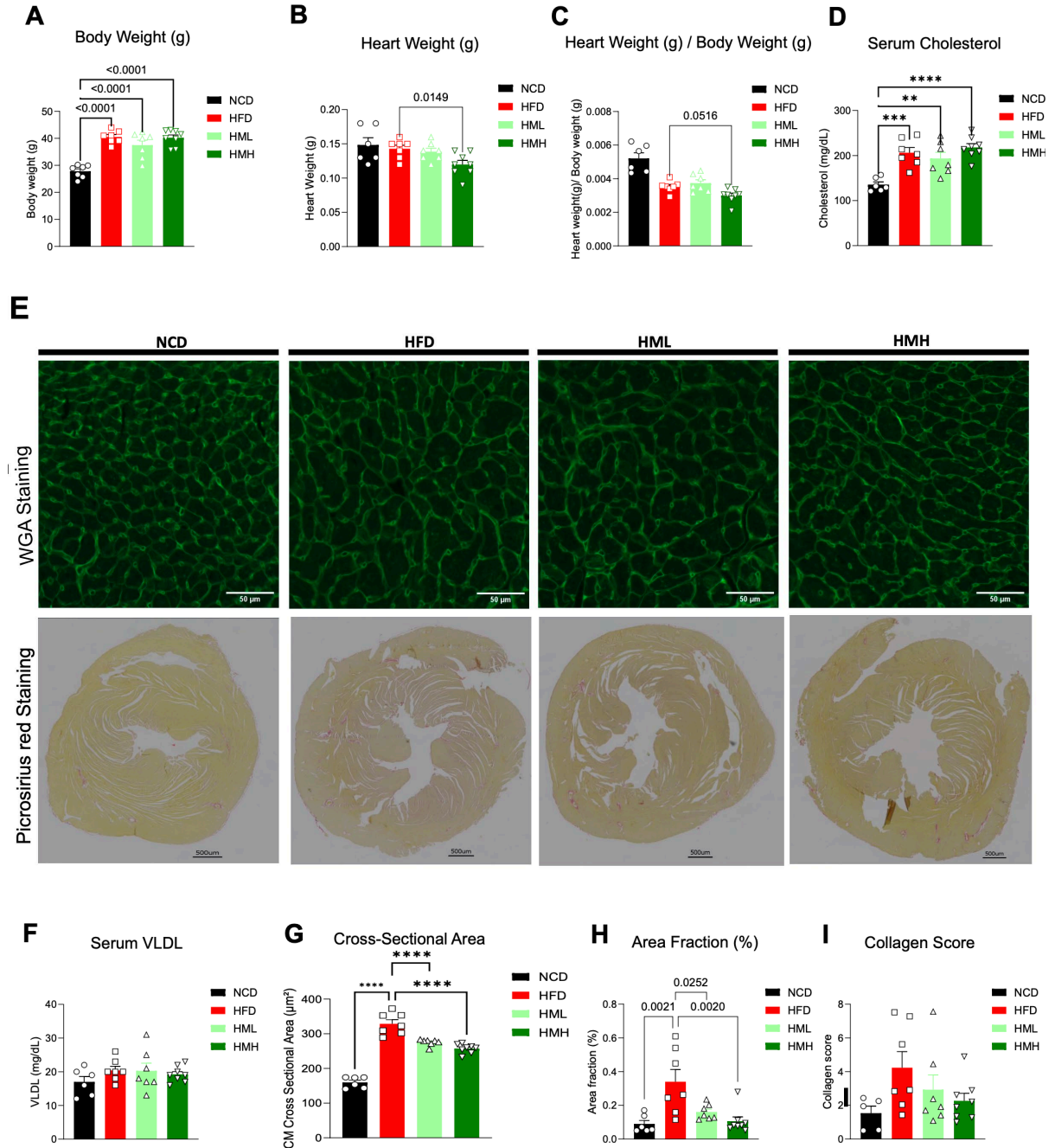


Figure 1. Matcha supplementation attenuates HFHSD–induced cardiomyocyte hypertrophy and fibrosis in mice. (A) Final body weight (g), (B) Final heart weight (g), (C) Ratio of heart weight to body weight, (D) Serum cholesterol concentrations (mg/dL), (E) Representative images of Wheat Germ Agglutinin (WGA) and Picro-Sirius Red stained heart cross-sections, (F) Serum VLDL, (G) Quantification of cardiomyocyte cross-sectional area (μm^2), (H) Quantification of collagen area fraction (%), (I) Semi-quantitative collagen score. Data are presented as mean $\pm$ SEM. Statistical significance is indicated as * $p < 0.05$, $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

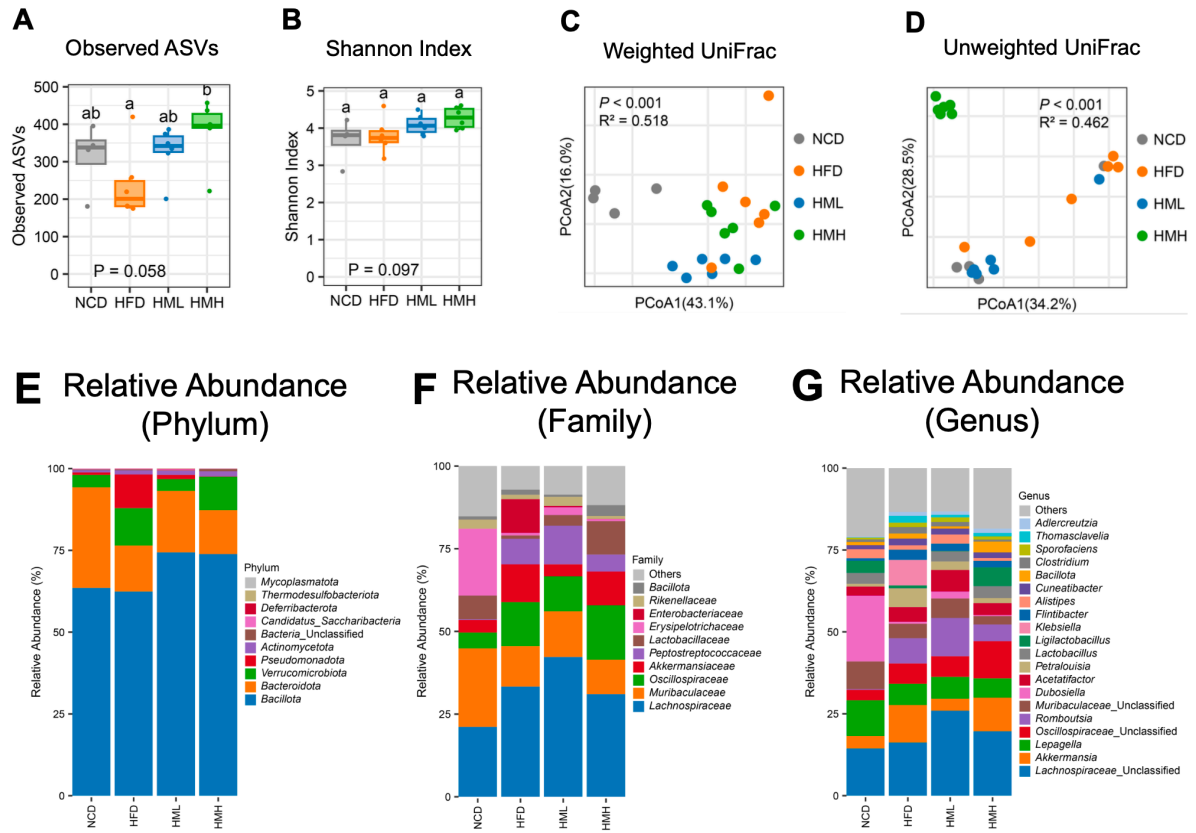


Figure 2. Matcha supplementation modulates gut microbiota diversity and composition in HFHSD–fed mice. Alpha diversity of the gut microbiome measured by (A) Observed ASVs and (B) Shannon Index. Beta diversity evaluated by Principal Coordinate Analysis (PCoA) using (C) Weighted UniFrac and (D) Unweighted UniFrac distances. Relative abundance of gut microbiota taxa at the (E) Phylum, (F) Family, and (G) Genus levels across the different dietary groups. (Note: NCD = Normal Chow Diet, HFD = High-Fat Diet, HML = High-Fat + 0.2% Matcha, HMH = High-Fat + 1% Matcha).

4. Discussion and Conclusions

The findings of this study demonstrate that dietary matcha supplementation successfully attenuates HFHSD-induced cardiac hypertrophy and fibrosis in mice. Matcha supplementation reduces cardiomyocyte hypertrophy and fibrosis induced by HFHSD-mediated obesity, counteracting the cardiometabolic dysfunction typically caused by poor diets. Furthermore, matcha supplementation significantly reshapes the gut microbiota.

These findings suggest that matcha improves cardiometabolic health potentially by modulating the gut microbiota along the gut-heart axis. Future studies are needed to determine whether these reductions in hypertrophy translate into enhanced cardiac function. Additionally, further research is required to determine whether the observed microbiota changes exert causal effects on cardiovascular disease risk.

5. Summary

This study investigated the therapeutic potential of matcha green tea on cardiometabolic dysfunction. Male C57BL/6 mice fed a high-fat, high-sugar diet supplemented with 0.2% or 1% matcha for 10 weeks showed significantly reduced heart weights, cardiomyocyte cross-sectional area, and collagen deposition compared to HFHSD controls. Matcha also successfully altered the gut microbiome by increasing beneficial bacteria and reducing pathogenic taxa. Ultimately, dietary matcha supplementation attenuated HFHSD-induced cardiac hypertrophy, likely through mechanisms involving the gut-heart axis.

6. Appendices

6a. Acknowledgements

This research was supported by the USDA (2024-67018-42461) and the Oklahoma Center for the Advancement of Science & Technology (OCAST, HR23-060). I would like to extend my sincerest gratitude to Dr. and Mrs. Niblack for their generous financial support and sponsorship of the Niblack Research Scholars program. A special thank you to Dr. Christine Johnson, Associate Vice President for Research, for her guidance and direction throughout the program. Finally, I would like to thank my Faculty Sponsor, Dr. Yoo Kim, my Graduate Student Mentor, Gopal Lamichhane, and my co-authors Jong-Beom Jin, Guo Jiaqing, Da-Yeon Lee, Jiyoung Bae, and Guolong Zhang for their mentorship, assistance, and collaboration on this project.

6b. Papers Published

Moss, C., Lamichhane, G., Jin, J.-B., Jiaqing, G., Lee, D.-Y., Bae, J., Zhang, G., and Kim, Y., 2026. Matcha Supplementation Attenuates High-Fat, High-Sugar Diet-Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice. Manuscript under review in *Nutrients*.

Moss, C., Lamichhane, G., Jin, J.-B., Jiaqing, G., Lee, D.-Y., Bae, J., Zhang, G., and Kim, Y., 2026. Matcha Supplementation Attenuates High-Fat, High-Sugar Diet–Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice. Poster presented at the American Society for Nutrition (ASN) NUTRITION 2026 Conference, Washington, D.C., July 2026.

Moss, C., Lamichhane, G., Jin, J.-B., Jiaqing, G., Lee, D.-Y., Bae, J., Zhang, G., and Kim, Y., 2026. Matcha Supplementation Attenuates High-Fat, High-Sugar Diet–Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice. Poster presented at the Oklahoma State University Undergraduate Summer Research Expo, Stillwater, OK, July 2026.

Moss, C., Lamichhane, G., Jin, J.-B., Jiaqing, G., Lee, D.-Y., Bae, J., Zhang, G., and Kim, Y., 2026. Matcha Supplementation Attenuates High-Fat, High-Sugar Diet–Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice. Poster presented at the Oklahoma State University Undergraduate Research Symposium, Stillwater, OK, April 2026.

6c. Literature Cited

Abel, E. D., Litwin, S. E., and Sweeney, G., 2008. Cardiac remodeling in obesity. *Physiological Reviews*, 88(2): 389-419.

Kochman, J., Jakubczyk, K., Antoniewicz, J., Mruk, H., and Janda, K., 2020. Health benefits and chemical composition of matcha green tea: A review. *Molecules*, 26(1): 85.

Tang, W. H. W., Kitai, T., and Hazen, S. L., 2017. Gut microbiota in cardiovascular health and disease. *Circulation Research*, 120(7): 1183-1196.

Matcha Supplementation Attenuates High-Fat, High-Sugar Diet-Induced Cardiomyocyte Hypertrophy by Reshaping the Gut Microbiota in Mice

Cody Moss

Mentored by Dr. Yoo Kim and Gopal Lamichhane

Niblack Research Scholars Program | Department of Nutritional Sciences

Why Examine Matcha and the Gut-Heart Axis?

High-Fat, High-Sugar Diet (HFHSD)

Obesity promotes cardiac hypertrophy, fibrosis, and cardiometabolic dysfunction.

Gut Microbial Disruption

Diet-induced changes in gut microbiota may influence cardiovascular outcomes through the gut-heart axis.

Matcha

Contains L-theanine and polyphenols associated with cardiometabolic and gut health benefits.

Study Question

Can dietary matcha attenuate HFHSD-induced cardiac hypertrophy and fibrosis while reshaping the gut microbiota?

Hypothesis

Dietary matcha reduces HFHSD-induced cardiac remodeling by modulating the gut microbiome.

Abel et al., 2008 – Tang et al., 2017 – Kochman et al., 2020

Experimental Design

Animals & Intervention

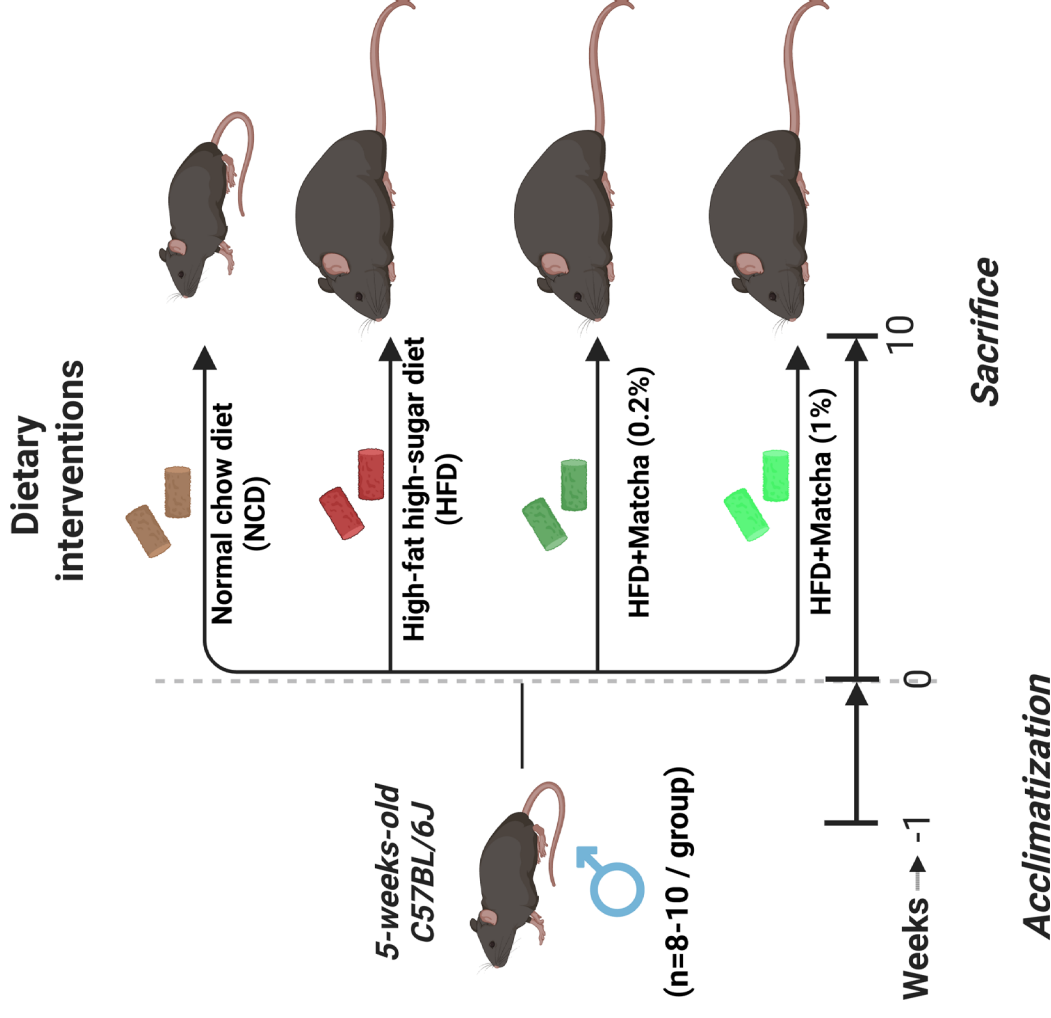
5-week-old male C57BL/6J mice
n = 8-10 per group
10-week dietary intervention

Four Diet Groups

- **NCD** Normal Chow Diet
- **HFD** High-Fat, High-Sugar Diet
- **HML** HFD + 0.2% Matcha
- **HMH** HFD + 1% Matcha

Measurements

Body and heart weight, serum lipids, WGA and Picro-Sirius Red histology, and fecal 16S rRNA sequencing



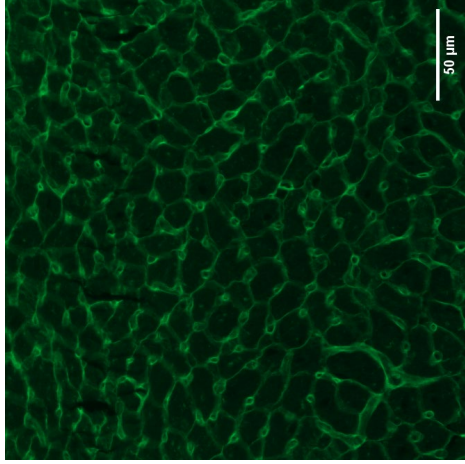
Cardiometabolic Effects of Matcha



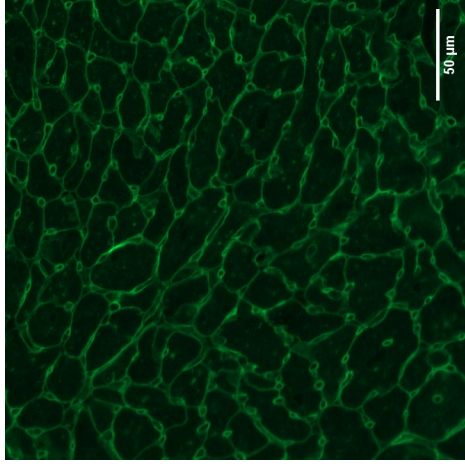
NCD = Normal Chow Diet HFD = High-Fat, High-Sugar Diet HML = HFD + 0.2% Matcha HMH = HFD + 1% Matcha

Matcha Attenuated Cardiomyocyte Hypertrophy

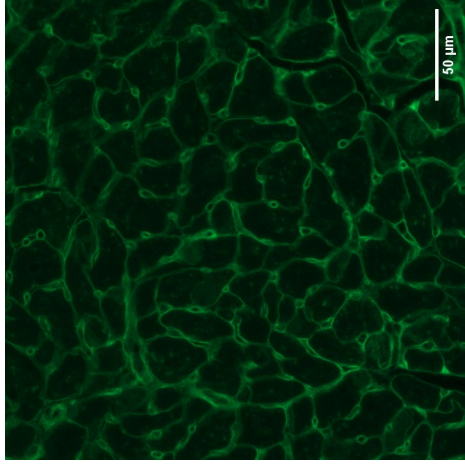
NCD



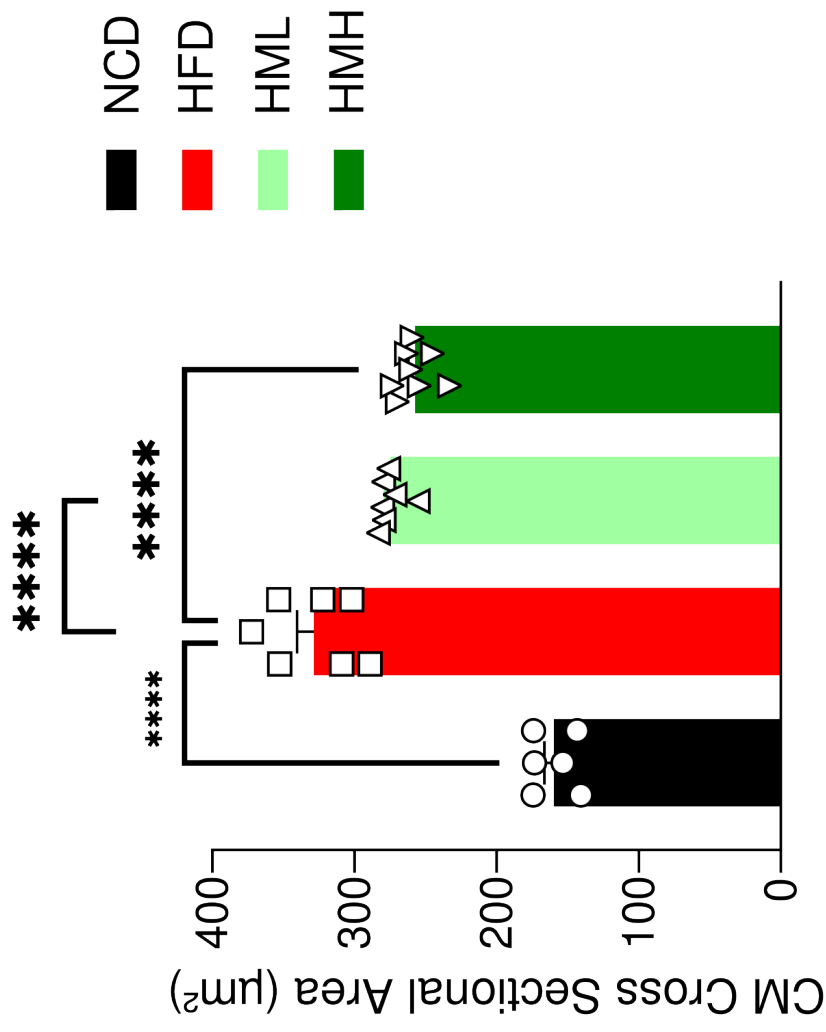
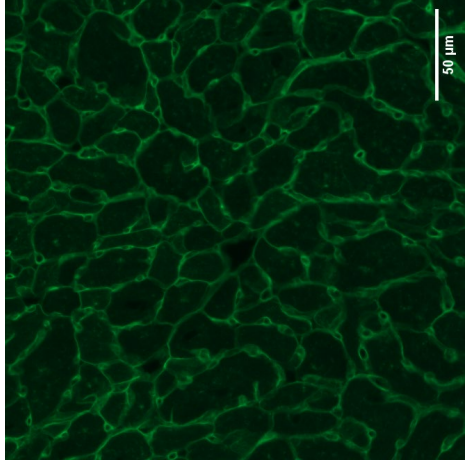
HFD



HML



HMH



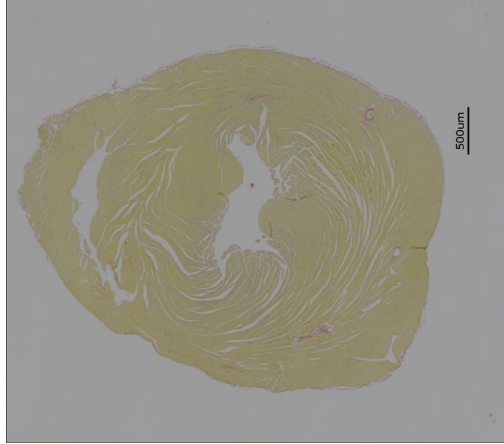
Both matcha doses significantly reduced cross-sectional area relative to HFD

Wheat Germ Agglutinin (WGA) Staining

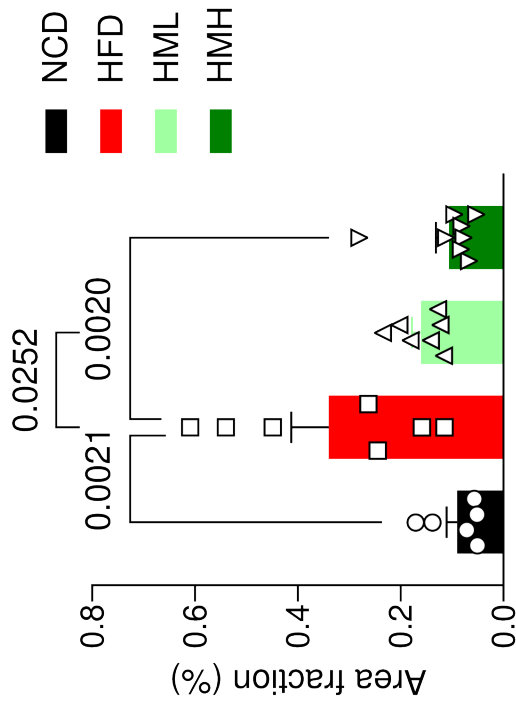
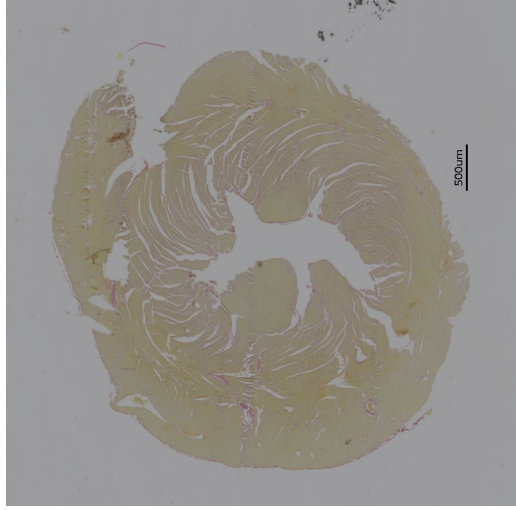
Matcha Reduced Cardiac Collagen Deposition

Picro-Sirius Red Staining

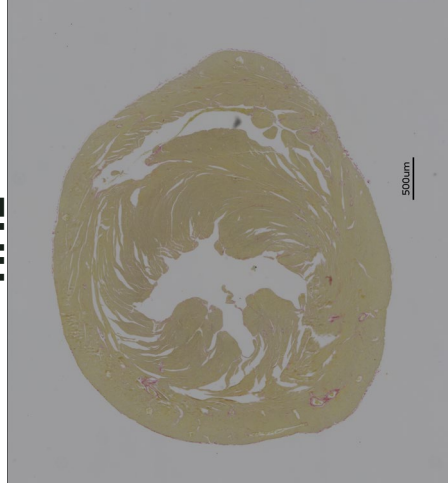
NCD



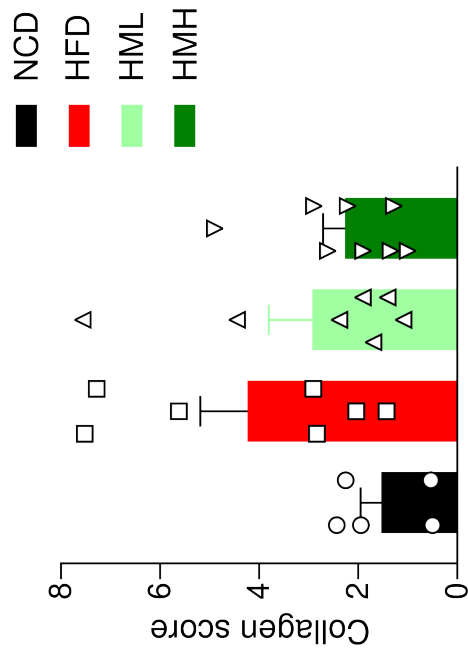
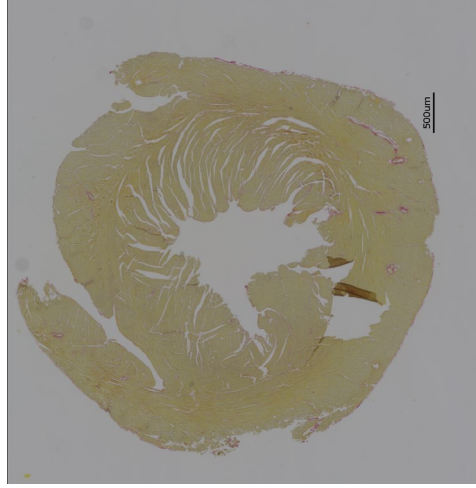
HFD



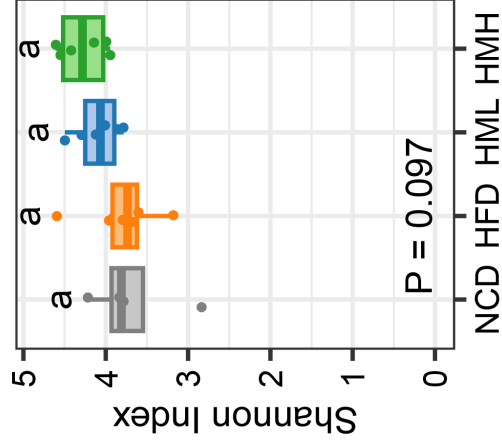
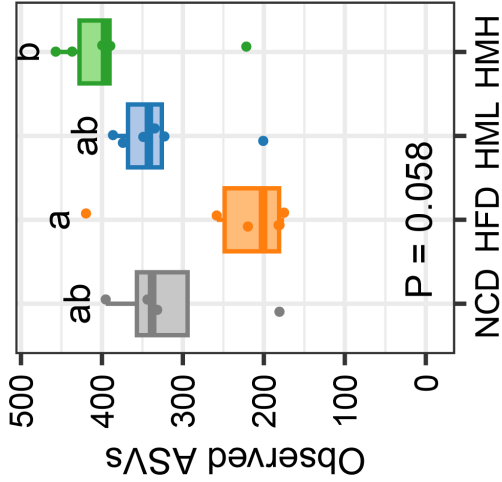
HML



HMH

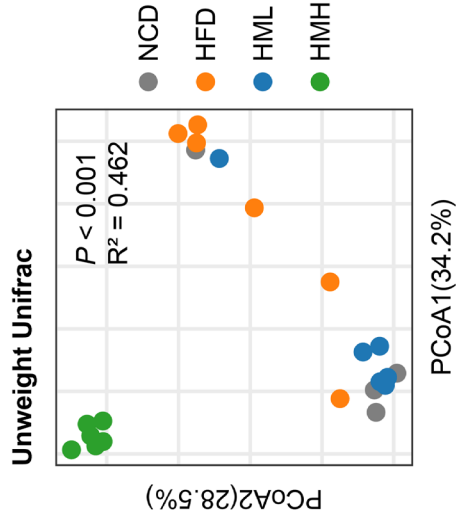
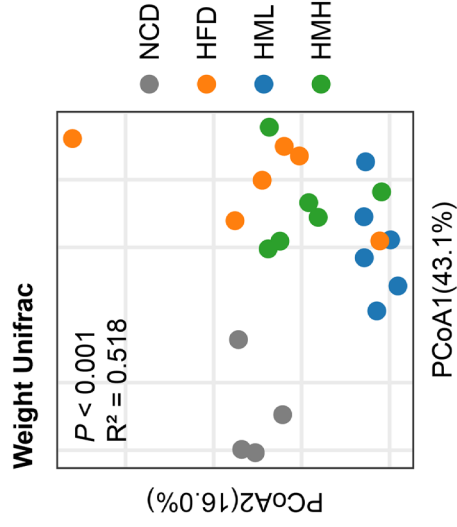


Matcha Shifted Gut Microbial Diversity and Community Structure



Alpha Diversity

Observed ASVs and Shannon diversity trended upward with matcha, but overall p values were 0.058 and 0.097.

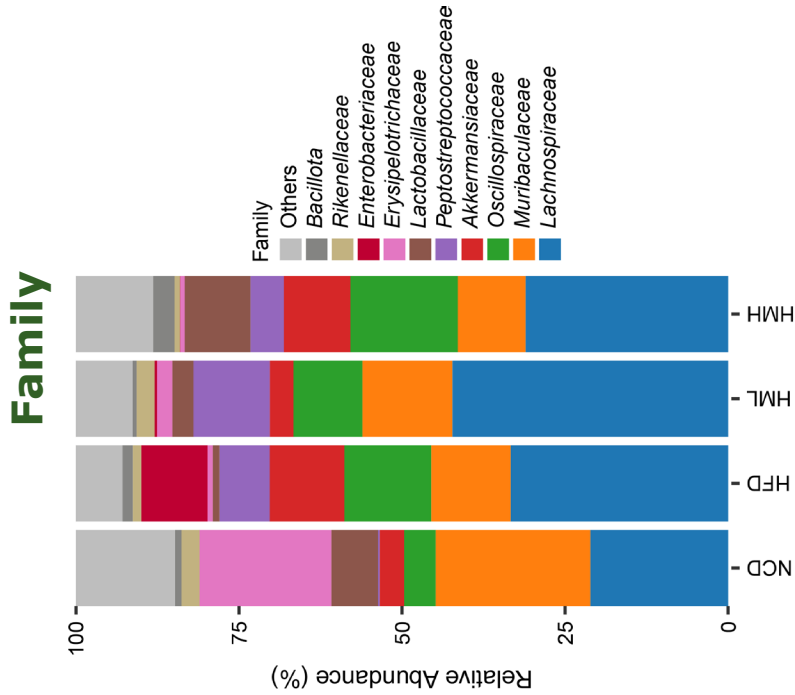


Beta Diversity

Community composition differed by diet for weighted and unweighted UniFrac distances ($p < 0.001$).

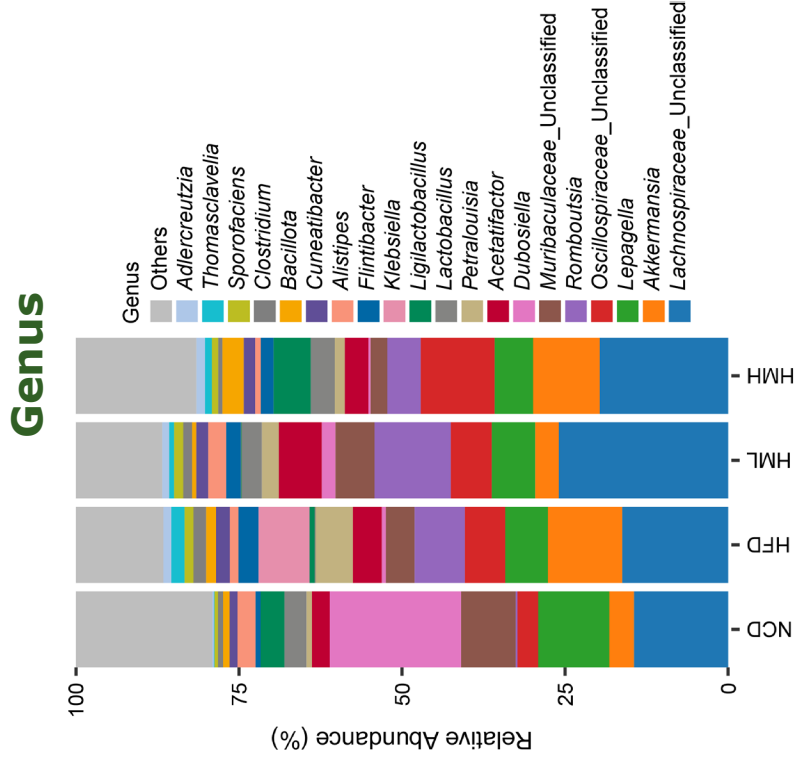
NCD = Normal Chow Diet HFD = High-Fat, High-Sugar Diet HML = HFD + 0.2% Matcha HMH = HFD + 1% Matcha

Matcha Altered Microbial Taxonomic Composition



More Abundant With Matcha:

Ligilactobacillus, *Lactobacillus*, and *Oscillospiraceae*
(Beneficial Taxa)



Less Abundant With Matcha:

Enterobacteriaceae and *Klebsiella*
(Potentially Pathogenic Taxa)

NCD = Normal Chow Diet HFD = High-Fat, High-Sugar Diet HML = HFD + 0.2% Matcha HMH = HFD + 1% Matcha

Conclusion

Matcha attenuated HFHSD-induced cardiac remodeling and shifted gut microbial communities in male mice.

Evidence

- Lower heart weight with 1% matcha
- Reduced cardiomyocyte cross-sectional area
- Reduced collagen area fraction
- Significant shifts in beta diversity

Interpretation

The parallel cardiac and microbiome changes support a possible gut-heart mechanism, but this study does not establish causality or human efficacy.

Thank you, Dr. and Mrs. Niblack!
Questions?

Supported by the Niblack Research Scholars Program.
Also supported by USDA (2024-67018-42461) and OCAST (HR23-060).

SESSION TWO:

MaKayla Friend

“Evaluating Soil Physical Properties of Stillwater Soils: The Role of Biochar Amendments”

Research Area: Biology

Faculty Sponsor: Dr. Benedicte Bachelot

Graduate Student Mentor: Jonathan Medellin

Omar Ameen

“Haloalkylation of Arylcyclohexenes via Carbenium Ion Formation: Application of Light-Mediated Transducer Catalysis”

Research Area: Chemistry

Faculty Sponsor: Dr. Jimmie Weaver

Graduate Student Mentor: Osaïd Alkhamayseh

Nichollette DuCom

“Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers”

Research Area: Physics

Faculty Sponsor: Dr. Eric Benton

Graduate Student Mentor: Conner Heffernan & Garrett Thornton

Evaluating Soil Physical Properties of Stillwater Soils: The Role of Biochar Amendments

MaKayla Friend

Department of Environmental Science

Faculty Sponsor: Dr. Benedicte Bachelot

Graduate Student Mentor: Nimalka Weerasuriya

ABSTRACT

Promoting soil health and stimulating natural soil processes that have been depleted over time are the goal of current agriculture practices, including the use of organic amendments. Biochar application has been associated with an increase in soil organic carbon content, suggesting improved microbial activity. In addition to soil biochemistry, biochar might also influence soil physical properties. Biochar use was also associated with increasing microbial community size, due to the addition of microsites on biochar particles and organic carbon. Besides soil biochemistry, biochar might also influence soil physical properties due to its influence on the fate of contaminants as well as nutrient and water absorption by plants. Agricultural systems also heavily rely on the use of biocides, which could indirectly alter soil health. My aims of this study are (1) to evaluate the impacts of different biochar amendments on soil properties, and (2) to assess the possible interaction between biochar amendments and the use of biocides on soil health. We analyzed biomass, infiltration rate, mean weight diameter of aggregates, percent destruction of aggregates, and root colonization rates across differing biochar and biocide treatments. We found that poultry litter biochar had more of an effect on soil health and plant community dynamics than BSG biochar, or no biochar. We found that biocide treatments did not significantly influence soil health or plant communities. We did not observe any significant interaction between biocide and biochar treatments, which provides more insights into how land management practices may alter soil health and productivity.

1. Introduction

As the world's population exponentially grows year after year, food security becomes an increasingly important task for agricultural systems. We need to increase yield while decreasing land needed for cultivation and decreasing negative impacts on the environment. Healthy soils have the potential to increase crop yields, provide environmental benefits, and sequester terrestrial carbon. Promoting soil health and stimulating natural soil processes that have been depleted over time is the goal of new agriculture practices, such as the use of biochar. Biochar is a material made of organic

waste partially combusted. It is therefore rich in carbon and has been used as a soil primer. Biochar has a prominent role in the carbon cycle and terrestrial carbon sequestration potential (Khan et al., 2024). Biochar can have other outcomes on the soil properties, such as higher water retention capabilities and nutrient retention (Khan et al., 2024). Biochar implementation has also been associated with an increase in soil organic carbon content, suggesting improved soil carbon sequestration (El-Naggar, Awad, Tang et al., 2018). Biochar use was also associated with increasing microbial community size, due to the addition of microsites on biochar particles and organic carbon (Gul, Whalen, Thumas, et al., 2015). Besides soil biochemistry, biochar might also influence soil physical properties such as bulk density, soil porosity, field capacity, and nutrient adsorption. This is important as soil physical properties influence the fate of contaminants as well as nutrient and water absorption by plants (Dhaliwal, et al., 2019). Biochar implementation may not occur on organic land management practices only, as different land management practices have different intended impacts. Biocide amendments, such as fungicide, insecticide, and molluscicide, are commonly used in agricultural, forest, and pastureland management practices. Agricultural systems heavily rely on the use of biocides for pest control (United States Geological Survey, 2019). In this study, I am interested in investigating the impacts of biochar on soil physical properties in a system using biocides. Specifically, I have two aims. Aim 1: evaluate the impacts of different land management practices (biochar and biocide) on soil physical and plant community properties. Aim 2: access the possible correlation between biochar amendments and the use of biocides on soil physical properties and health.

2. Experiment Details

Experimental Site and Design: The study utilizes an existing experimental collaborative research site, BugNet at James K. McPherson Botanical Preserve. McPherson Preserve is owned and operated by the College of Arts and Science at Oklahoma State University, and is located at 36.099° N and -97.204° W. The BugNet experiment consists of three blocks (repetitions) of 8 5 meter x 5 meter plots which have been receiving one of 8 biocide treatment combinations for the last four years. The fungicide treatment utilizes Ortiva (Azoxystrobin) and Score (Difenoconazol). The insecticide treatment utilizes Karate Zeon (Lambda-Cyhalothrin). The molluscicide treatment utilizes generic slug bait (1.25% ferric phosphate). This project used three quadrants (1 meter x 1 meter) within each plot of the experiment. In each plot, two different feedstocks, spent brewer's grain (BSG) and poultry litter, were converted to biochar and applied to two of the three quadrants. BSG and poultry litter feedstock was collected from Iron Monk Brewery (Stillwater, OK, USA) and a local Tulsa egg farm (Sand Springs, OK, USA). Firewood was collected from downed trees and mulched into 5-10 cm wood chips. Biochar feedstocks were oven-dried (90 °C, 14 days) and placed into a 15-gallon metal drum with a thermocouple attached. Biochar feedstock was heated

to 300-500 °C for 3-5 hours, depending on the feedstock material. 1 L of biochar was applied to each 1 m² quadrant. One quadrant served as a control treatment with no biochar applied to it.

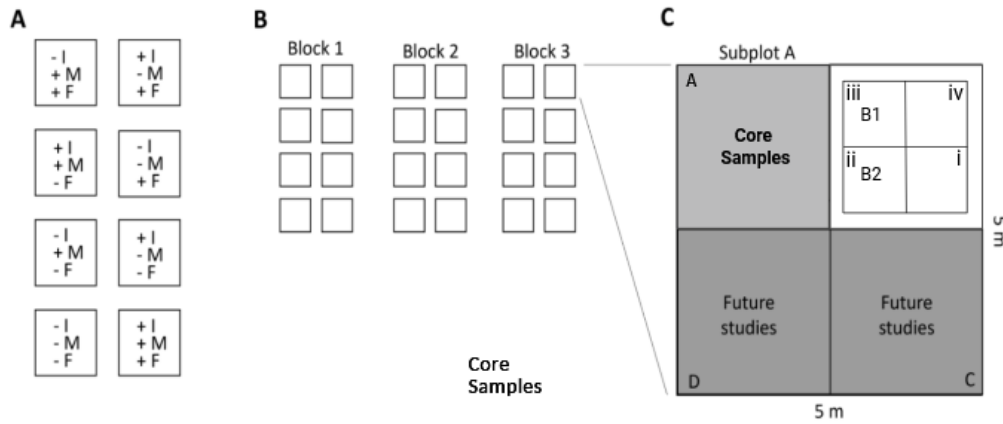


Figure 1: A graphical representation of the experimental site and design. A) shows the block design. B) shows the three randomized blocks. C) shows the subplots (A,B,C,D) and quadrants (i,ii,iii,iv). B1 represents the BSG biochar. B2 represents the poultry litter biochar. Quadrant iv served as the control treatment (no biochar).

Physical properties sampling: Soil aggregate stability, aboveground biomass, root colonization, and infiltration rate was assessed in all three quadrants. Soil aggregate stability was analyzed using dry and wet sieving methods to determine the percent destruction of aggregates and mean weight diameter of aggregates (Xukai et al., 2020). Dry sieving soil data was obtained by determining the fraction of soil particles at each sieve size in a 100-gram soil samples (<4 mm, 2-4 mm, 1-2 mm, 0.5 mm-1 mm, 0.25 mm-0.5 mm, <0.25 mm). Wet sieving soil data was obtained by placing a piece of filter paper (100 mm) on top of the previous sieve set, running a faucet of 1 L/min DI water, and oscillating the sieve set at 30-50 rpm. The fraction of soil particles at each sieve size in a 100-gram soil sample was recorded. Infiltration rate was analyzed using a double ring infiltrometer (13 cm diameter) and the infiltration ring was placed flush against the bare soil surface. Then, water was dispersed into the outer ring before filling the inner ring. The water drained (in cm) per unit of time was recorded, and each repetition ended after 5 minutes.

Biological properties sampling: Biomass was determined by collecting above ground biomass samples in the same subquadrant (5x10cm) in each experimental quadrant. Then, biomass samples were oven-dried (90 C for 48 hours) and weighed to determine aboveground plant biomass. Root colonization samples were collected from little bluestem, *Schizachyrium scoparium*, individuals in each subplot for single biocide treatments (I+,M+,F+,Control,I+M+F+). One individual plant was collected and divided into three representative root samples. Root samples were cleared with 10% KOH for 1

hr at 90 °C and acidified with 1% HCl for 5 min before staining. Roots were stained with 0.05% Trypan blue for 10 min at 110 °C. Root samples were transferred into 30% lactic acid before analysis. We utilized 3 equal sections from each root sample to determine percent arbuscular mycorrhizal fungi (AMF) colonization using the modified gridline method (McGonigle et al, 1990), under x200 magnification (Hirox KH 7700, Tokyo, Japan). Fungal hyphae, arbuscules, vesicles, and fungal spores of AMF counted towards total root colonization rate.

Statistical Analysis: Statistical analysis was performed using R-STUDIO (ver. 2025-05.0-526). We used analysis of variance (Kenward-Roger and TukeyHSD) and reported significance at 0.001, 0.01, and 0.05 significance levels, with the experimental block as a random effect. When appropriate, multiple comparison adjustments were employed for statistical grouping. To determine interactions, significance levels were displayed for all biochar and biocide interactions. Significant differences between means were estimated at 95% confidence intervals. For spider plots, the data was normalized into z-scores, which determined how many standard deviations from the mean a treatment type was.

3. Results

Aim 1: Management practices differently influenced soil health

Biochar Treatments: PDA did not significantly differ across biochar treatments (p-value>0.05). B1 biochar had a mean of 25.9%, B2 biochar had a mean of 21.3%, and the control had a mean of 26.2% (Figure 1). There was no statistically significant difference across treatment groups for PDA. MWD did not significantly differ across biochar treatments (p-value>0.05). B1 biochar had a mean of 2.02 mm, B2 had a mean of 2.18 mm, and the control had a mean of 1.94 mm (Figure 2). There was no statistical difference across treatment groups for MWD. Biomass did not significantly differ across biochar treatments (p-value>0.05). B1 biochar had a mean of 19.2 g, B2 biochar had a mean of 22 g, and the control had a mean of 19.1 g (Figure 3). There was no statistical difference across the treatment groups for biomass. Infiltration rate did not significantly differ across biochar treatments (p-value>0.05). B1 biochar had a mean of 1.75 log (cm per hour), B2 biochar had a mean of 1.72 log (cm per hour), and the control had a mean of 1.76 log (cm per hour) (Figure 4). There was no statistical difference across treatment groups for infiltration rate. Root colonization rates did significantly differ across biochar treatments (p-value=0.09). B1 biochar had a mean of 27.9%, B2 biochar had a mean of 32.5% and the control had a mean of 25.7% (Figure 5). B2 biochar and the control were significantly different (p-value=0.09). B1 biochar was not statistically different from B2 biochar or the control treatment.

Figure 1: Percent Destruction of Aggregates (in %)

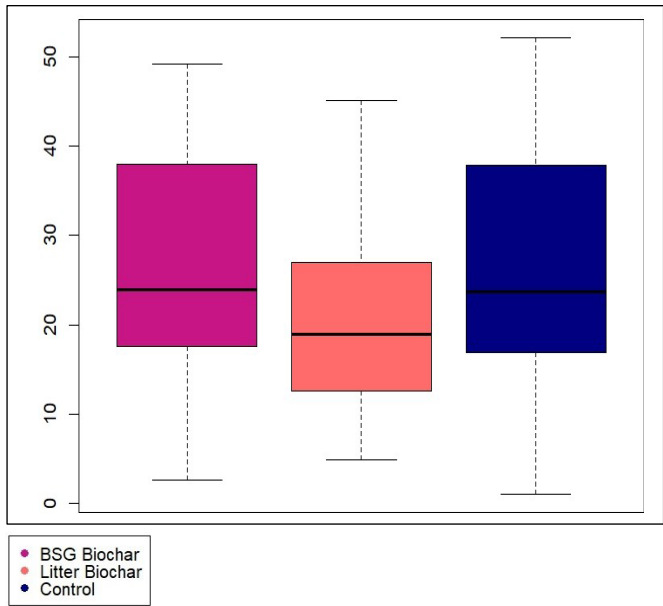


Figure 2: Mean Weight Diameter (in mm)

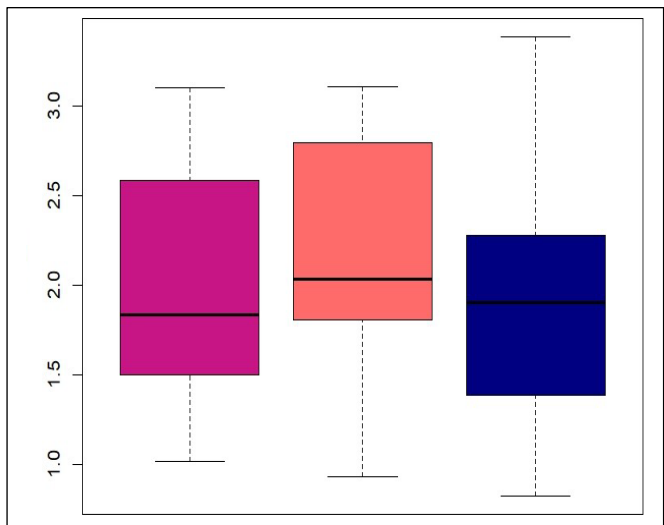


Figure 3: Aboveground plant biomass (in grams)

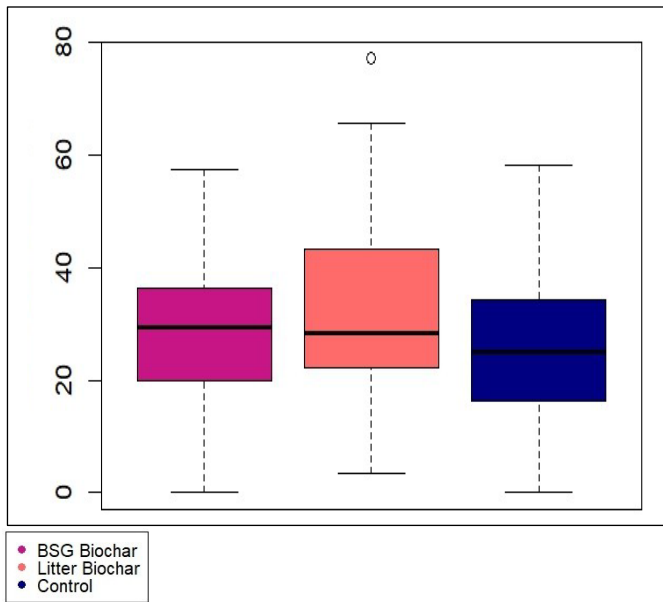


Figure 4: Infiltration rate (in cm per hour)

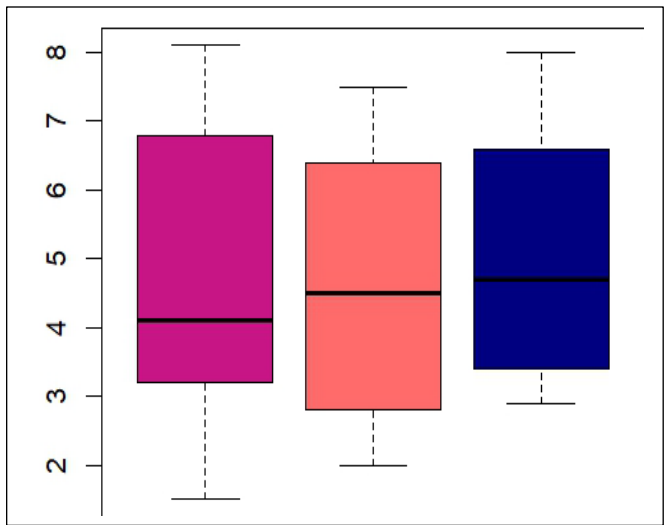


Figure 5: Root Colonization Rate (in %)

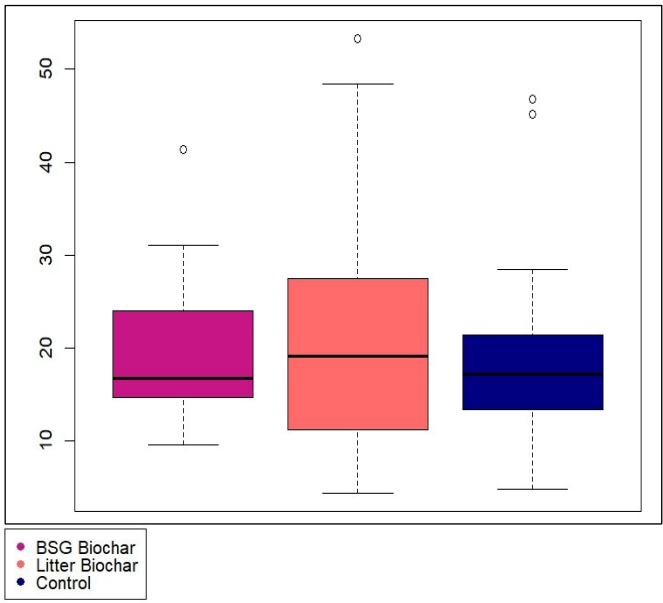


Figure 6: Spider plot of Biochar Treatments (in z-scores)

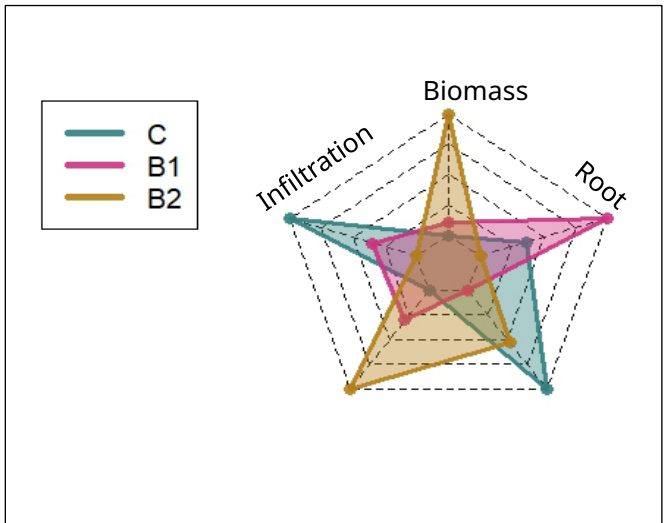


Figure 7: Percent Destruction of Aggregates (in %)

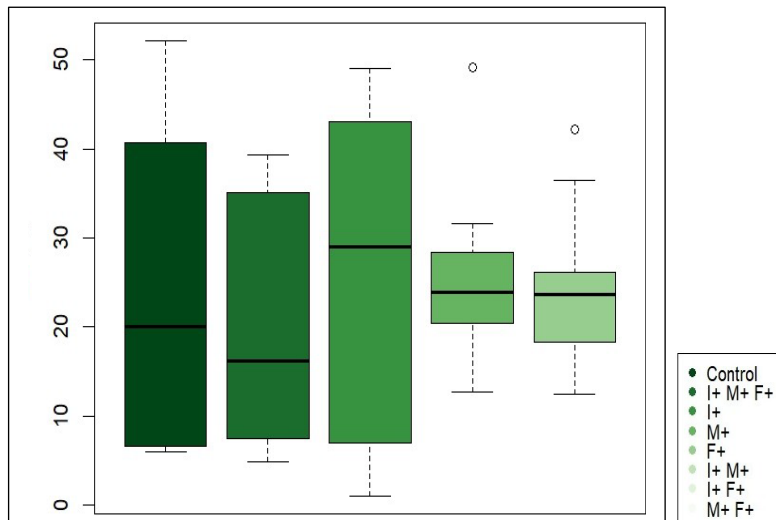


Figure 8: Mean Weight Diameter (in mm)

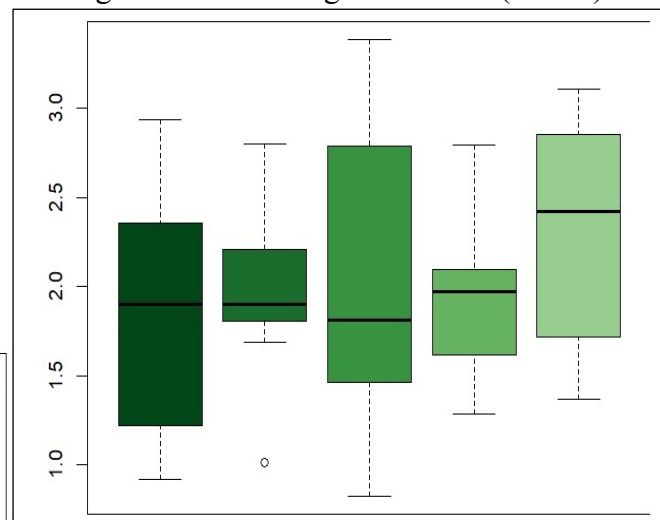


Figure 9: Aboveground Plant Biomass (in grams)

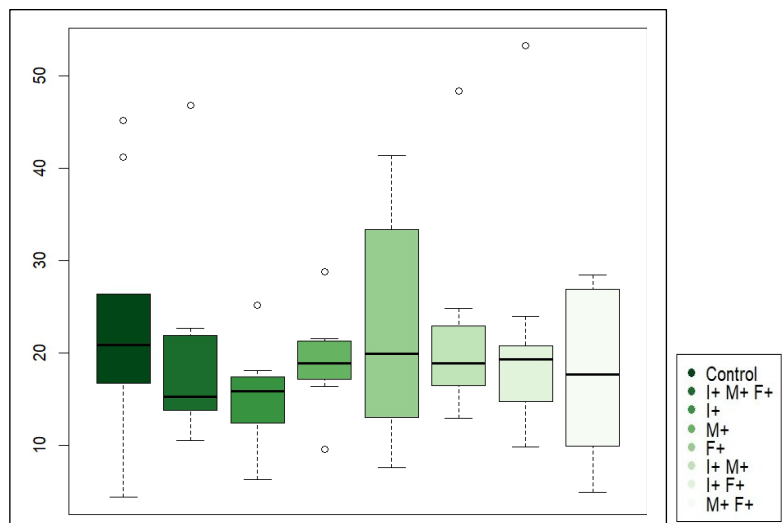


Figure 10: Infiltration Rate (in cm per hour)

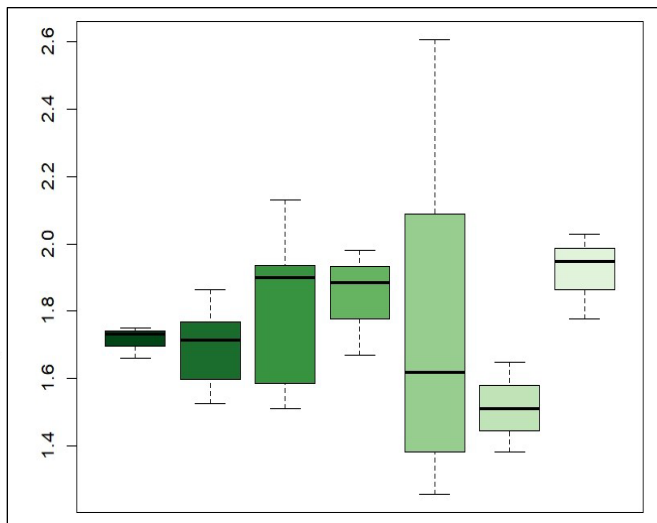


Figure 11: Root Colonization Rate (in %)

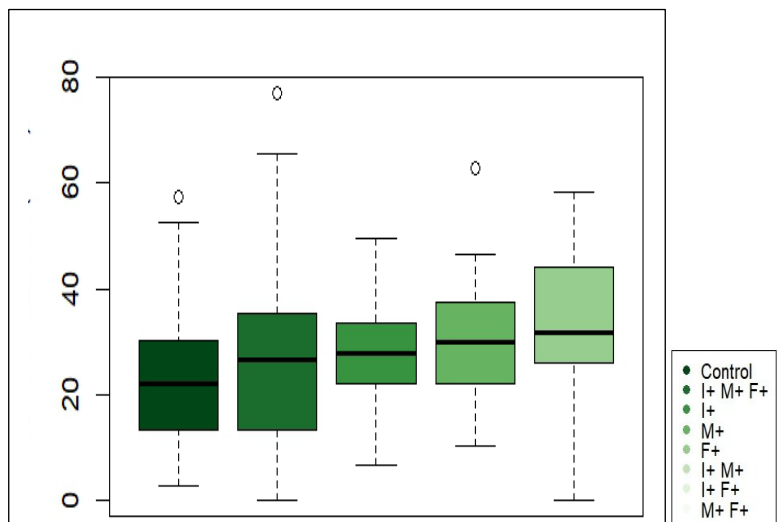
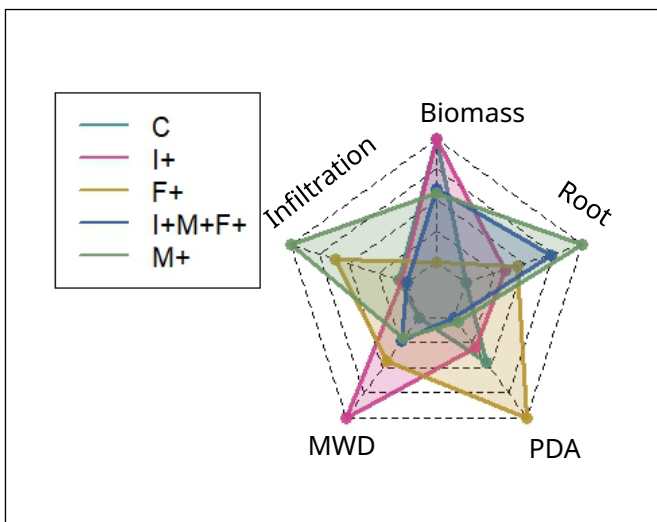


Figure 12: Spider plot of Biocide Treatments (in z-scores)



Biocide Treatments: PDA did not statistically differ across biocide treatments. F+ had a mean of 19.6%, M+ had a mean of 24.8%, I+M+ had a mean of 25.8%, I+ had a mean of 26.5%, and the control had a mean of 25.7% (Figure 7). There was no statistical difference across the treatment groups for PDA. MWD did not significantly differ across biocide treatments ($p\text{-value}>0.05$). F+ had a mean of 2.35 mm, I+ had a mean of 2.08 mm, I+M+F+ had a mean of 1.98 mm, M+ had a mean of 1.97 mm, and the control had a mean of 1.87 mm (Figure 8). There was no statistically significant difference across the treatment groups for MWD. Biomass did not significantly differ across biocide treatments ($p\text{-value}>0.05$). I+ had a mean of 15.4 g, M+F+ had a mean of 18.2 g, M+ had a mean of 19.2 g, I+M+F+ had a mean of 19.4 g, I+F+ had a mean of 21.1 g, F+ had a mean of 22.2 g, I+M+ had a mean of 22.2 g, and the control treatment had a mean of 22.2 g (Figure 9). Infiltration did not significantly differ across biocide treatment ($p\text{-value}>0.05$). I+M+F+ had a mean of log 1.68 (cm per hour), F+ had a mean of 1.76 log (cm per hour), I+ had a mean of 1.79 log (cm per hour), M+ had a mean of 1.85 log (cm per hour), I+ F+ had a mean of 1.51 log (cm per hour), M+F+ had a mean of 1.92 log (cm per hour), and the control had a mean of 1.68 log (cm per hour) (Figure 10). Root colonization rates did not statistically differ across biocide treatments. F+ had a mean of 27.7%, I+ had a mean of 26.8%, M+ had a mean of 32.9%, I+M+F+ had a mean of 30.4%, and the control had a mean of 23.6% (Figure 11). There was no significant difference across the treatment groups for root colonization rates.

Aim 2: There was no interaction between biocide and biochar on soil health

We performed pair comparisons across all biochar and biocides treatments and did not detect any significant effect of multiple treatments. Most comparisons had high p -values, which may mean a different statistical test is needed, or the sample size is too small to compare two factors.

4. Discussion and Conclusions

This study highlights the benefits of poultry litter biochar on soil health. Poultry litter biochar had the highest biomass, MWD, and root colonization rates (Figure 6). Poultry litter biochar also had the lowest infiltration rate and PDA across biochar treatments. Root colonization rates were significantly higher in the poultry litter biochar treatment, when compared to the control treatment. We propose this is due to the high phosphorus content of poultry litter manure, which served as the main feedstock for this treatment. Phosphorus is a limiting nutrient in tallgrass prairie ecosystems due to the soil parent material (Hein et al., 2002). Previous literature also finds higher root colonization rates and plant biomass with AMF inoculum (Sawant, 2023). BSG biochar was more like the control treatment than poultry litter biochar treatment. BSG biochar also had a smaller particle size, which could have led to higher rates of loss, when compared to poultry litter

biochar. Previous literature finds BSG biochar to increase soil organic matter (SOM), which can improve soil aggregation (Yoo et al., 2021).

In contrast to the biochar treatments, the biocide treatments had little effects on soil health. However, there were some interesting differences across biocides. Insecticide treatment had the highest biomass and MWD, due to less plant herbivory and larger plant belowground root biomass. Molluscicide treatment had the highest root colonization rate and infiltration rate, which suggests it is different from other biocides. Overall, the molluscicide treatment had the highest positive effect on soil health out of all the biocides, most likely due to its solid, pelletized form applied directly on the soil surface. Fungicide and insecticide treatments were foliar applications, which have more interactions with soil-water dynamics. Biocide treatments have also been established for multiple years, which could explain significance in biocide treatments, but not biochar treatments.

Finally, contrary to our expectations, the combination of biochar and biocide treatments did not have any significant interaction between treatment responses. Biomass, infiltration rate, root colonization rate, MWD, and PDA were not significantly affected by biochar and biocide treatment at the same time. We performed multiple statistical tests to ensure the issue was not statistical methodology, but the experimental design could have used more plot replications of biochar within each randomized block. Biocide treatment was applied to four of the subplots, so the addition of biochar to those additional subplots and additional samples could have produced different results.

5. Summary

My work provides insight into the benefits associated with the use of biochar for soil health and whether these benefits hold for biocides. Poultry litter biochar significantly improves AMF colonization rates in little bluestem, a keystone species in tallgrass prairies. Improved plant resource acquisition via AMF networks can improve soil biological health, and in return improve plant community dynamics. Future research into soil amendments should include emphasis on plant and microbe communities, which may alter or impact soil health. The increasing demand for biocides to control biological problems, exasperated by rapid land use change, coincides with an overall decrease in soil carbon across the world's arable soils. Biochar, and other organic soil amendments have continually increased in popularity for land management practices, and our research seeks to fill our understanding of its impacts on soil ecology. Soil types, such as the soil orders present in Central Oklahoma, represent a large population of the country's most agriculturally productive soils, encompassing a global 12% of all arable soils (Anderson and Lafond, 2010). Better understanding of the complicated dynamics between soil chemistry, physics, and biology could provide more opportunities for soil and land conservation. Our experiment therefore serves as a bridge between soil ecology and human impact, which can lead to more restoration initiatives.

6. Appendices

6a. Acknowledgements

I would like to issue a special thank you to my faculty and graduate mentors, Dr. Bachelot, Jonathan, and Nimalka, who helped me conduct my research and fostered my academic growth this past year. I would also like to thank Dr. and Mrs. Niblack for providing me with this scholarship opportunity, because none of this would be possible without your generosity. Lastly, I would like to thank my father for helping me construct my biochar barrel system for my project, and for inspiring me to pursue scientific research. None of this would be possible without everyone's help!

6b. Papers Published

Friend, MaKayla. Oklahoma Academy of Science Oral Poster Presentation

Friend, MaKayla. Oklahoma State University Undergraduate Research Symposium Oral Poster Presentation

6c. Literature Cited

Anderson, D., and Lafond, G. 2010. Global Prospective on Arable Soils and Major Soil Associations. *Prairie Soil & Crops Journal*. 3:1-8.

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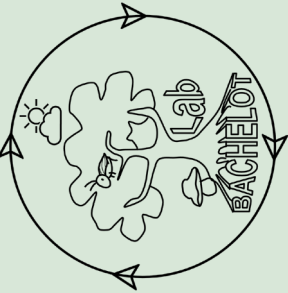
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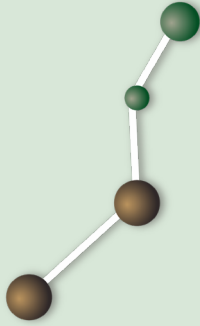
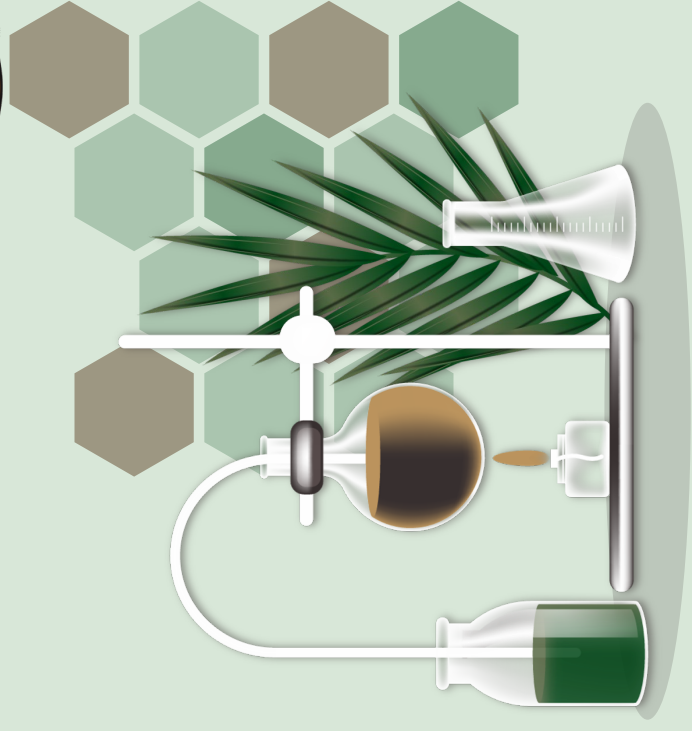
Niblack Presentation



By MaKayla Friend *B.S. in Environmental
Science, M.S. Student in Plant and Soil Sciences*

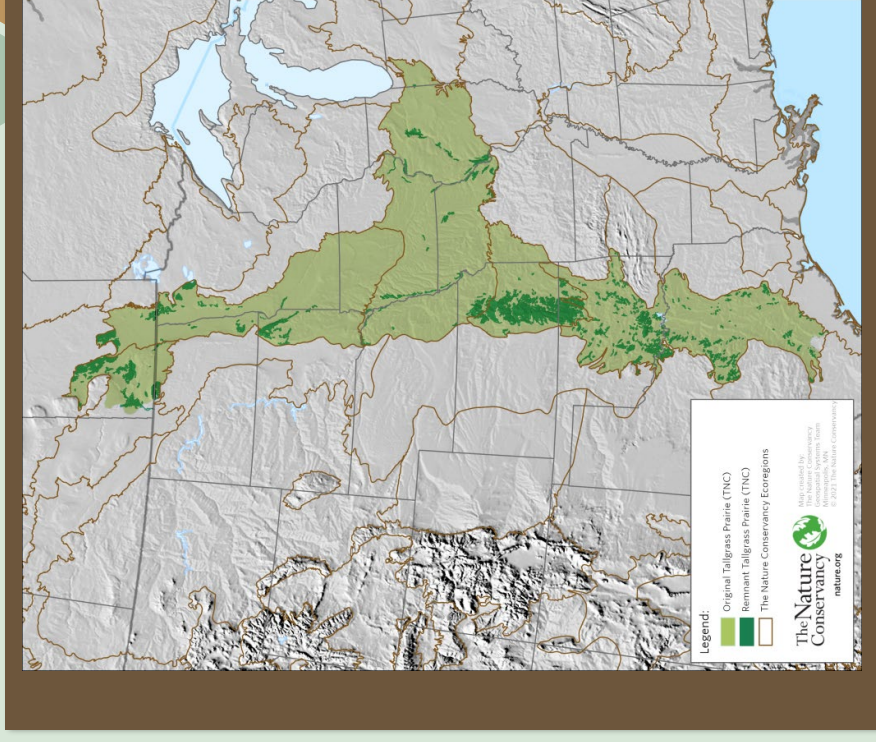
Faculty Mentor: Dr. Benedicte Bachelot

Graduate Student Mentors: Jonathan Medellin
and Nimalka Weerasuriya

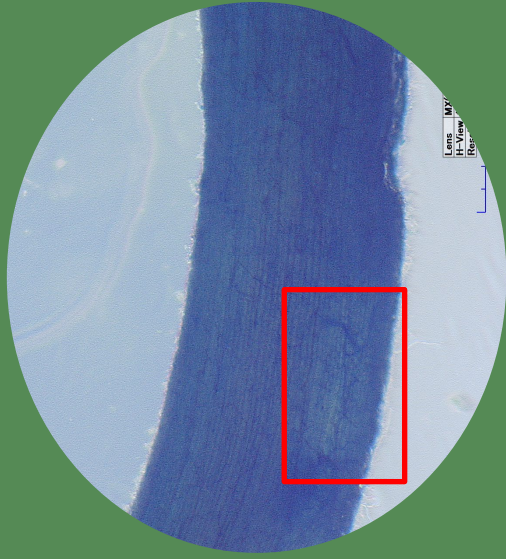


Tallgrass Prairies

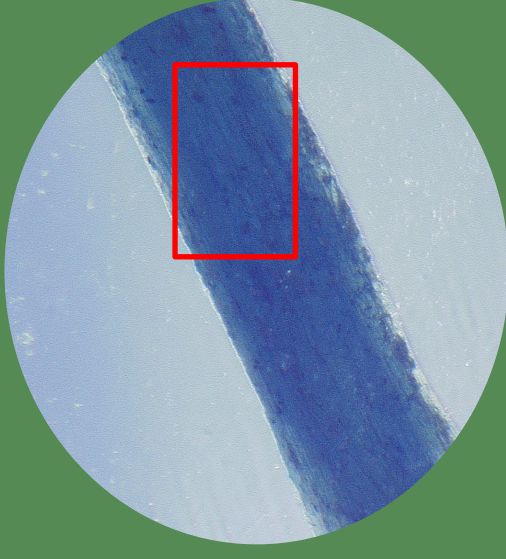
- Dominated by grasses, shrubs, and forbs
- Less than 4% of tallgrass prairies remain intact as functioning ecosystems
- Mesic temperature and low rainfall



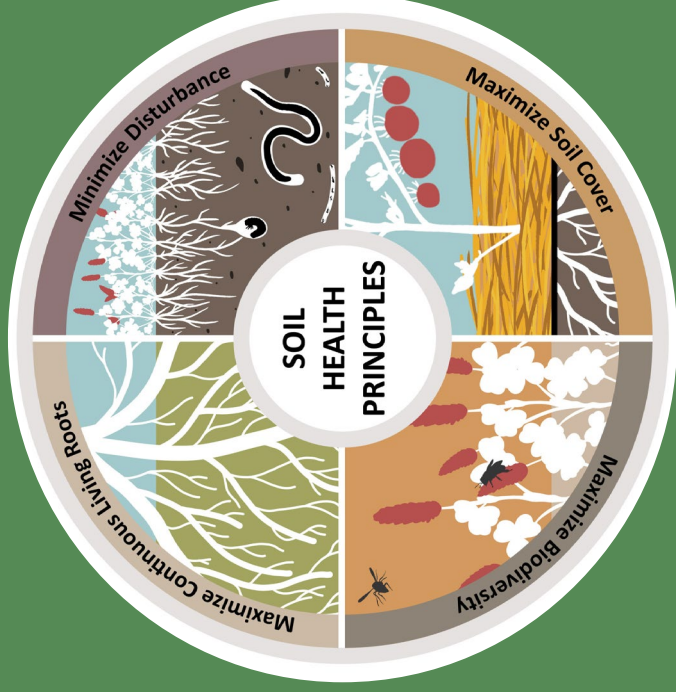
Healthy Soils are Living



Arbuscular
Mycorrhizal
Fungi (hyphae)



Arbuscular
Mycorrhizal Fungi
(arbuscules)

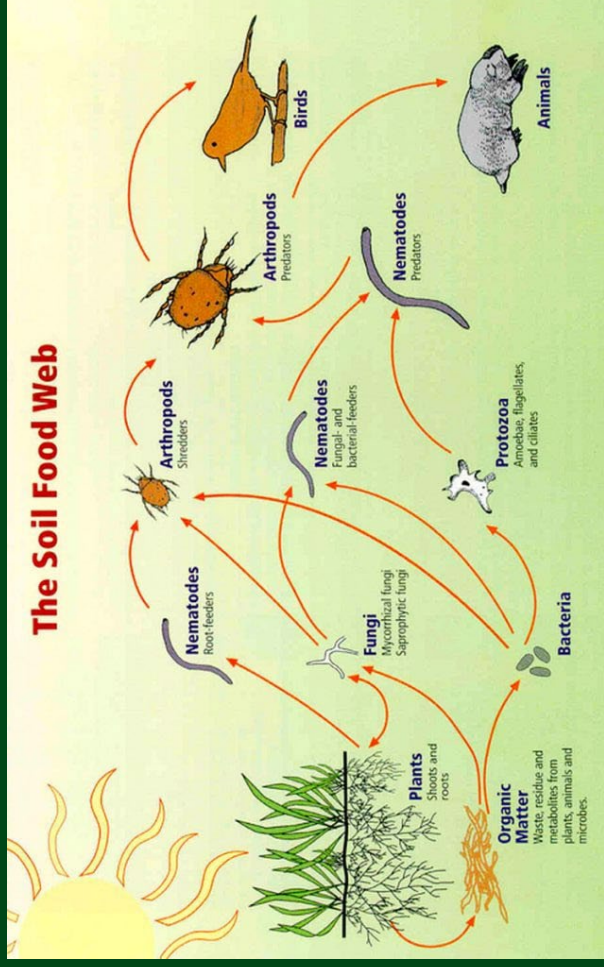


Biocide and Biochar

- Biochar is proposed to increase soil health
- Insecticide, fungicide, and molluscicide are used to lower predator abundance and increase forage quality



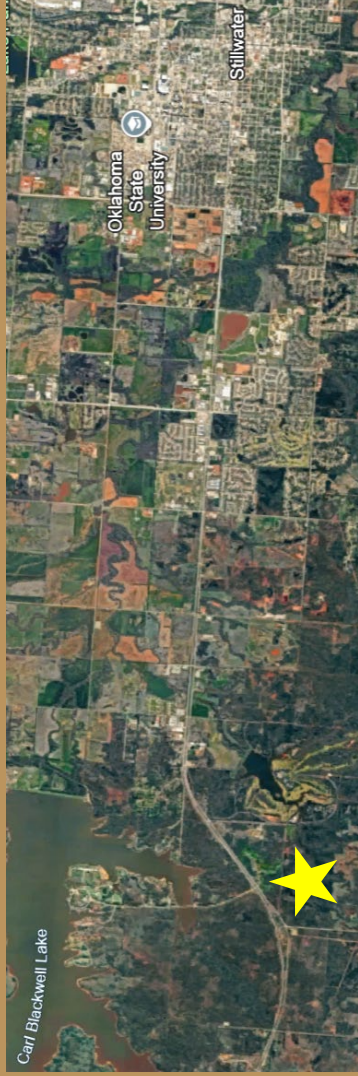
B1 biochar



B2 biochar

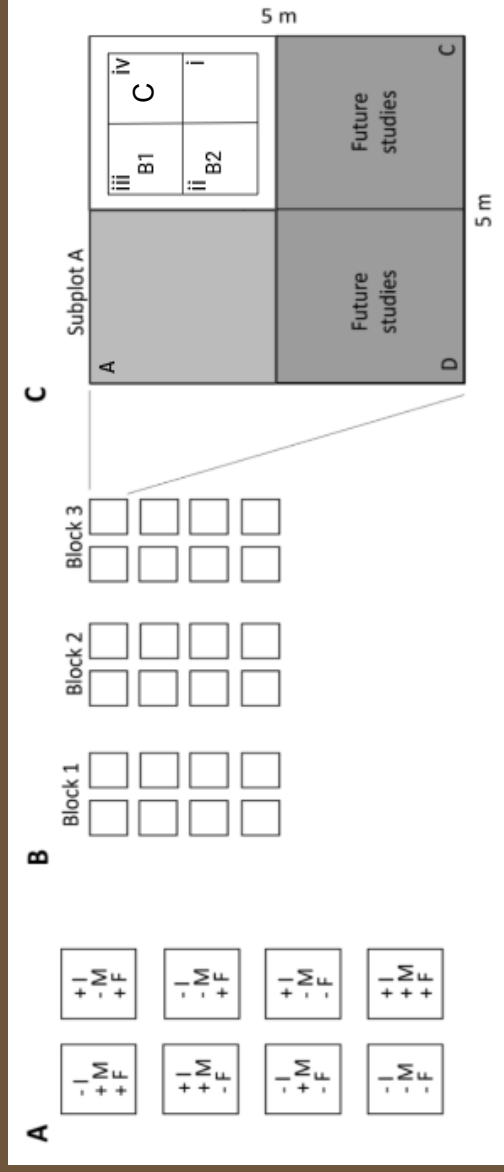
McPherson Preserve

- 200 acres of remnant tallgrass prairie ecosystem
- Serves as an experimental site for BugNet (international scientific collaboration network)

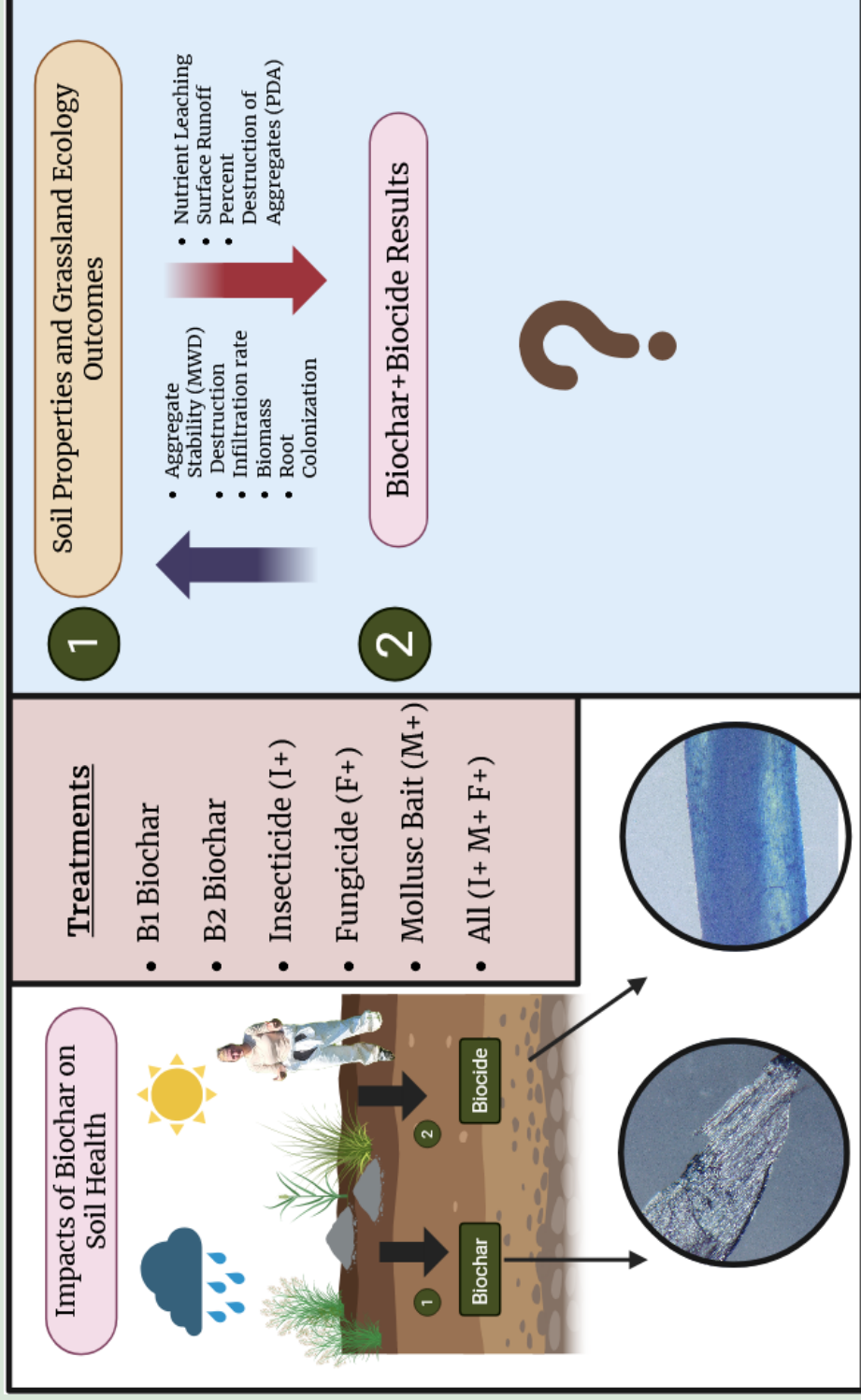


BugNet

- Operating at McPherson since 2022
- Randomized block design with 2 subplots dedicated to biochar
- Collect environmental samples and plant community data during growing season (May - August)



Goals and Objectives



Biomass Production and Pyrolysis

B1 (BSG) Biochar



B2 (Poultry Litter) Biochar

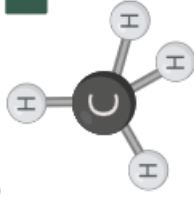


Inner Configuration

Outer Configuration



Byproducts and Applications



Biochar, bio-oils, and gases

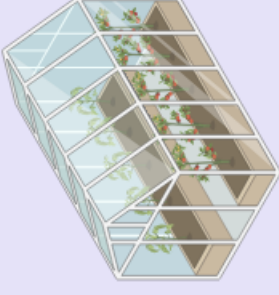
Soil fertility



Soil health



Greenhouse Production



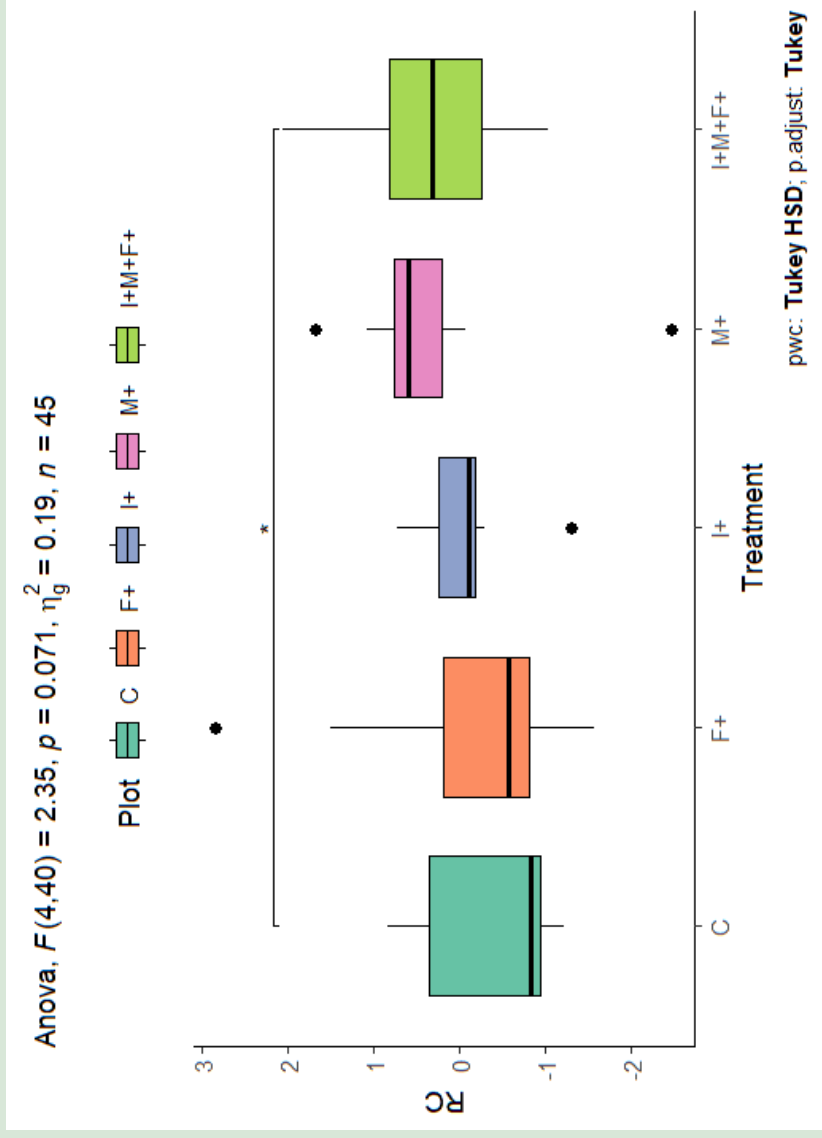
Environmental Sampling

- Double-Ring Infiltrrometer to assess rate of infiltration
- 3 individuals (3 replicates) of *Schizachyrium scoparium* roots, stained with Trypan blue and analyzed using Palmer cell counting method to assess root colonization rate



Biocides increase root colonization

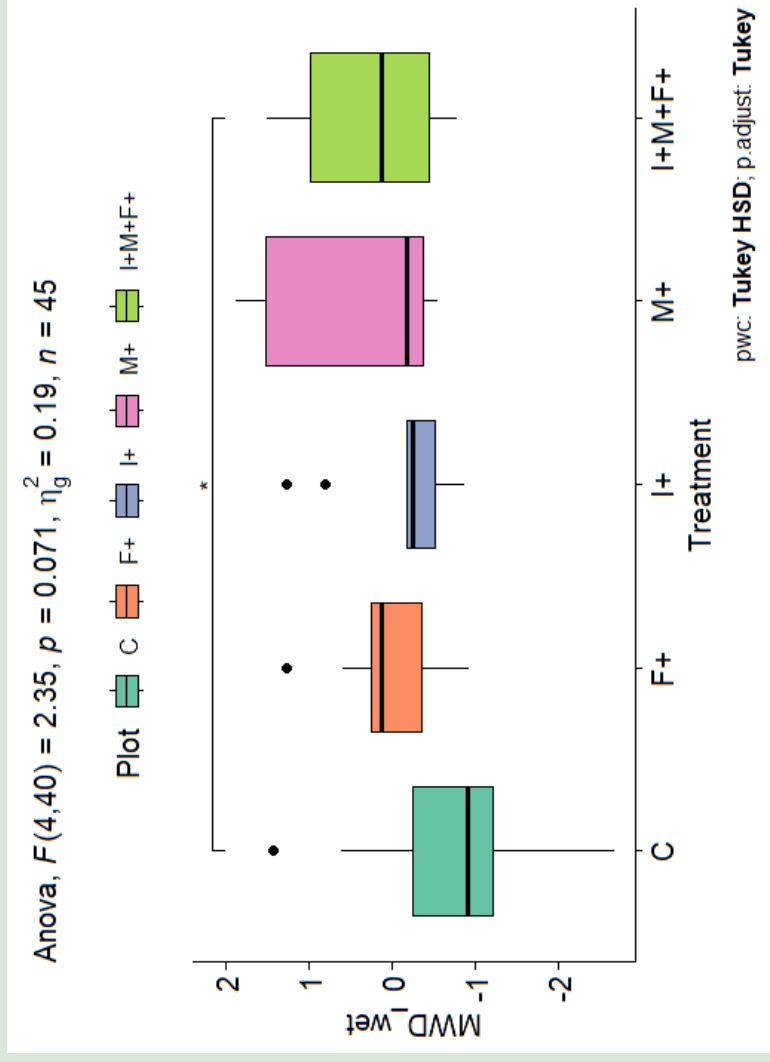
- Root Colonization was highest in M+ treatment, small herbivories lower mycorrhizal colonization



“Herbivory of an invasive slug in a model grassland community can be affected by earthworms and mycorrhizal fungi” Trounev, R, et al.

Enemy suppression increases aggregation

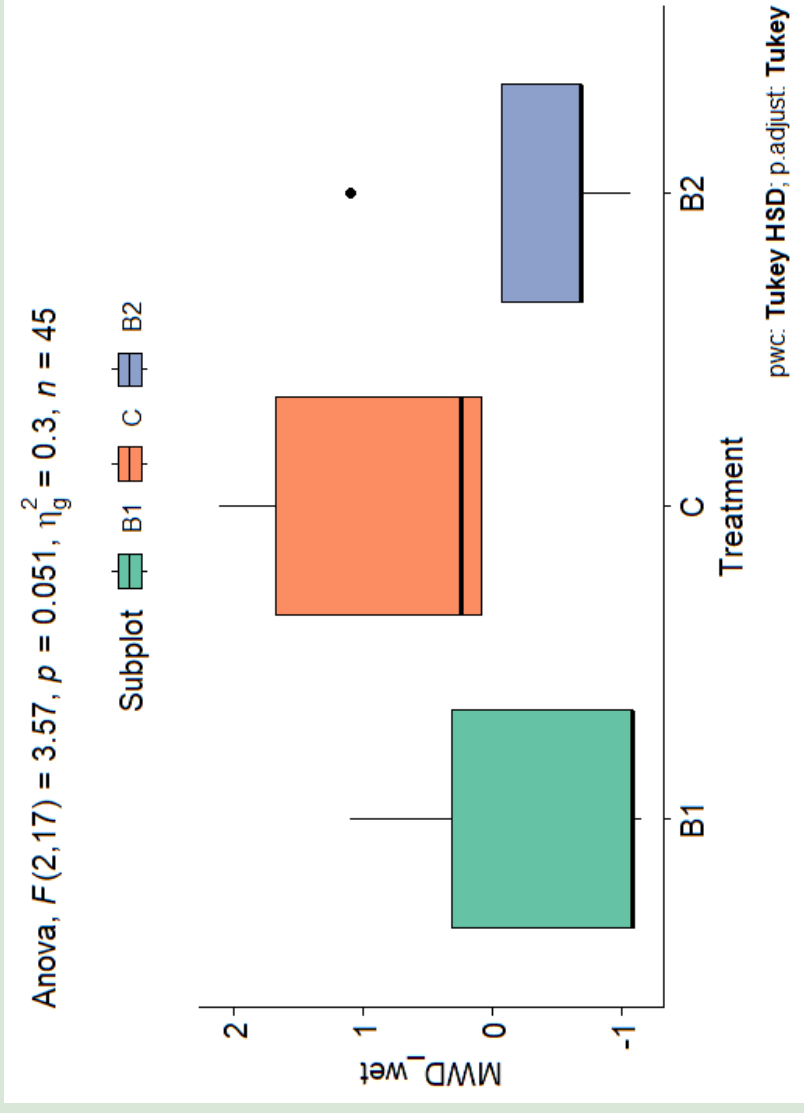
- Wet Mean Weight Diameter highest in F+ treatment, fungal pathogens could reduce beneficial soil-forming fungi



“Enhancement of soil aggregation and physical properties through fungal amendments under varying moisture conditions” Angulo, V, et al.

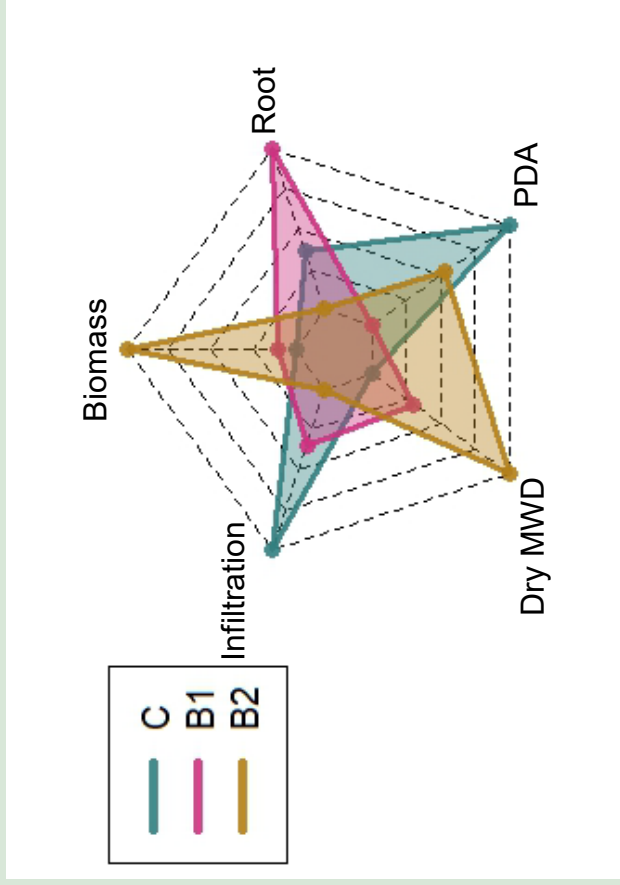
Biochar conserves soil water

- Short infiltration rate highest in control, biochar increases short-term soil water conservation

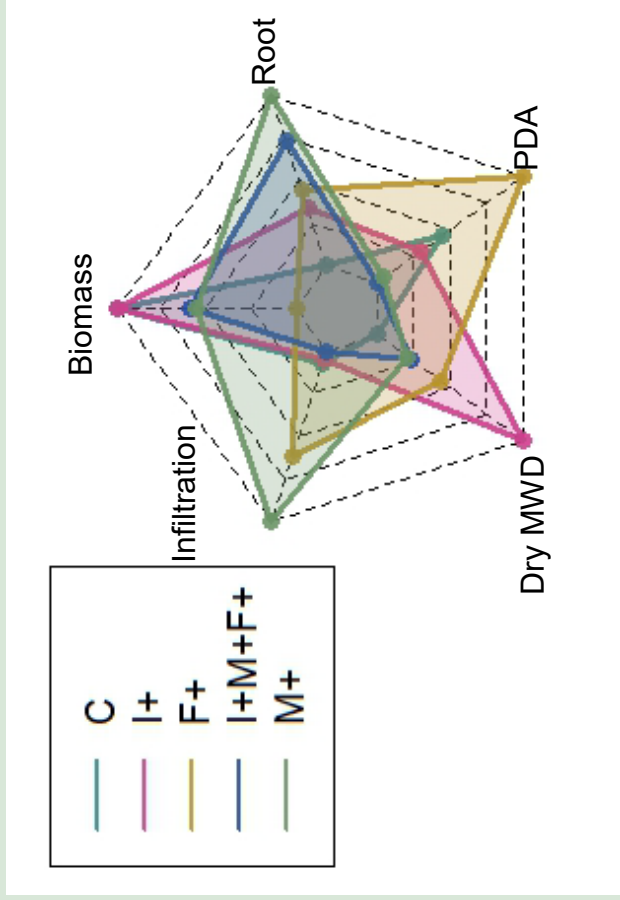


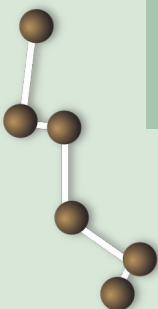
“Evaluating the Impact of Combined Application of Biochar and Compost on Hydro - physical Properties of Loamy Sand Soil” Al - Omran, A, et al.

Summary spiderplots



B1 biochar- spent grains
B2 biochar- poultry litter





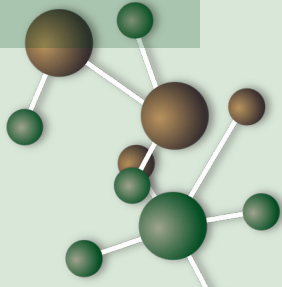
Conclusion



- Biochar does impact soil water conservation during short - term infiltration
 - Biocide does impact aggregate size under water stress and AMF colonization rate
 - No significance or trend across biochar and biocide interactions
- 

Future Directions

- Ferrous biochar surface chemistry (future research)
- Timespan, ageing, and application rate of treatments
- Method of application, homogenization, and pyrolysis rates



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Dr. Niblack and Mrs. Niblack

Faculty Mentor: Dr. Bachelot

Graduate Mentors: Nimalka Weerasuriya and Jonathan Medellin

My Lab Group: Sarah Cope, Elizabeth Merkov, Cassidy Brandt



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Haloalkylation of Arylcyclohexenes via Carbenium Ion Formation: Application of Light-Mediated Transducer Catalysis

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Faculty Sponsor: Dr. Jimmie D. Weaver

Graduate Student Mentor: Osaid Alkhamayseh

ABSTRACT

This project investigates the use of visible-light-mediated photoisomerization of arylcyclohexenes to generate strained *trans*-cyclohexene intermediates that can be used for haloalkylation of arylcyclohexene frameworks. My primary role was to synthesize arylcyclohexene starting materials and support optimization and scope studies for the haloalkylation reaction. Optimization identified 0.25 mol% Ir(dFppy)₃, 20 mol% collidine•HPF₆, dichloromethane, blue light, room temperature, and an argon atmosphere as effective conditions. Scope studies showed that electron-withdrawing aryl substituents on the cyclohexene generally increased product formation, whereas electron-donating groups and some expanded-ring substrates reduced productive haloalkylation. The halogenated-substrate study also revealed a selectivity trend of Br > Cl >> F. Overall, these results support a mild visible-light approach for installing polyhaloalkyl units onto arylcyclohexene frameworks and provide a foundation for expanded substrate scope, product characterization, and mechanistic studies. Beginning in January 2026, I transitioned from experimental work on the haloalkylation project to contributing to an invited Chemical Review article focused on the photoisomerization of cycloalkenes. This review examines the historical development, mechanistic basis, and synthetic applications of light-mediated *cis/trans* cycloalkene photoisomerization. My work on the review helped me understand the broader context of light-mediated transducer catalysis, where photonic energy is converted into molecular strain and then used to promote productive chemical reactivity.

1. Introduction

Cyclic alkenes are useful platforms for studying the conversion of light energy into chemical strain energy. In the Weaver group, *cis*-arylcyclohexenes are used as molecular transducers that convert photonic energy into molecular strain energy. After visible-light excitation of a photocatalyst, triplet energy transfer can convert a lower-energy *cis*-cycloalkene into a highly strained *trans*-cycloalkene. The existence, structure, and unusual strain of *trans*-cyclohexene derivatives has been investigated for decades, and prior studies report that these intermediates are short-lived but chemically useful when generated under controlled photochemical conditions.¹⁻⁶

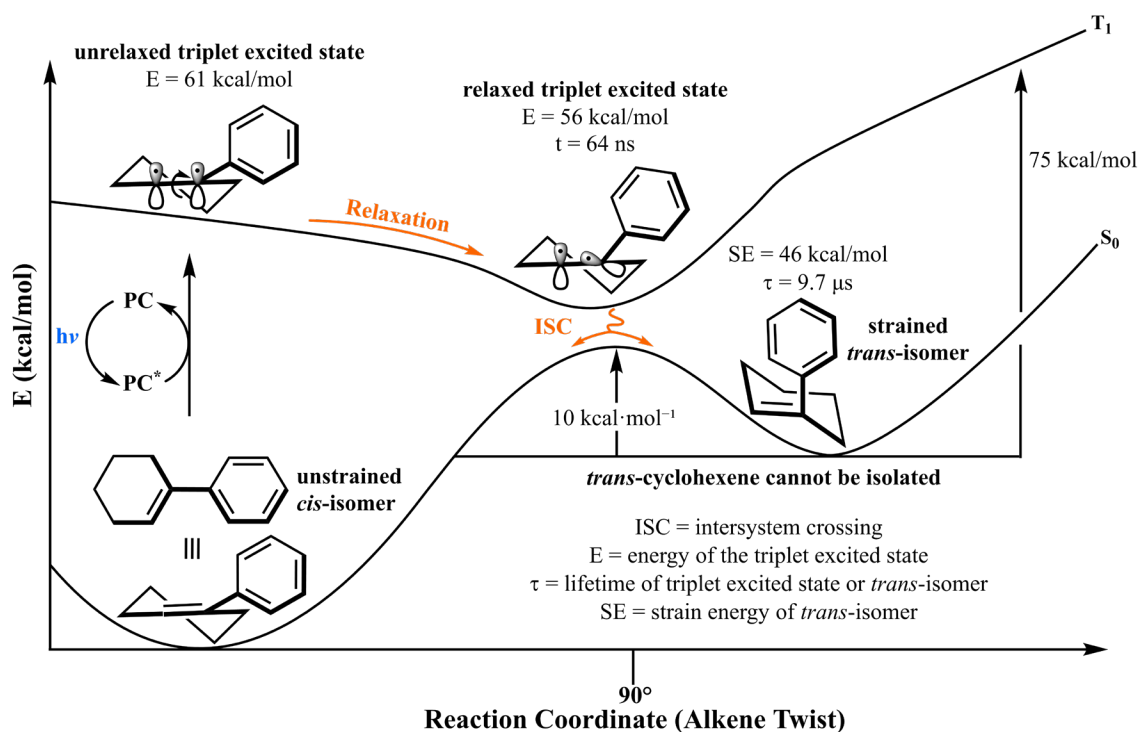


Figure 1. Photoisomerization energy diagram showing conversion of *cis*-phenylcyclohexene to the strained *trans* intermediate and the energetic basis for strain-release reactivity.

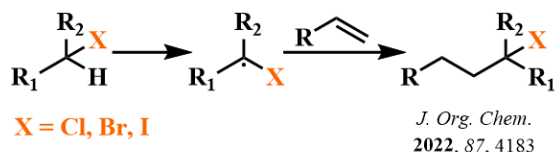
The central advantage of this platform is that the *trans* isomer stores enough strain energy to undergo reactions that are not normally accessible from the *cis* isomer. Visible-light approaches developed by the Weaver group have shown that *trans*-arylcyclohexenes can be generated under mild conditions and then used in ionic additions, including bridged ether formation, alcohol protection, CO₂ valorization, and Friedel-Crafts alkylation.⁷⁻¹¹ This strategy is significant because classical carbenium ion chemistry often relies on strongly acidic media, whereas this approach uses visible-light-mediated formation of strained *trans*-cyclohexene intermediates to access carbenium ions under remarkably mild conditions.¹²

The goal of my Niblack project was to help develop a related haloalkylation reaction using photoisomerized arylcyclohexenes and halogenated substrates. Polyhaloalkyl motifs are valuable because they can serve as versatile handles for further functionalization, but many existing approaches rely on radical generation through C–H or C–X bond cleavage (**Scheme 1A and B**). Those radical pathways are powerful but can suffer from selectivity challenges because haloalkyl radicals are highly reactive intermediates.^{13,14} Our project tests whether the light-generated *trans*-arylcyclohexene platform can provide a complementary route to polyhaloalkylated arylcyclohexenes under mild photochemical conditions (**Scheme 1C**).

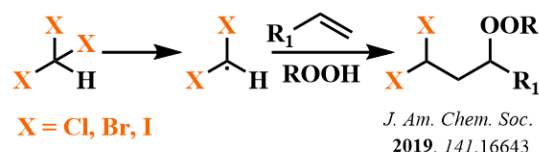
Scheme 1. Project background and rationale, including previous radical methods for installing polyhaloalkyl units and the light-driven arylcyclohexene platform

Methods to Introduce Polyhaloalkyl Units into Organic Molecules:

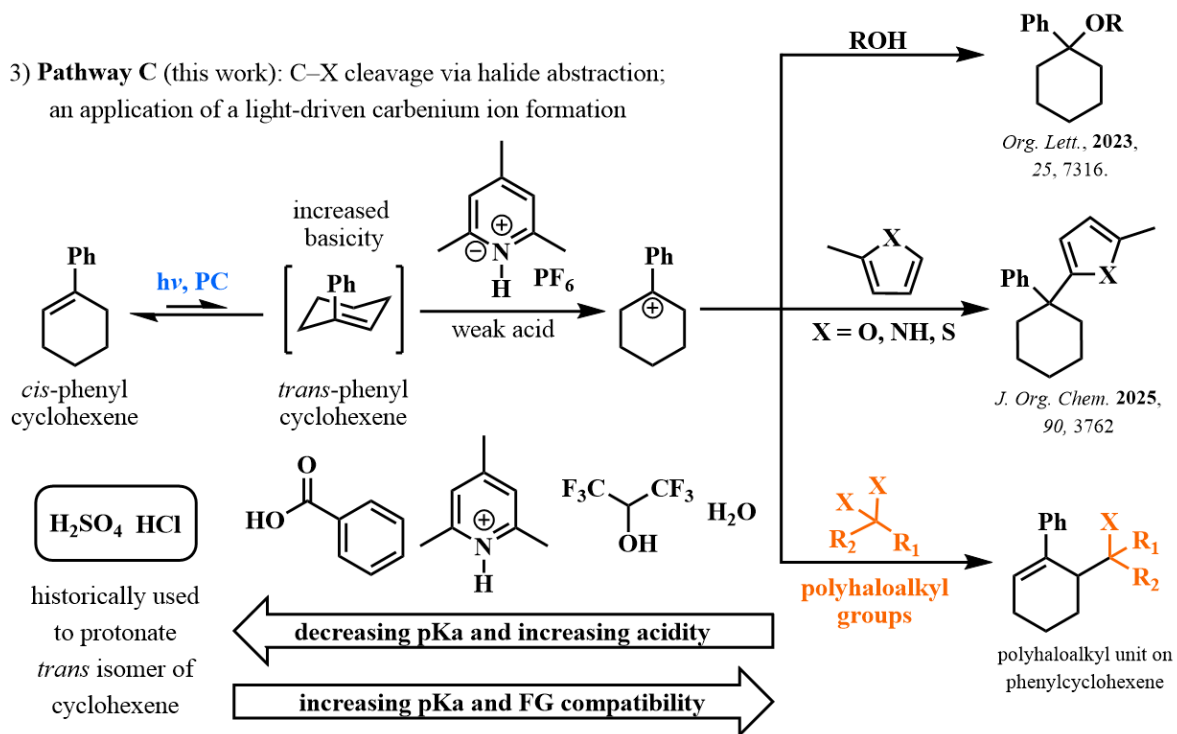
1) **Pathway A:** Breaking C–H Bond (Well Explored)



2) **Pathway B:** Breaking C–X Bond (Well Explored)



3) **Pathway C** (this work): C–X cleavage via halide abstraction;
an application of a light-driven carbenium ion formation



2. Experimental Details

The arylcyclohexene starting materials used in the photochemical studies were prepared through a two-step synthetic sequence. First, an aryl bromide was added to a cyclohexanone derivative by Grignard addition to form the corresponding tertiary alcohol. The tertiary alcohol was then converted to the desired arylcyclohexene through acid-catalyzed dehydration under Dean-Stark conditions. Each arylcyclohexene was purified via column chromatography before use. Photochemical reactions were performed on approximately 0.253 mmol scale using an arylcyclohexene and a halogenated substrate in dichloromethane or chlorobenzene under an argon atmosphere. Blue LED irradiation was used to activate the iridium photosensitizer and collidine•HPF₆ was the weak acid used for carbenium ion formation. Both components of this salt are important: collidine is sterically hindered enough to avoid trapping the carbenium ion after proton transfer, while PF₆[−] is a weakly coordinating, non-nucleophilic counterion that does not compete with the desired halogen-abstraction pathway. For example, pyridinium•PF₆ would generate less hindered pyridine, which could

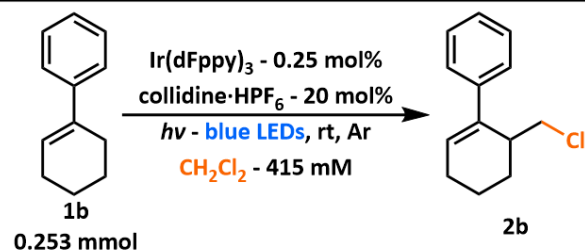
attack the carbenium ion, while collidine•HCl would introduce chloride, a more nucleophilic counterion that could also interfere with productive reactivity. Optimization experiments varied photocatalyst loading, acid loading, solvent composition, substrate concentration, water content, and temperature. Reaction progress and yields were evaluated primarily by NMR analysis, while product structures in the substrate-scope studies were supported by ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and GC(EI) analysis when available.

Beginning in January 2026, my role shifted from lab work on the haloalkylation project to literature-based work on a review paper focused on cycloalkene photoisomerization. The purpose of this review is to organize decades of work on how *cis*-cycloalkenes are converted into strained *trans*-cycloalkenes and to explain how the field has progressed from direct UV irradiation to milder sensitized and visible-light-mediated approaches. For this portion of the project, I read and interpreted primary literature on UV photoisomerization, excited state energy transfer, ring-size effects on *trans*-cycloalkene strain, and synthetic applications of strain-loaded intermediates. This work required me to connect fundamental photochemical principles to modern applications in light-mediated transducer catalysis. ChemDraw schemes, figures, and tables were prepared to represent key ideas from the text, strengthening my ability to translate complex photochemistry concepts into clear visual figures. Through this work, I became more proficient in literature searching, evaluating primary sources, using ChemDraw, and scientific writing. Ultimately, this review gave me a clearer understanding of how my haloalkylation project fits into the broader development of photochemical strategies that convert light energy into molecular strain and use that strain to drive new reactivity.

3. Results

An optimization study was conducted to identify reaction conditions that maximized productive haloalkylation. Under the optimized conditions, the desired product was observed in 60% NMR conversion after 4.3 h and 86% after 21 h. Increasing the collidine•HPF₆ loading to

Table 1. Optimization study for visible-light-mediated arylcyclohexene haloalkylation

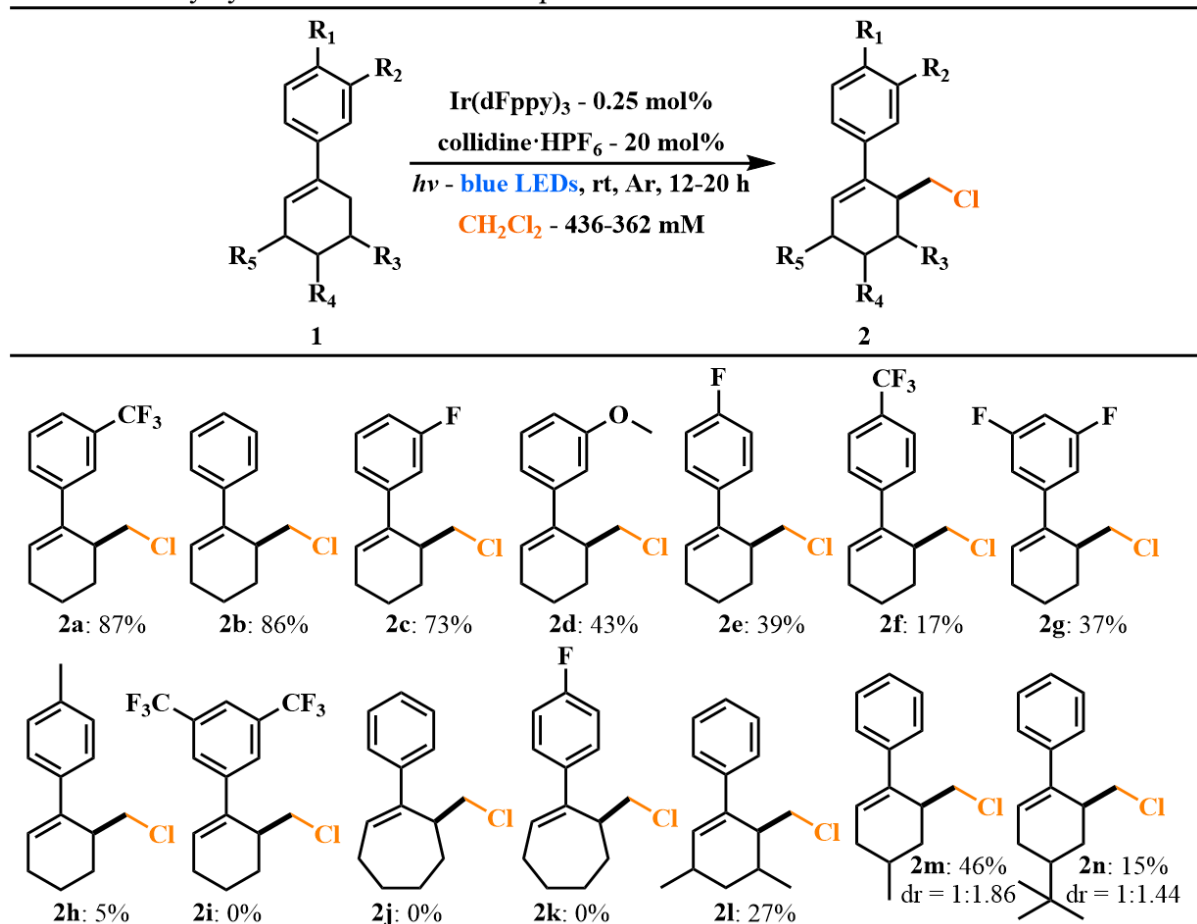


Entry	Modification of above reaction	NMR conversion (%)	
		4.3 h	21 h
1	None	60	86
2	100 mol % coll.HPF ₆	5	18
3	5 mol % coll.HPF ₆	0	12
4	33 equiv water	11	28
5	DCM/DCE (50V:50V)	3	41
6	DCM/DCE (25V:75V)	0	10
7	208 mM of SM	22	35
8	104 mM of SM	11	16
9	50 °C	0	3
10	70 °C	0	0
11	0.125 mol % PC	37	21
12	1 mol % PC	43	0

100 mol% strongly suppressed product formation, while lowering the salt loading to 5 mol% also reduced reactivity. The lower conversion at 5 mol% may reflect the deleterious effect of trace water on the reaction system, where 20 mol% collidine·HPF₆ is sufficient to overcome small amounts of water present, but 5 mol% is not. Adding water, changing solvent ratio, varying substrate concentration, increasing temperature, or changing photocatalyst loading generally reduced conversion relative to the optimized condition.

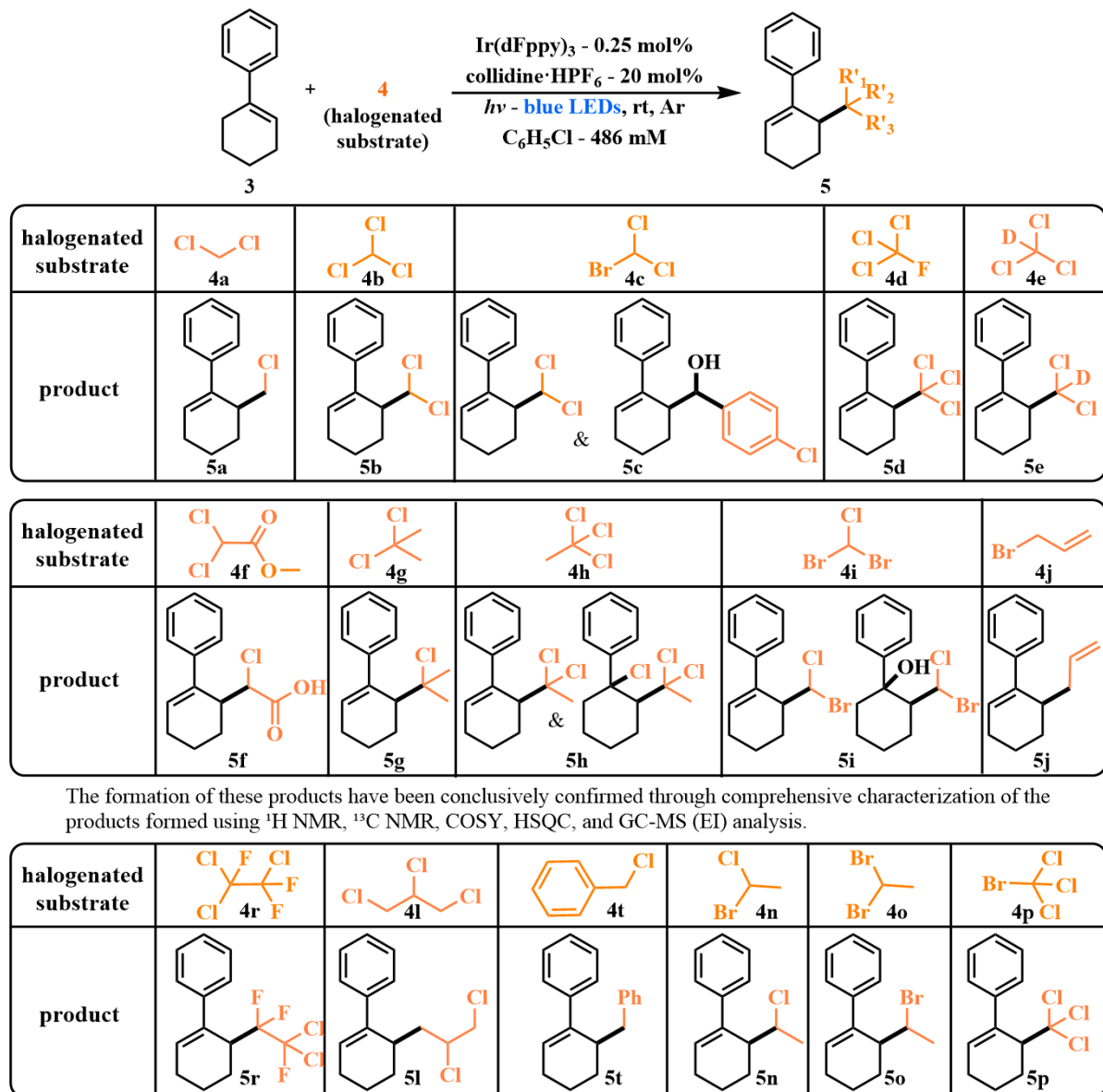
The arylcyclohexene scope revealed a clear substituent effect. A *m*-CF₃ substrate and the parent phenylcyclohexene gave high NMR yields, whereas electron-donating and highly deactivating substitution patterns were less effective. For example, the *m*-CF₃ substrate gave 87% yield, the parent substrate gave 86%, and the *m*-F substrate gave 73%. In contrast, the *m*-OMe substrate gave 43%, the *p*-CF₃ substrate gave 17%, and the methyl-substituted substrate gave 5%. The bis-CF₃ substrate did not produce the desired product likely due to excessive carbenium-ion destabilization caused by the two strongly electron-withdrawing substituents. Cycloheptene-derived substrates did not provide productive haloalkylation under the screened conditions. Our hypothesis is that the corresponding *trans*-cycloheptene intermediates contain less strain energy than *trans*-cyclohexenes, making them less basic and therefore less susceptible to protonation by the relatively weak acid present under the reaction conditions.

Scheme 2. Arylcyclohexene substrate scope



The halogenated-substrate scope expanded the reaction beyond a single halogen donor and allowed preliminary analysis of halide selectivity. Products from chlorinated, brominated, fluorinated, deuterated, allylic, and carbonyl-containing substrates were either confirmed or tentatively assigned by available NMR and GC(EI) data. The most important empirical trend was the halogen abstraction selectivity in the order Br > Cl >> F. This trend provides a practical design rule for future substrate selection, although the mechanism responsible for the observed selectivity still requires further investigation.

Scheme 3. Halogenated-substrate scope



The formation of these products have been conclusively confirmed through comprehensive characterization of the products formed using ^1H NMR, ^{13}C NMR, COSY, HSQC, and GC-MS (EI) analysis.

These substrates are still under investigation to confirm product formation. Based on crude ^1H NMR data, there are indications that the desired product is being formed.

4. Discussion and Conclusions

The optimization and substrate-scope data show that the haloalkylation reaction is sensitive to reaction conditions, aryl substitution, aliphatic ring structure, and the identity of the halogenated substrate. Electron-withdrawing substituents generally improved productive reactivity, provided that they were not so extreme as to prevent protonation, whereas electron-donating groups and expanded-ring substrates were less effective. The halogen-selectivity trend, $\text{Br} > \text{Cl} \gg \text{F}$, was especially useful because it provides a practical design rule for future reactions with mixed-halogen substrates. Overall, these results are best interpreted as structure–reactivity relationships that support the involvement of strained photoisomerized intermediates and carbenium-ion reactivity, although the detailed mechanism responsible for product formation and selectivity, as well as the high observed selectivity in the regioselectivity of the resulting alkene all still requires further study. Compared with established radical approaches to polyhaloalkylation, this method offers a complementary visible-light pathway for accessing polyhaloalkylated arylcyclohexenes under mild conditions and challenges the prevailing dogma about utility of carbenium ion chemistry.^{13,14}

My transition into the review-paper portion of this project was valuable because it connected the haloalkylation results to the larger field of cycloalkene photoisomerization. Our review can serve as a useful resource for researchers interested in applying cycloalkene photoisomerization to new reactions. This work is significant because it brings together scattered literature and highlights how light can be used to generate strain-loaded intermediates that provide potential energy that enables productive chemical reactivity. In doing so, the review has the potential to guide future reaction design, help researchers identify gaps in the field, and support the continued development of light-mediated transducer catalysis. Beyond the chemistry itself, contributing to this review strengthened my ability to evaluate primary literature, prepare ChemDraw schemes, and communicate photochemical concepts in a clear scientific fashion.

Future work should focus on improving product isolation, expanding the halogenated-substrate scope, and testing whether the $\text{Br} > \text{Cl} \gg \text{F}$ selectivity trend holds across broader substrate classes. Targeted experiments such as Stern–Volmer quenching, quantum-yield measurements, isotope-effect studies, trapping experiments, and competition experiments would help clarify the factors responsible for product formation and selectivity. The method should also be tested on more complex targets to evaluate its synthetic utility.

5. Summary

This Niblack project developed a visible-light-mediated approach for using arylcyclohexenes in halogen abstraction from halogenated compounds. My experimental role was to synthesize arylcyclohexene starting materials and support optimization and scope studies. The optimized conditions used low photocatalyst loading, collidine•HPF₆, dichloromethane, blue LEDs, room temperature, and argon. Electron-withdrawing aryl substituents generally improved reaction efficiency, while electron-donating groups and expanded-ring substrates were less effective. The substrate study showed halide selectivity in

the order Br > Cl >> F. After my experimental work on this project ended in January 2026, I continued contributing to the broader research direction through work on a Weaver Lab review paper about cycloalkene photoisomerization and light-mediated transducer catalysis. Together, these experimental and literature-based contributions establish a foundation for mild, visible-light polyhaloalkylation of arylcyclohexenes while leaving the detailed product-forming mechanism to future targeted experiments.

6. Appendices

6a. Acknowledgements

I gratefully acknowledge Dr. and Mrs. Niblack and the Niblack Research Scholars Program for supporting undergraduate research and making this work possible. I thank Dr. Jimmie Weaver for serving as my faculty sponsor and for his mentorship, Osaid Alkhamayseh for graduate mentorship and day-to-day guidance in the laboratory, and the Weaver Lab for their continued support and feedback throughout this project. I also thank Keaton Greenhaw for working alongside me in the synthesis of several arylcyclohexene starting materials used in this project. I acknowledge the OSU Department of Chemistry and financial support from the National Institutes of Health through NIH NIGMS grant R35GM139613.

6b. Papers Published

No papers have been published from this work as of the date of this report. The review article on cycloalkene photoisomerization is in preparation and will be submitted in the coming weeks, but because it is not yet published, it is not listed here as a published paper.

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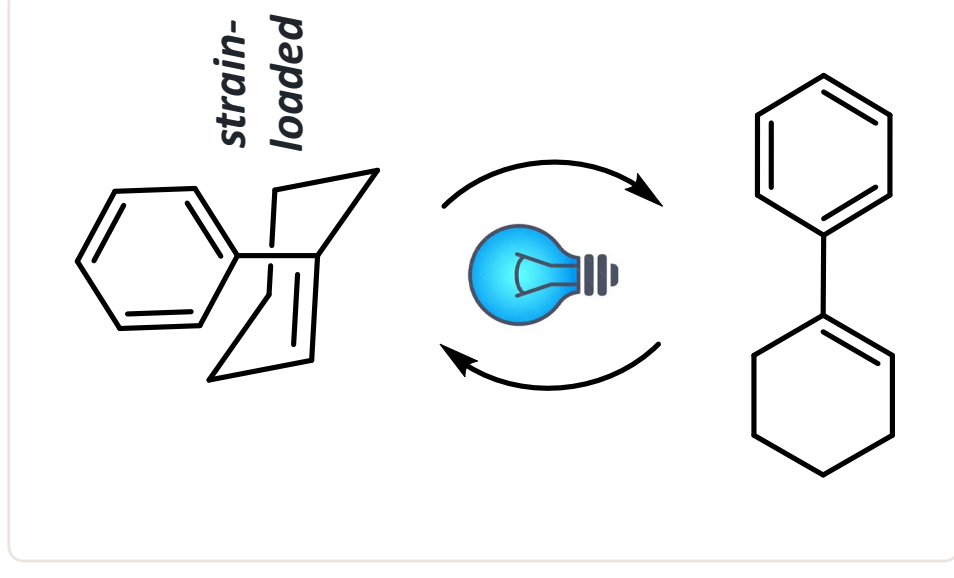
Haloalkylation of Arylcyclohexenes via Carbenium Ion Formation

Application of Light-Mediated Transducer Catalysis

Omar Ameen

Department of Chemistry • Oklahoma State University

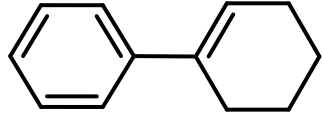
Faculty Sponsor: Dr. Jimmie D. Weaver
Graduate Mentor: Dr. Osaid Alkhamayseh



Using Light to Transduce Energy into Molecular Strain

The core idea behind light-mediated transducer catalysis

1. Ground-state alkene



ring comfortably accommodates the *cis* alkene

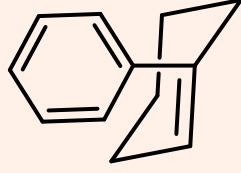
Cis-phenylcyclohexene is relatively low in energy in its low-strain, nonreactive configuration.

cis-cyclohexene

$h\nu + \text{PC}$



2. Strain-loaded *trans* isomer



ring strains to accommodate the *trans* alkene

strain energy: ~46 kcal/mol
 ≈ 1.4 kcal/mol $\rightarrow$ 10 $\times$ equilibrium shift at rt

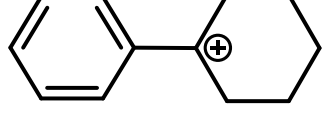
Triplet energy transfer converts the alkene into a strained *trans*-isomer.

trans-cyclohexene

acid (H^+)



3. Strain-release reactivity



ring strain is released through chemical reactivity

Releasing that stored energy can open reaction pathways that are difficult from the normal *cis* alkene.

new reactivity

LIGHT $\rightarrow$ MOLECULAR STRAIN $\rightarrow$ CHEMICAL REACTIVITY

Research Question

Can strain give access to carbenium ions under unusually mild conditions?

THE CHALLENGE

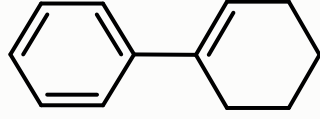
Carbenium ions are powerful synthetic intermediates.

But accessing them often relies on strongly acidic or otherwise harsh conditions. At the same time, alkyl halides are useful synthetic handles but activating their C–X bonds often relies on highly reactive radical pathways.

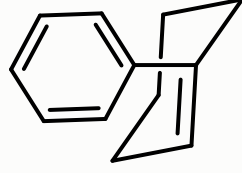
Question:

Can strain-induced carbenium ions promote halide abstraction from alkyl halides to enable a mild haloalkylation method?

OUR PLATFORM



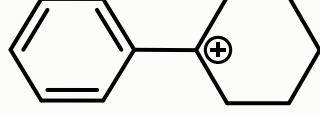
hν, PC



(+H⁺)



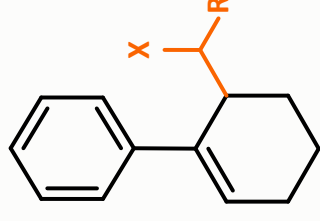
weak acid



halogen abstraction



alkyl halide



X = F, Cl, Br

haloalkylated product

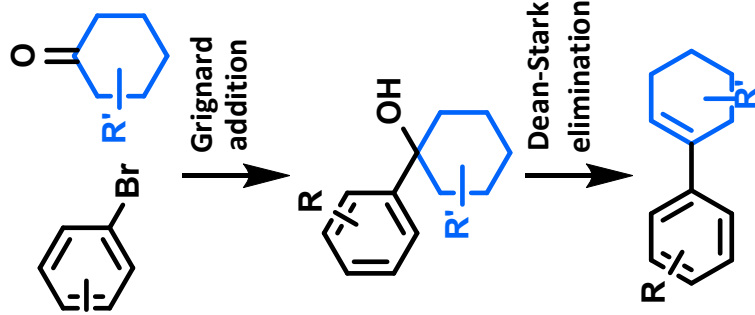
Objectives

- Generate carbenium ions under mild, strain-driven conditions.
- Develop a haloalkylation method using alkyl halides and arylcyclohexenes.
- Determine how substrate structure influences chemical reactivity and selectivity.

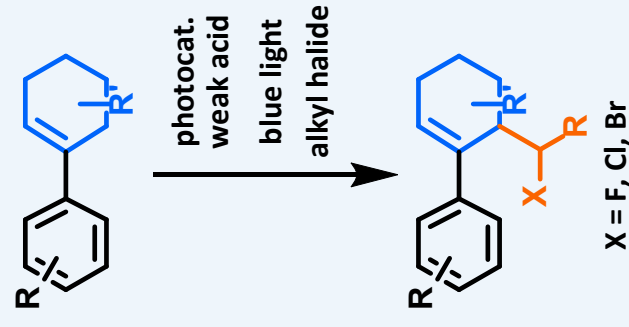
What I Did

From starting-material synthesis to reaction analysis

1. Build the substrates



2. Run photochemistry



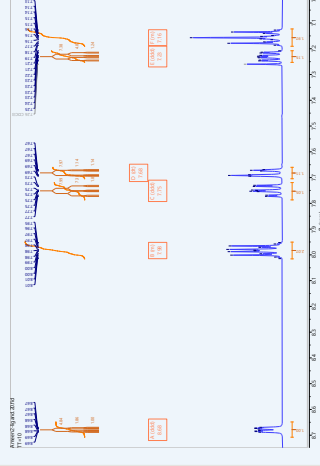
3. Optimize + scope

- Optimize conditions:**
- photocatalyst loading
 - acid loading
 - solvent
 - concentration
 - temperature

- Explore scope:**
- aryl substituents
 - aliphatic substituents
 - alkyl halide structure

4. Analyze data

- Analyze by NMR spectroscopy + GC-MS to:**
- quantify yield
 - elucidate product structures
 - compare substrate reactivity
 - identify selectivity trends



My role: substrate synthesis • optimization • scope studies • reaction analysis

Optimization of Reaction Conditions

Small changes in conditions often sharply reduced productive haloalkylation

OPTIMIZED CONDITIONS

86%

yield at 21 h

- Ir(dFppy)₃ • 0.25 mol%
- collidine·HPF₆ • 20 mol%
- CH₂Cl₂ • blue LEDs • rt • Ar

Selected perturbations

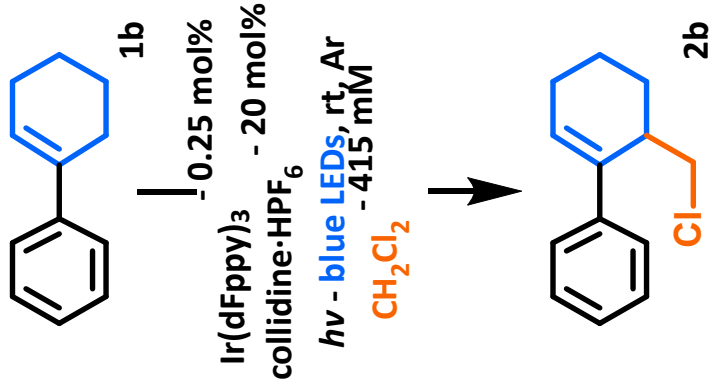
100 mol% acid **18%**

+ H₂O

28%

50 °C

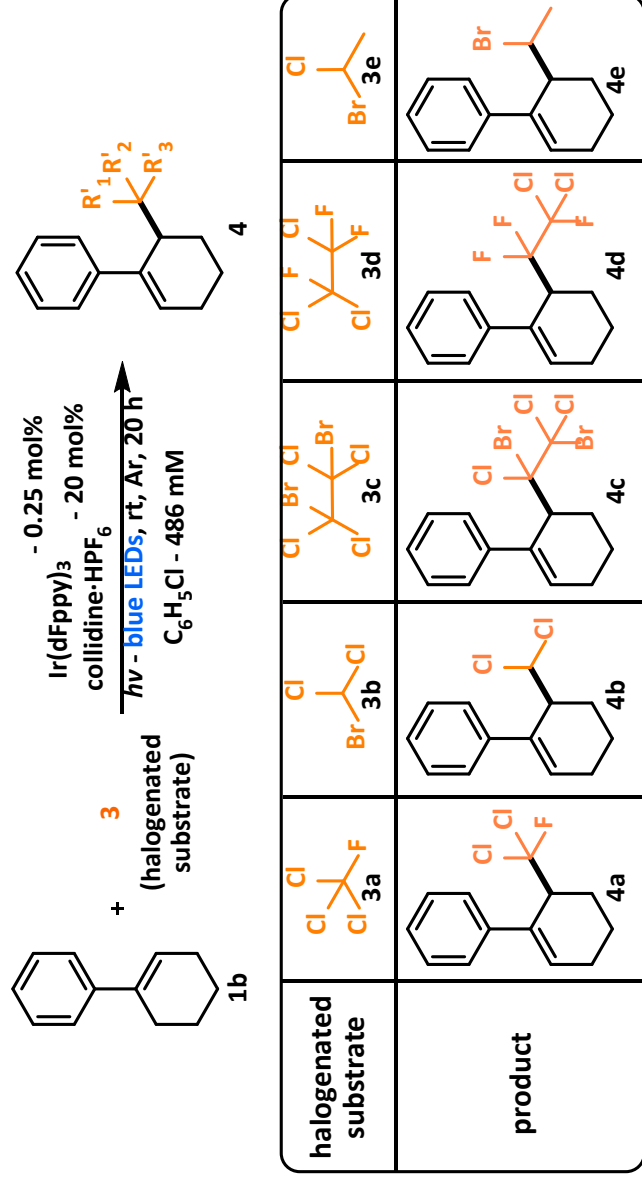
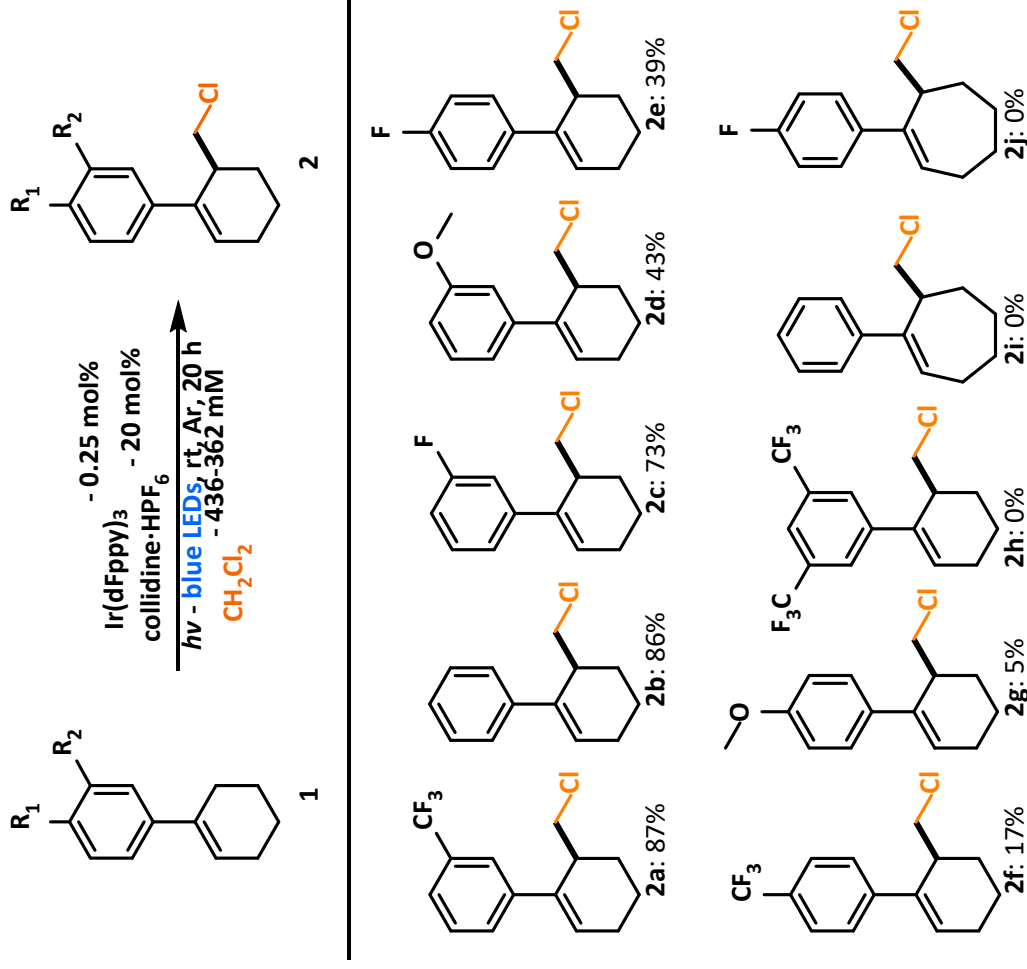
3%



Entry	Modification of left reaction	NMR conversion (%)	
1	None	60	86
2	100 mol % coll. HPF ₆	5	18
3	5 mol % coll. HPF ₆	0	12
4	33 equiv water	11	28
5	DCM/DCE (50V:50V)	3	41
6	DCM/DCE (25V:75V)	0	10
7	208 mM of SM	22	35
8	104 mM of SM	11	16
9	50 °C	0	3
10	70 °C	0	0
11	0.125 mol % PC	37	21
12	1 mol % PC	43	0

Reactivity Trends

Selected substrate scope produced clear structure–reactivity trends



Electronic trend

m-CF₃ **87%** m-OMe **43%**
p-CF₃ **17%** p-OMe **5%**

Moderate electron withdrawal
tends to favor productive reactivity

Halogen abstraction selectivity

Br ≈ Cl >> F

HCl/HBr reversibility may
influence product distribution.

What Did We Learn?

The project established a useful platform and several design rules

Mild access to reactivity

Visible light and molecular strain provide a route to carbenium-ion chemistry without relying on classical strongly acidic conditions.

Electronic effects

Electron-withdrawing substituents improved reactivity, while donating or strongly deactivating groups suppressed haloalkylation.

Mechanism still unclear

The observed halogen-dependent reactivity cannot yet be fully rationalized by a single consistent mechanistic pathway.

Important limitation

Reaction sensitivity: productive reactivity was strongly diminished by small changes in acid loading, solvent, or temperature, and required careful exclusion of air and moisture.

NEXT STEPS: mechanism • broader scope • complex substrates

From One Reaction to the Broader Field

My Niblack experience expanded into an invited review on strain-loadable molecules

SUBMITTED TO CHEMICAL REVIEWS

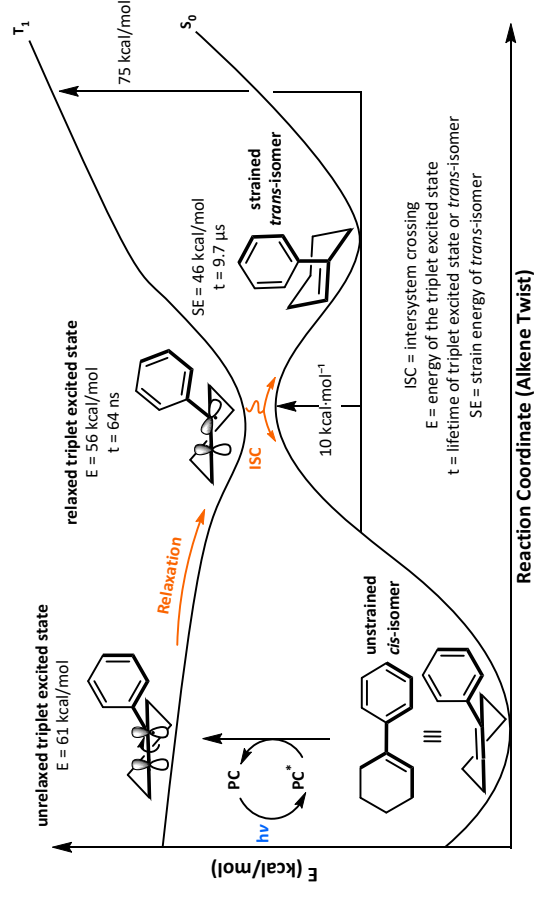
Strain-Loadable Cycloalkenes and Cycloalkenones

Driving Endergonic Reactions with Applications in Synthesis, Biorthogonal Coupling and Natural Products

CHEMICAL REVIEWS	64.2	109	Publication in chemistry's highest impact-factor journal*
	Impact Factor	CiteScore	

My contributions

- Primary-literature analysis
- ChemDraw schemes, tables, figures
- Scientific analysis and synthesis of decades of literature



What can strain energy enable?

Synthetic
methods

Chemical
biology

Natural-product
synthesis

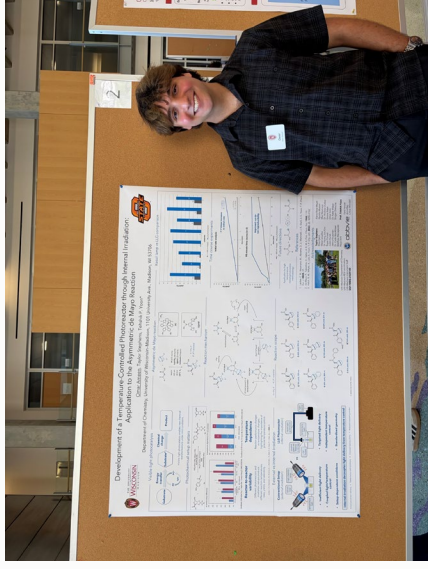
Dark endergonic
reactions

The review connects the same central idea from my project to a much larger body of photochemical chemistry.

What the Niblack Opportunity Made Possible

The program became a launching point for the next stages of my development as a researcher

The Niblack Research Scholars program gave me the laboratory skills, experimental independence, background, and confidence to take on other research problems.



Summer/Fall 2025

Niblack Research Scholar

Weaver Lab

Haloalkylation and organic photochemistry

Spring 2026

Chemical Reviews publication

Weaver Lab

Strain-loadable cycloalkenes and cycloalkenones

Summer 2026

UW–Madison REU

Yoon Group

Internally irradiated, temperature-controlled photoreactor

Thank You

The Niblack Research Scholars Program gave me more than the opportunity to complete a research project.

It gave me the opportunity to become a researcher.

RESEARCH

Hands-on ownership

PUBLICATION

Scientific writing

REU

Research independence

FUTURE

PhD + pharma R&D

With gratitude to Dr. and Mrs. Niblack, Dr. Jimmie Weaver, Dr. Osaid Alkhamayseh, the Weaver Lab, NMR facilities, the OSU Department of Chemistry.

Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers

Nicholette DuCom

Oklahoma State Department of Physics

Faculty Sponsor: Dr. Eric Benton

Graduate Student Mentors: Conner Heffernan and Garrett Thornton

ABSTRACT

During high-altitude transoceanic flights, frequent flyers are exposed to higher levels of ionizing radiation than they experience on the ground. This radiation is produced when cosmic rays interact with atmospheric nuclei, initiating nuclear interactions that produce cascading secondary particle avalanches. This is an effect known as cosmic ray air showers. Although most secondary radiation is absorbed at higher altitudes, some particles penetrate to aviation altitudes, where aircrew and frequent passengers may experience increased radiation exposure that can pose potential health risks. This project focuses on the development of a portable, handheld radiation detector designed to be carried by frequent flyers to measure exposure during long duration flights. The device utilizes a Geiger-Müller counter and a microcontroller to detect, process, and record ionizing radiation counts from the atmosphere. The detector has been tested on commercial airline flights and high-altitude balloon flights. The data collected from the detector have provided the E.V. Benton Radiation Physics Laboratory with successful computational models that can be used to refine the conversion of the count rate that is being measured to a standard unit of human radiation exposure and to benchmark CARI-7 predictions under real flight conditions. This work contributes to a more comprehensive understanding of radiation levels at aviation altitudes and further emphasizes the importance of continued monitoring for health and safety.

1. Introduction

Earth's atmosphere acts as a shield against galactic cosmic rays, which continuously bombard the planet from deep space. These high-energy particles consist primarily of protons; hydrogen nuclei, along with heavier nuclei extending up to uranium. When cosmic rays enter the atmosphere, they interact with atmospheric nuclei, producing cascades of

secondary particles through nuclear interactions. These cascades, known as cosmic ray air showers, include a mixture of particles such as muons, electrons, and gamma radiation.

While most secondary radiation is absorbed before reaching the Earth's surface, a significant portion persists at higher altitudes. As a result, radiation levels increase with altitude due to the reduced atmospheric shielding. This effect is relevant in the context of aviation, where aircraft routinely operate at altitudes of 30,000–40,000 feet. At these elevations, aircrew and frequent flyers are exposed to higher levels of ionizing radiation compared to ground level, raising concerns about long-term cumulative exposure.

Accurate measurement of radiation exposure at aviation altitudes is important for both scientific understanding and health risk assessment. The purpose of this detector is to collect radiation data during high-altitude flights in order to improve understanding of radiation levels in aviation environments and to enable comparison with existing computational models.

2. Experimental Details

An initial Geiger-Müller (GM) system was constructed using a commercially available CAJOE Geiger counter kit. The kit was interfaced with an Arduino Uno micro-controller system for data acquisition, shown in Figure 1. The system included a BME280 sensor that measures barometric pressure, humidity, and temperature, a DS3231 real-time clock (RTC), a micro-SD card module for data storage, and a 16x2 LCD display for real-time count rate. The initial prototype is shown in figure 2.

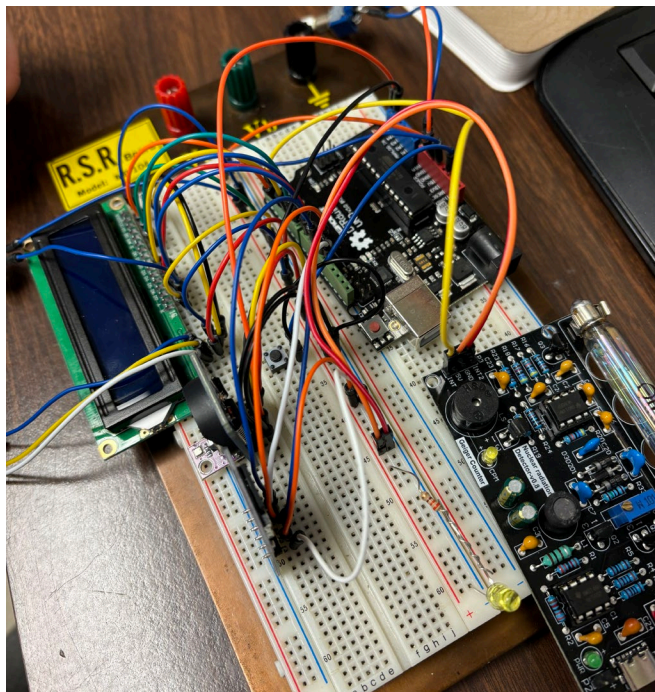


Figure 1. Initial breadboard setup of the CAJOE Geiger counter system interfaced with an Arduino Microcontroller.

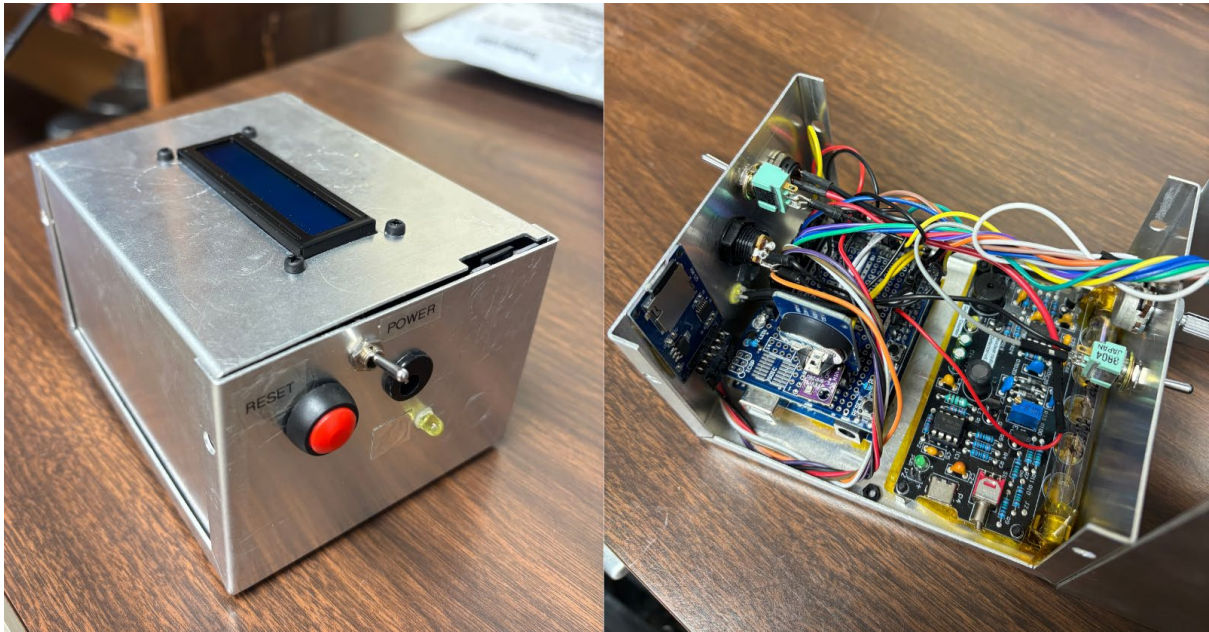


Figure 2. On the left is the outside of the first prototype. On the right is the inside of the enclosure. Shown is the CAJOE Geiger counter kit and Arduino Uno.

A custom Arduino script was developed using the Arduino IDE to log the time and day, atmospheric pressure, temperature, humidity, and counts per minute (CPM) to the SD card module at fixed time intervals. The script also allowed for real-time CPM and a running average (RA) of the count rate on the LCD display.

Following validation of the initial GM kit, a custom Geiger-Müller circuit was designed to improve portability and performance. The circuit was developed using KiCad for schematic capture and printed circuit board (PCB) layout. The design utilized a 9V input supply that was regulated to 5V using linear voltage regulators. A NE555 timer was configured to generate a pulsed DC signal, which drove a Cockcroft-Walton voltage multiplier to produce around 480V for the GM tube. Ionizing radiation is detected by the GM tube, which generates electrical pulses that are conditioned and transmitted to the Arduino micro-controller. These pulses are counted and displayed in real time by the Arduino. The schematic can be seen in figure 3.

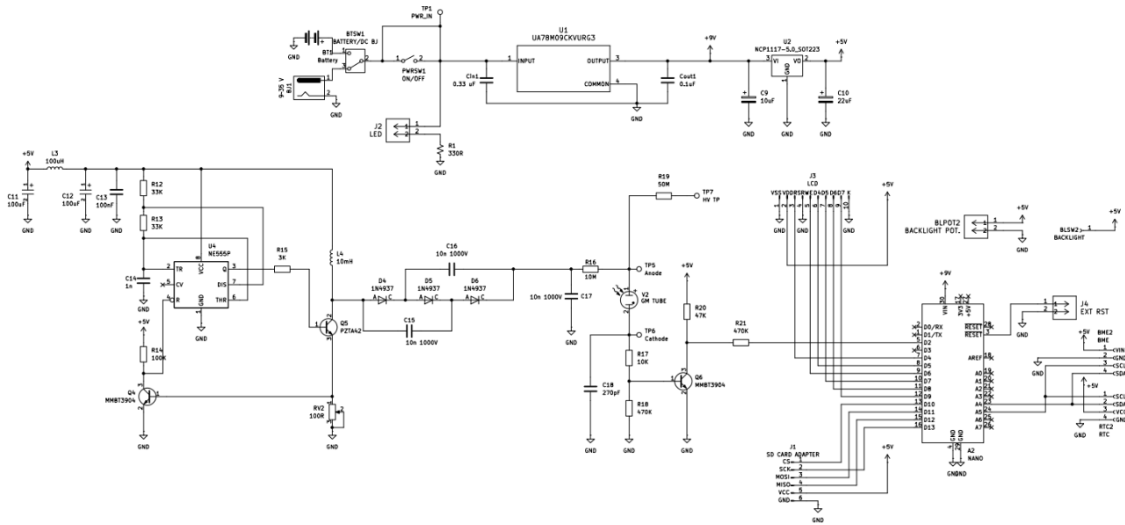


Figure 3. Schematic of the Geiger-Müller circuit. The circuit shows an input of 9V into two linear voltage regulators to maintain a constant voltage output. The constant voltage output then enters the NE555 timer, producing a pulsed signal into the Cockcroft-Walton voltage multiplier chain. The voltage is stepped up to a high voltage of 480V. This signals the Arduino software, which produces data logging and display.

Prior to PCB fabrication, the circuit was assembled and tested on a breadboard. Tests were made using a multimeter and oscilloscope to confirm proper voltage levels and pulse behavior. After successful validation, the PCB was fabricated, assembled, and integrated into a compact enclosure.

The detector's response to radiation was evaluated using a controlled Cesium-137 source to verify count detection and the system's functionality. Multiple design iterations were performed to improve reliability, reduce size, and enhance the detector's usability. These iterations included optimization of component selection, reduction of board dimensions, and transition from a metal to a plastic enclosure.

The finalized detector was deployed on high-altitude, long-duration commercial flights to collect radiation data, shown in figure 4. The flight data was recorded for analysis and comparison with CARI-7A, a computer program developed by the FAA that calculates the effective dose of galactic cosmic radiation received by an individual on an aircraft (Copeland, 2016).

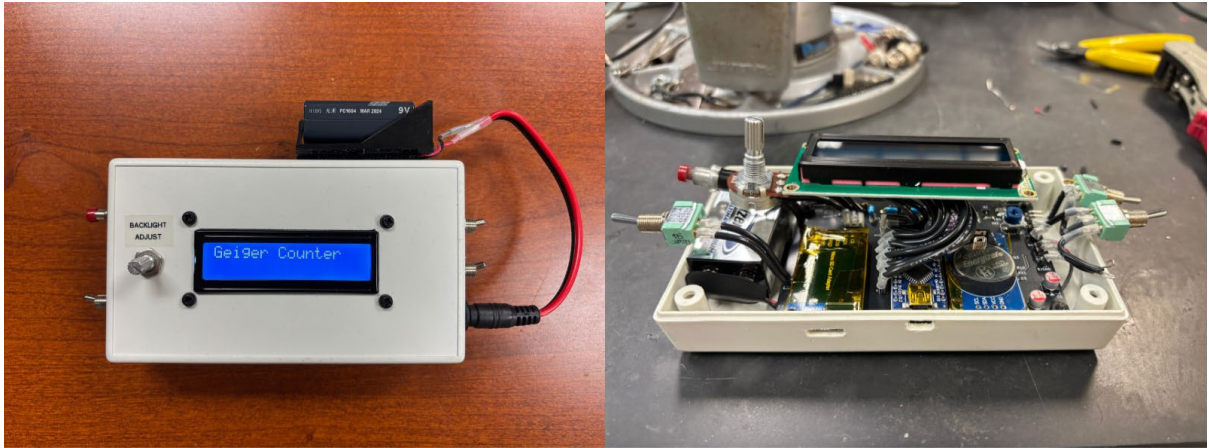


Figure 4. On the left is the outside of the final prototype used to collect data during high-altitude flights. The external battery compartment improved user accessibility but will be redesigned in future iterations. On the right is the inside of the final prototype. The battery can be seen initially in the enclosure.

3. Results

The ionizing radiation count rates were recorded during multiple high-altitude commercial flights using both the OSURPL detector and a GMC-800 Geiger counter. Both detectors exhibited similar trends across all phases of flight. This is shown in a commercial flight to London in figure 5. Figure 7 shows the flight path for AAL154 to London. The calculated conversion factors were $2.43 \times 10^{-4} \mu\text{Sv/count}$ ($\pm 20.75\%$) for the OSURPL detector and $2.47 \times 10^{-4} \mu\text{Sv/count}$ ($\pm 20.9\%$) for the GMC-800. The average ratio of absorbed dose, H^*10 to count rate across all flights is shown in figure 6.

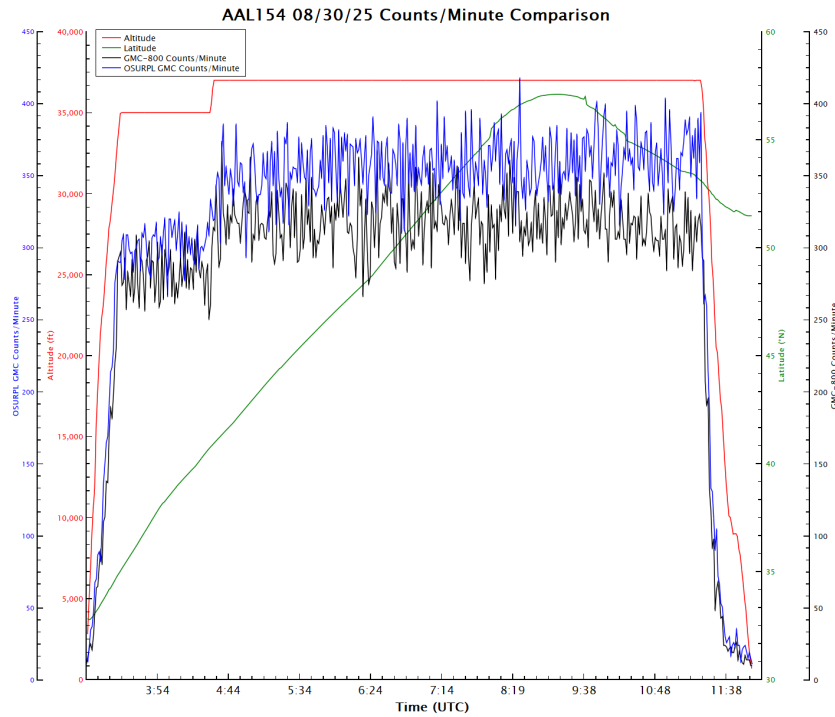


Figure 5. Counts per minute (CPM) recorded during a flight AAL154 to London as a function of time, comparing the OSURPL detector (blue) and GMC-800 (black). Flight altitude (red) and latitude (green) are also shown. Both detectors exhibit increasing count rates with altitude, stable behavior during cruise, and a sharp decrease during descent.

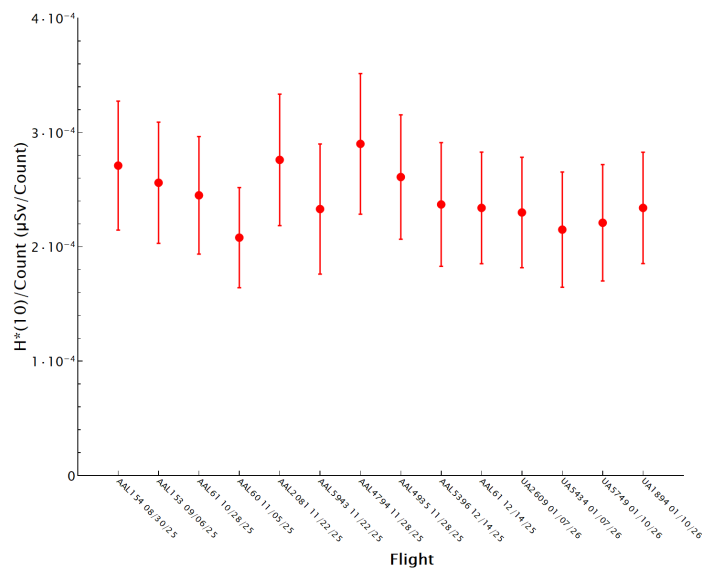


Figure 6. Average ratio of absorbed dose H^*10 to count $\mu\text{Sv}/\text{count}$ all altitudes for each flight. Values remain relatively consistent across flights, indicating stable detector response under varying flight conditions.

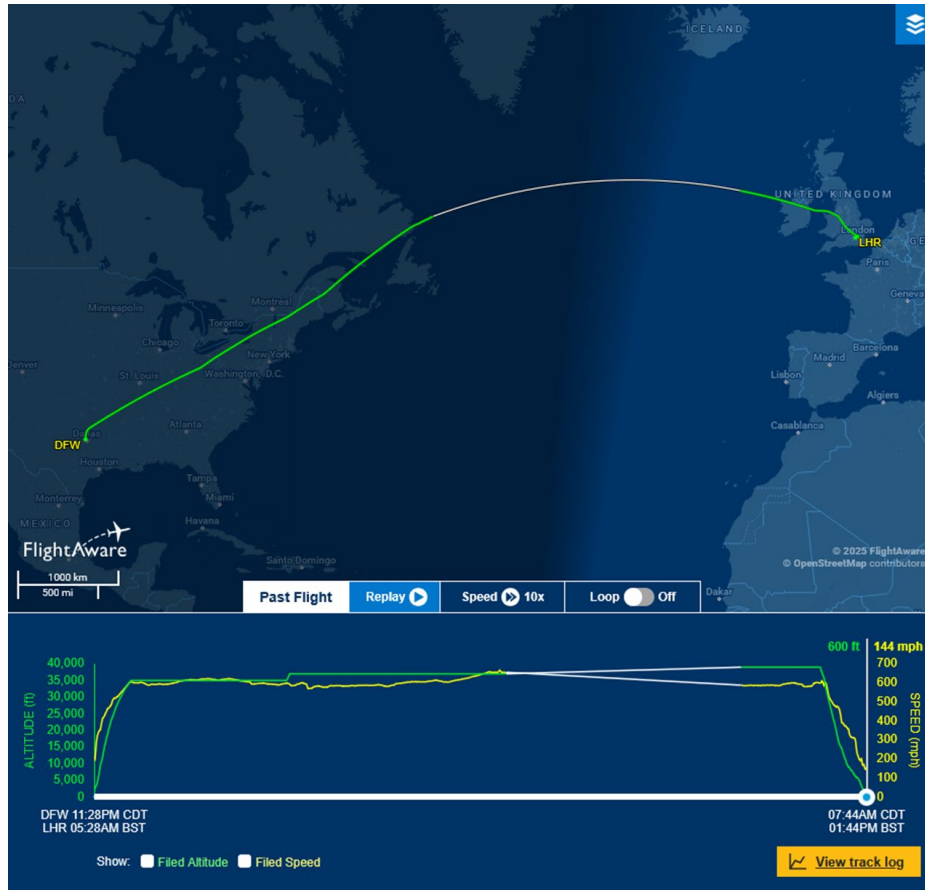


Figure 7. Shows a geodesic flight path of AAL154 on 08/30/2025 from Dallas/Fort Worth International Airport (DFW) to London Heathrow International Airport (LHR).

4. Discussion and Conclusions

Discuss in detail the meaning (interpretation) of your results, how your results relate to the existing (prior) literature, and what you conclude. Discuss what future work needs to be done based on your findings. The observed increase in count rate with altitude is consistent with the expected behavior of cosmic radiation in the atmosphere. As altitude increases, a greater flux of secondary particles from cosmic ray air showers reach the detector. The constant count rate observed during cruise is also consistent with the aircraft maintaining a nearly constant altitude, resulting in stable radiation exposure conditions.

The close agreement between the OSURPL detector and the GMC-800 Geiger counter indicates that the detector is capable of reliably measuring ionizing radiation during flights. The similarity in response across all phases of flight suggests that the detector's electronics, high-voltage system, and data acquisition method are functioning as intended. The calculated conversion factors for both detectors were in strong agreement, with values of $2.43 \times 10^{-4} \mu\text{Sv/count}$ ($\pm 20.75\%$) and $2.47 \times 10^{-4} \mu\text{Sv/count}$ ($\pm 20.9\%$).

The small difference between these values suggests that the detector provides a comparable estimate of radiation exposure when calibrated against established models. The large uncertainties can likely be attributed to the fact that Geiger-Müller tubes do not provide energy discrimination, which can limit the ability to relate the count rate to absorbed dose without calibration. The use of computational models such as CARI-7A provides a useful reference for estimating effective dose during flight.

Additional challenges were observed during early high-altitude balloon testing, where initial detector designs failed to produce reliable data. These issues were likely due to the high-voltage supply stability, reduced performance of the Geiger-Müller tubes, and the interference from the placement of the LCD display and control switches. As a result, later design iterations focused on improving high-voltage regulation, signal conditioning, and component selection. These modifications contributed to reliable performance on later commercial flights.

During one of the last flights, data loss occurred due to failure of the SD card's storage. To fix this, future designs will incorporate redundant data storage through multiple SD card modules, along with higher reliability SD cards to enhance reliability.

Overall, the results demonstrate that the detector is capable of tracking changes in radiation exposure at aviation altitudes and produces measurements that are consistent with a commercial reference instrument.

5. Summary

A portable Geiger-Müller-based radiation detector was successfully designed, constructed, and tested for measuring ionizing radiation at aviation altitudes. The detector demonstrated consistent performance during high-altitude commercial flights, with measured count rates increasing with altitude and remaining stable during cruise conditions.

Comparison with a reference GMC-800 Geiger counter showed strong agreement in detector response, and the calculated conversion factors were found to be consistent between the two systems. These results indicate that the detector is capable of reliably measuring radiation exposure during high-altitude flights.

The detector provides a practical and user-friendly solution to monitoring radiation levels in aviation environments. Future work will focus on improving the system's design, component selection, and further reducing measurement uncertainty through additional testing and data collection.

6. Appendices

6a. Acknowledgements

The author thanks: Dr. John and Heidi Niblack, Dr. Kenneth Sewell, and Dr. Christine Johnson for their help during the Niblack Research Scholars Program; faculty mentor Dr. Eric Benton, for his help and guidance throughout this project; Dr. Martin Yang for his assistance and guidance; graduate students, Garrett Thornton and Conner Heffernan for their guidance and assistance; and Kyle Copeland of the Civil Aerospace Medical Institute (CAMI), FAA, Oklahoma City, OK for his contributions to CARI-7A.

6b. Papers Published

DuCom, N. “Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers.” Poster Presentation at the Undergraduate Research Symposium, 2025, April 22, Oklahoma State University-Stillwater.

DuCom, N. “Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers.” Poster Presentation at the 2025 Undergraduate Summer Research Expo, 2025, July 24, Oklahoma State University-Stillwater.

DuCom, N. “Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers.” Poster Presentation at the Undergraduate Research Symposium, 2026, April 21, Oklahoma State University-Stillwater.

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Personal Radiation Dosimeter for Pilots, Flight Attendants, and Frequent Flyers

Nichollette DuCom

E.V. Benton Radiation Physics Laboratory
Oklahoma State University Department of Physics

Introduction

Background

- Galactic cosmic rays (GCR) constantly bombard the Earth's atmosphere.
- Cosmic rays interact with atmospheric nuclei, causing cosmic ray showers.
- Significant portion of ionizing radiation persists at high altitudes.
- Important in aviation, aircraft operates at 30,000-40,000 feet.
- Aircrew and frequent flyers are exposed to higher levels of ionizing radiation.

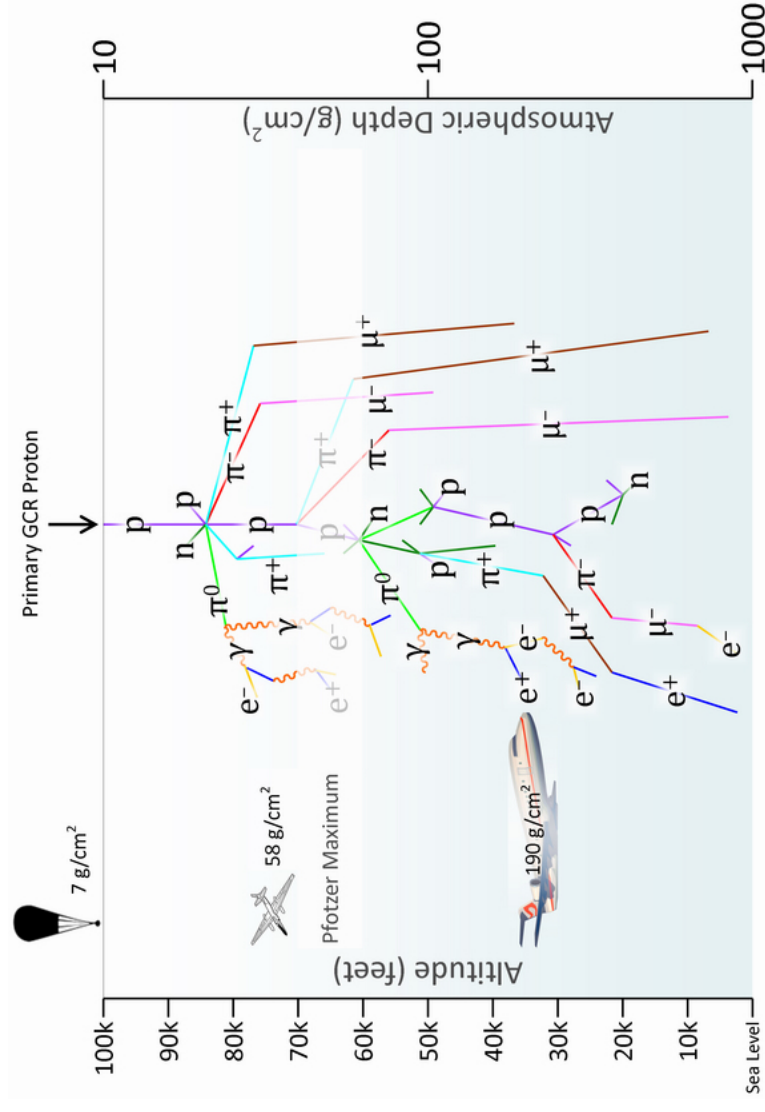


Figure 1. Cosmic ray air shower illustration. These showers consist of secondary particles shown above which includes protons, neutrons, pions, muons, electrons, and photons.

Introduction

Objectives

- Design and develop a low-cost, portable radiation detector that utilizes a Geiger-Müller tube.
- Measure ionizing radiation at aviation altitudes.
- Collect radiation data during high-altitude flights and compare to computational models.
- Improve understanding of radiation level in aviation environments.

Methods

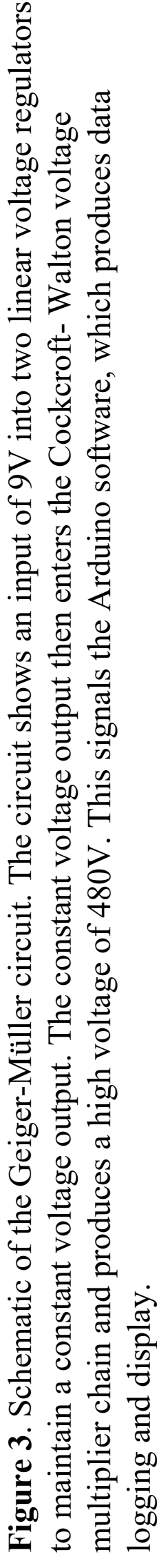
System Design

- Geiger-Müller (GM) tube for radiation detection.
- Arduino microcontroller for data acquisition.
- Pressure, temperature, and humidity sensor (BME280).
- Data logging:
 - Micro-SD card module.
 - Real time clock (RTC) module
- Real-time display of counts per minute on LCD.



Figure 2. Shows the systems components: printed circuit board (PCB), micro-SD card module, RTC, switches, reset, and a background potentiometer.

Circuit Design



Testing and Results



Figure 6 and 7. Final prototype used to collect data during high-altitude commercial flights.

Testing and Results

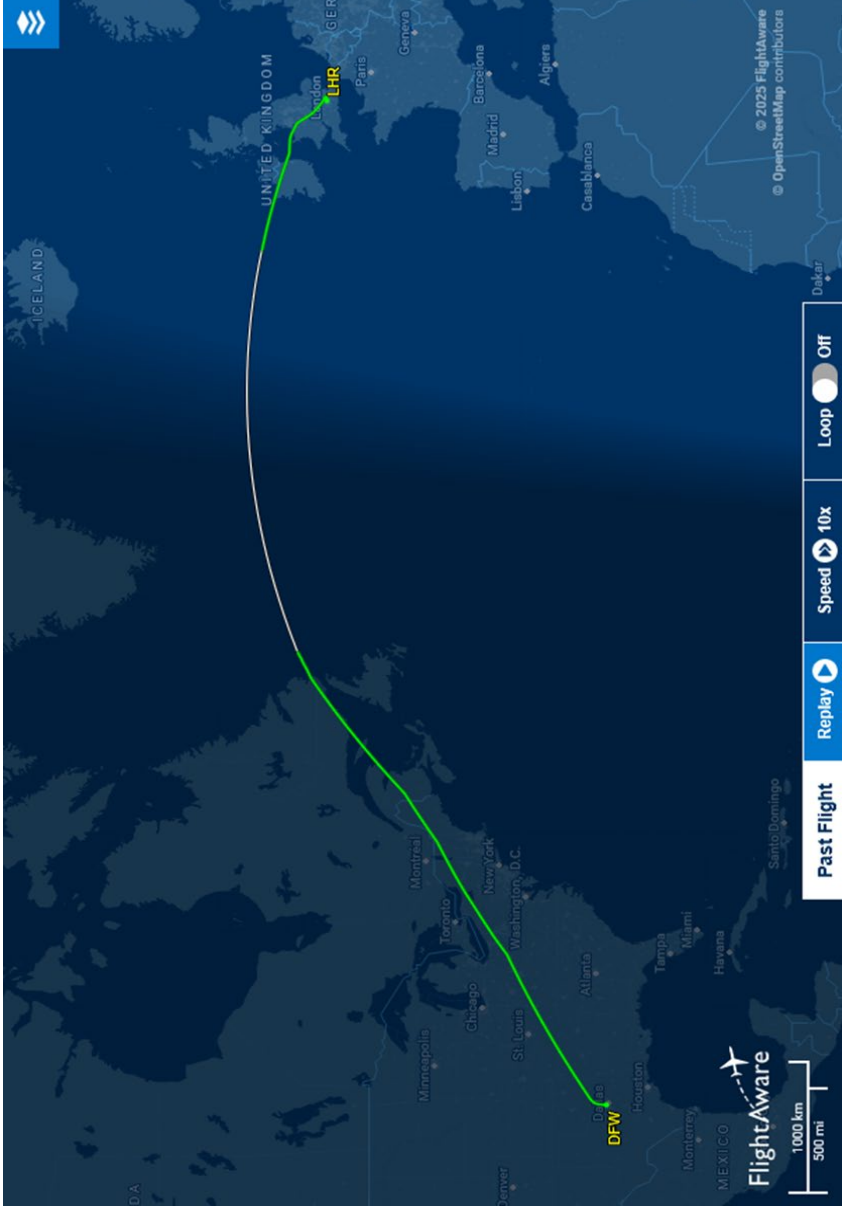


Figure 4. Shows a geodesic flight path of AAL154 on 08/30/2025 from Dallas/Fort Worth International Airport (DFW) to London Heathrow International Airport (LHR).

- Data collected on commercial flights.
- Compared:
 - OSURPL detector vs GMC-800.
- Observations:
 - Count rate increases with altitude.
 - Stable during cruising altitude.
 - Decreases during descent.

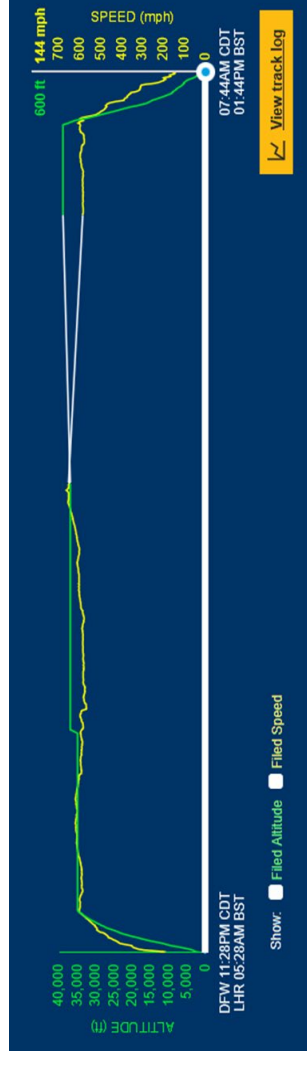


Figure 5. Flight profile showing altitude and speed as a function of time from AAL154.

Testing and Results

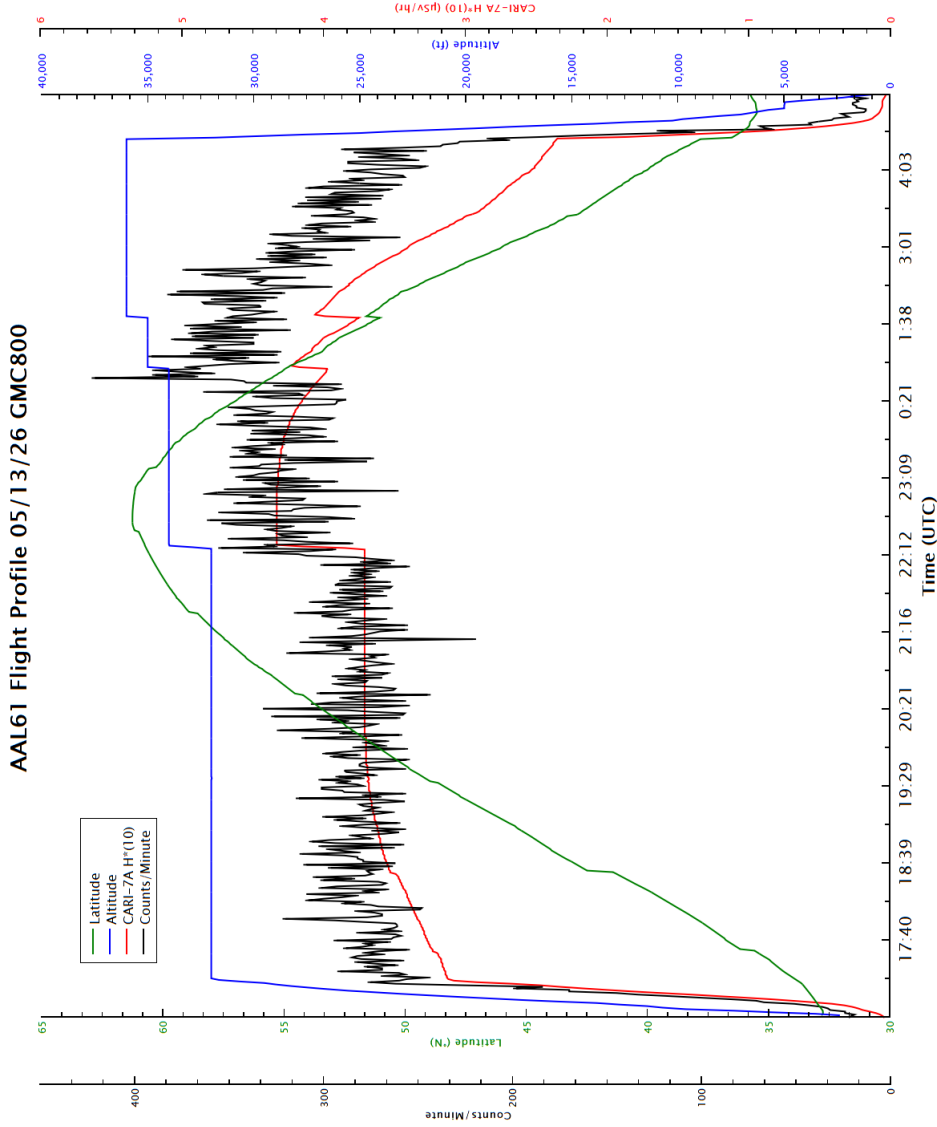


Figure 7. Counts per minute (CPM) recorded during a flight AAL61 to Japan as a function of time.

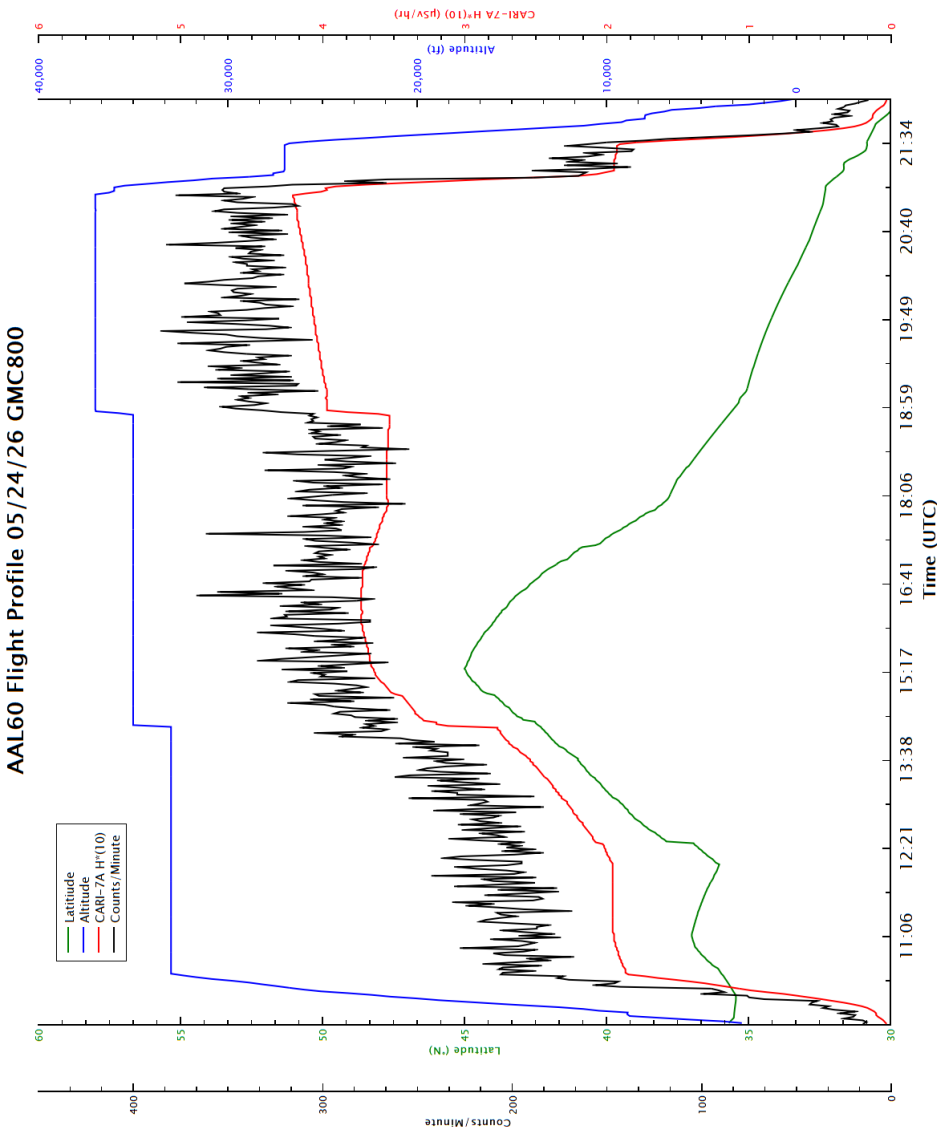


Figure 7. Counts per minute (CPM) recorded during a flight AAL60 from Japan as a function of time.

Testing and Results

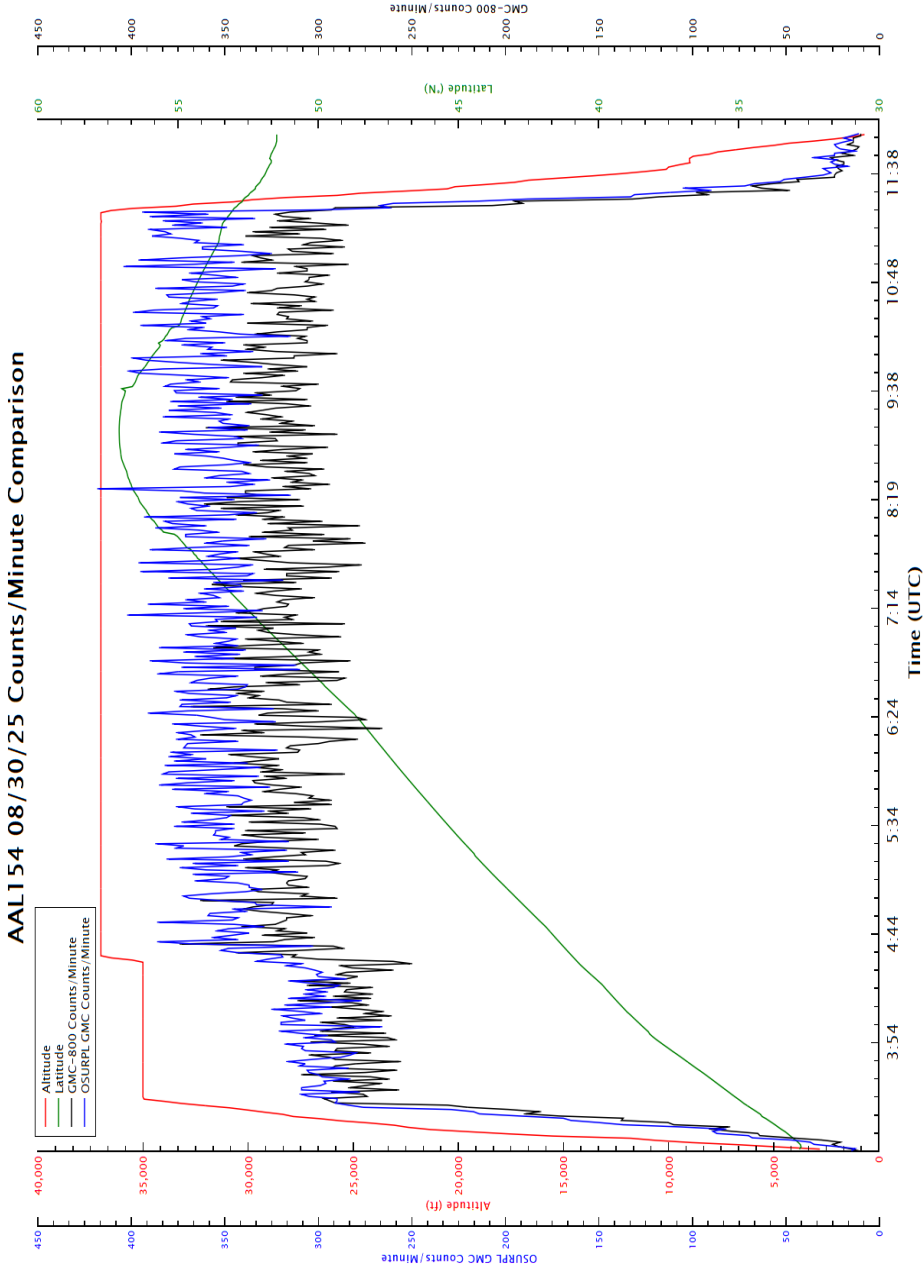


Figure 7. Counts per minute (CPM) recorded during a flight AAL154 to London as a function of time, comparing the OSURPL detector (blue) and GMC-800 (black).

Testing and Results

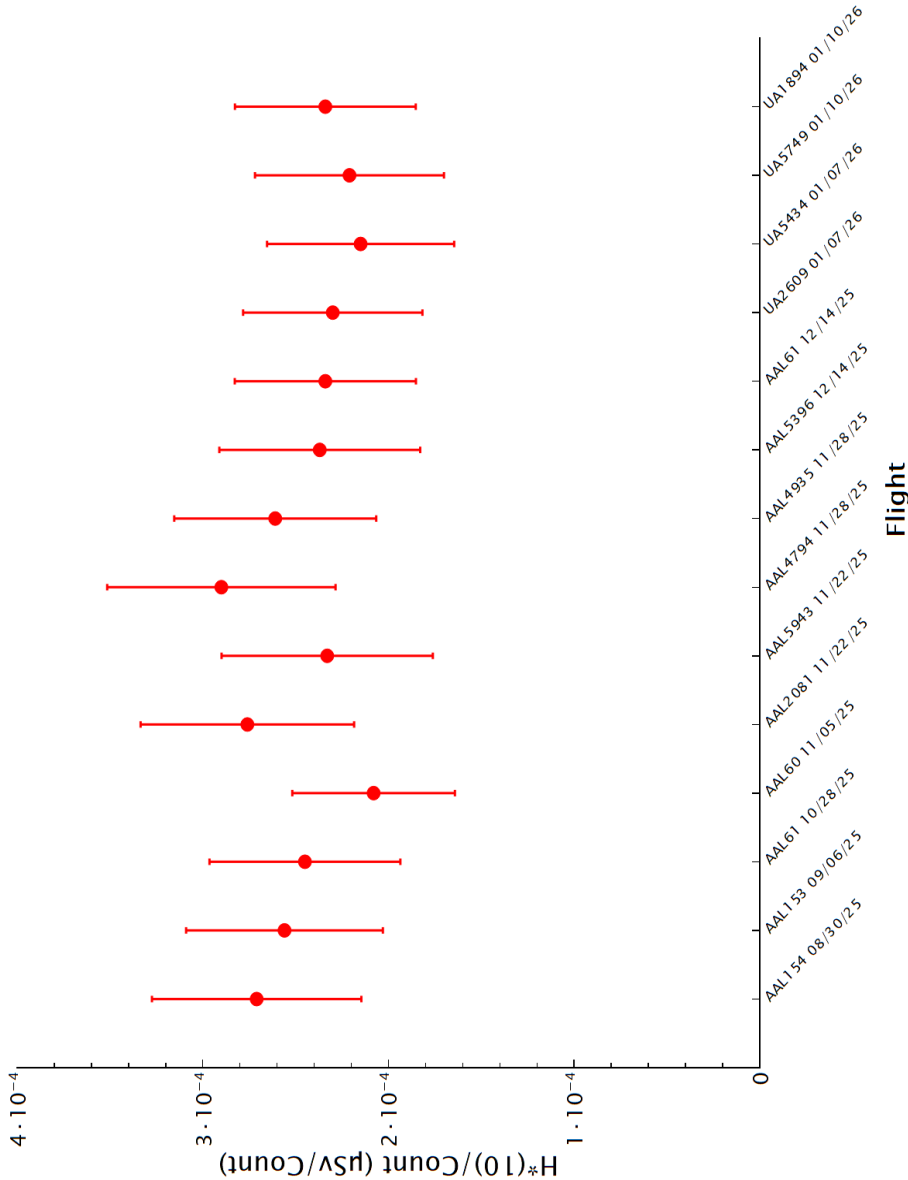


Figure 7. Average ratio of absorbed dose H^*10 to count $\mu\text{Sv}/\text{count}$ all altitudes for each flight. Values remain relatively consistent across flights, indicating stable detector response under varying flight conditions.

- Flight data compared to CARI-7A
- Conversion factors:
 - OSURPL: $2.43 \times 10^{-4} \mu\text{Sv}/\text{count}$ (± 20.75).
 - GMC-800: $2.47 \times 10^{-4} \mu\text{Sv}/\text{count}$ (± 20.9).
- Strong agreement between detectors.
- **Consistent across all flights.**

Discussion

- Observed increase in count rate matches expected behavior.
- Detector shows:
 - Reliable performance.
 - Stable measurements.
- Limitations:
 - GM tubes lack energy discrimination.
 - High uncertainty
- Future Improvements:
 - More compact design.
 - Improved signal conditioning.
 - Incorporate redundant data storage.

Conclusions

- Successfully designed, constructed, and tested a portable Geiger-Müller radiation detector.
- Detector demonstrated consistent and reliable performance during high-altitude flights.
- The OSURPL detector's memory system gives it a distinct advantage over commercial alternatives.
- Flight data enabled refinement of count rate $\rightarrow$ radiation dose conversion ($\mu\text{Sv/hr}$).
- Results provide valuable real-world validation for CARL-7A model.

Acknowledgments

- Deepest gratitude to Dr. John and Heidi Niblack, Dr. Kenneth Sewell, and Dr. Christine Johnson for their help during the Niblack Research Scholars Program.
- Sincere thanks to faculty mentor Dr. Eric Benton for his invaluable guidance throughout this project.
- Special thanks to Dr. Martin Yang for his assistance and guidance.
- Many thanks to graduate students Garrett Thornton and Conner Heffernan for their guidance and assistance.
- Thanks to Kyle Copeland of the Civil Aerospace Medical Institute (CAMI),

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SESSION THREE:

Chole Tolle

“UV-Treatment of Contaminants of Emerging Concern in Wastewater”

Research Area: Biosystems and Agricultural Engineering

Faculty Sponsor: Dr. Kiranmayi Mangalgi

Graduate Student Mentor: Ali Shahidi Nejad

Noor Atiyeh

“Catalytic Conversion of Cyclopentanone to Sustainable Aviation Fuel Precursors”

Research Area: Chemical Engineering

Faculty Sponsor: Dr. Hong Je Cho

Graduate Student Mentor: Tauhid Khan

Rachel Davis

“Multifactorial Evaluation of Structural Stability of Sodium Alginate-Gelatin Hydrogels for Dental Applications: Effects of pH, Crosslinking, and Fabrication Techniques”

Research Area: Chemical Engineering

Faculty Sponsor: Dr. Sundar Madhally

Graduate Student Mentor: xx

UV-Treatment of Contaminants of Emerging Concern in Wastewater

Chole Denise Tolle

Department of Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Kiranmayi Mangalgiri

Graduate Student Mentor: Ali Shahidi Nejad

ABSTRACT

The persistence of macrolide antibiotics in wastewater presents a growing environmental and public health concern due to their role in promoting antimicrobial resistance (AMR). Antibiotics such as azithromycin and gamithromycin are frequently detected in municipal and agricultural wastewater because conventional treatment systems are often unable to remove them fully. Their continued release into aquatic environments contributes to the development of resistant bacterial populations and ecological disruption. This study investigated the degradation of azithromycin and gamithromycin using ultraviolet (UV) treatment and advanced oxidation processes (AOPs) involving UV and hydrogen peroxide (UV/H₂O₂). Experiments were conducted using a bench-scale carousel-type UV reactor equipped with eight low-pressure UV lamps emitting at 254 nm. Treatment conditions included solution pH values of 4, 7, and 10 and hydrogen peroxide concentrations of 0.3, 0.5, 1, and 2 mM. (0.3, 0.5, 1, and 2 mM). UV dose was standardized using phosphate ferrioxalate actinometry to determine photon flux accurately. Results showed that direct UV photolysis alone resulted in less than 10% degradation, confirming that azithromycin is resistant to UV-only treatment. However, the addition of hydrogen peroxide significantly improved degradation by generating hydroxyl radicals. The highest removal efficiency of 58.76% was seen at pH 10 with 2 mM H₂O₂. These findings show that UV/H₂O₂ treatment is a more effective strategy for removing persistent antibiotics from wastewater and that pH plays a critical role in treatment performance. Future work will include testing gamithromycin and evaluating treatment performance in more complex water matrices.

1. Introduction

Contaminants of emerging concern (CECs) are compounds increasingly detected in aquatic environments that may pose risks to both environmental and human health. Among these contaminants, antibiotics have received considerable attention because of their persistence in wastewater and their contribution to antimicrobial resistance (AMR). Macrolide antibiotics such as azithromycin and gamithromycin are widely used in both human and veterinary medicines to treat bacterial infections. Due to their extensive use, these compounds are often

detected in wastewater treatment plant effluents, agricultural runoff, and surface waters. Conventional wastewater treatment systems are designed primarily to remove nutrients, suspended solids, and pathogens, and are often ineffective at completely removing pharmaceutical contaminants. As a result, residual antibiotics can enter aquatic environments where they may contribute to ecological toxicity and the spread of antibiotic-resistant bacteria.

The presence of antibiotics in environmental waters is particularly concerning because antimicrobial resistance has become one of the most significant global public health threats. Continuous exposure of microorganisms to low concentrations of antibiotics can promote the selection of resistant strains, reducing the effectiveness of clinically important antibiotics. Because of these concerns, improving the removal of antibiotics during wastewater treatment has become an important research priority. The United States Environmental Protection Agency and researchers worldwide have identified pharmaceutical contaminants as emerging pollutants that require improved treatment technologies to prevent long-term environmental impacts.

Macrolide antibiotics are among the most often detected pharmaceutical contaminants in aquatic environments due to their extensive use in human and veterinary medicine. Azithromycin is commonly prescribed for respiratory, skin, and bacterial infections, while gamithromycin is widely used in livestock production, particularly in cattle (Zouli, 2024; Wang et al., 2023). Because humans and animals do not completely metabolize these compounds, significant portions are excreted and later enter wastewater treatment systems. Studies have reported the presence of macrolides in wastewater treatment plant influent and effluent, surface waters, sediments, and agricultural runoff. Their continuous introduction into the environment raises concerns regarding persistence, ecological toxicity, and the development of antimicrobial resistance.

Ultraviolet (UV) disinfection is commonly used in wastewater treatment facilities to inactivate pathogens; however, UV irradiation alone is often insufficient to degrade stable pharmaceutical compounds, such as macrolide antibiotics. Earlier studies have shown that azithromycin exhibits limited direct photolysis under standard UV treatment conditions due to its low UV absorbance and stable molecular structure (Zouli, 2024). Our earlier research has similarly shown that direct UV photolysis resulted in less than 10% degradation of azithromycin, confirming the limited effectiveness of UV-only treatment.

Advanced Oxidation Processes (AOPs) are water treatment technologies that generate highly reactive species, primarily hydroxyl radicals ($\bullet\text{OH}$), capable of degrading persistent organic contaminants that are resistant to conventional treatment methods. They have appeared as promising alternatives for removing persistent contaminants from water. Among these technologies, UV/ H_2O_2 systems are particularly effective because they generate highly reactive hydroxyl radicals ($\bullet\text{OH}$) through the photolysis of hydrogen peroxide. These radicals can rapidly oxidize complex organic molecules and degrade contaminants resistant to conventional treatment methods. Cuerda-Correa et al. (2020) described advanced oxidation processes as among the most effective technologies for antibiotic removal, owing to their

ability to generate powerful oxidizing species capable of degrading persistent pharmaceutical contaminants. Similarly, Zhang et al. (2023) emphasized that advanced redox processes offer sustainable approaches for water treatment by enhancing the degradation of contaminants that conventional systems do not remove. Hübner et al. (2024) further reported that hydroxyl radical-based advanced oxidation processes have become increasingly important for wastewater treatment because they can effectively degrade contaminants that are poorly removed by conventional biological treatment systems.

Recent studies have proved the effectiveness of UV/H₂O₂ systems for degrading antibiotics. Wang et al. (2023) reported that H₂O₂-based advanced oxidation processes are among the most promising technologies for antibiotic removal due to their strong oxidation potential and rapid degradation of pharmaceutical contaminants. Zouli (2024) reported that increasing hydrogen peroxide concentrations significantly improved azithromycin degradation, particularly under alkaline conditions where hydroxyl radical production was enhanced. Complete degradation of azithromycin was achieved within 60 minutes under optimized treatment conditions, highlighting the potential of advanced oxidation processes for pharmaceutical removal. Although substantial research has focused on azithromycin, fewer studies have investigated gamithromycin degradation. Because gamithromycin is widely used in livestock production, understanding its environmental fate and degradation behavior is important for managing agricultural wastewater.

Although UV/H₂O₂ treatment has shown promise for degrading pharmaceutical contaminants, treatment efficiency can be influenced by several environmental factors. Water quality constituents, such as bicarbonate, chloride, nitrate, and natural organic matter, may compete with target contaminants for hydroxyl radicals, thereby reducing degradation efficiency. Hübner et al. (2024) noted that water matrix constituents can significantly influence the performance of advanced oxidation processes by scavenging reactive radicals and reducing oxidant availability. Bicarbonate and carbonate species can act as radical scavengers, while natural organic matter may absorb UV light and decrease oxidant availability.

The goal of this study was to evaluate the effectiveness of UV photolysis and UV/H₂O₂ advanced oxidation processes for degrading azithromycin and gamithromycin in water. Emphasis was placed on determining how hydrogen peroxide concentration and pH influence degradation efficiency.

2. Experimental Details

Azithromycin and gamithromycin solutions were prepared at a target concentration of 1 mg/L using a 10 mM phosphate buffer. Experimental solutions were adjusted to pH 4, 7, or 10 to evaluate the influence of water chemistry on degradation performance. These pH values were selected to represent acidic, neutral, and alkaline conditions commonly encountered in environmental and wastewater treatment systems. Although both compounds belong to the macrolide antibiotic class, gamithromycin has structural differences that may influence its reactivity toward UV/H₂O₂ treatment and hydroxyl radical oxidation. Therefore, both

antibiotics were evaluated to compare their degradation behavior under identical treatment conditions.

Phosphate buffer solutions (10 mM) were prepared and adjusted to pH 4, 7, and 10 using acid or base additions. These pH conditions were selected to represent acidic, neutral, and alkaline water environments commonly encountered in wastewater treatment systems. The influence of pH on degradation efficiency was evaluated under identical UV and UV/H₂O₂ treatment conditions.

Degradation experiments were conducted using a bench-scale 254 nm carousel UV reactor to evaluate both direct UV photolysis and UV/H₂O₂ advanced oxidation processes (AOPs), as shown in Figure 1. The UV dose was standardized using phosphoric ferrioxalate actinometry to quantify photon flux and ensure consistent treatment conditions across experiments. This method measures the photoreduction of ferric ions (Fe³⁺) to ferrous ions (Fe²⁺), followed by complexation with o-phenanthroline and spectrophotometric measurement at 510 nm. A quantum yield of 1.25 was applied, and the average photon flux was calculated as $1.87 (\pm 0.03) \times 10^{-5}$ Einstein L⁻¹ s⁻¹.



Fig 1. carousel-type UV reactor used for UV photolysis and UV/H₂O₂ advanced oxidation experiments. UV dose was standardized using ferrioxalate actinometry to ensure consistent treatment conditions.

For UV/H₂O₂ experiments, hydrogen peroxide was added at concentrations of 0.3, 0.5, 1.0, and 2.0 mM before UV exposure. Aliquots of 6 mL were transferred into quartz tubes and exposed to controlled UV irradiation intervals. Samples were collected over 30 minutes at multiple reaction times to monitor degradation progress. All experiments were conducted in duplicate to ensure reproducibility. For advanced oxidation experiments, sodium thiosulfate was added immediately after irradiation to quench residual oxidants and prevent further degradation reactions. Samples were transferred into amber liquid chromatography (LC) vials, thoroughly mixed, and stored at -20°C until analysis. Storage in amber vials minimized photodegradation and preserved sample integrity before instrumental analysis.

Residual concentrations of azithromycin and gamithromycin were quantified using liquid chromatography–tandem mass spectrometry (LC-MS/MS), a sensitive analytical technique capable of detecting trace levels of pharmaceutical contaminants in water. LC-MS/MS separates compounds based on chromatographic retention and identifies analytes using mass-to-charge ratios, allowing accurate quantification of antibiotic concentrations before and after treatment.

Degradation performance was evaluated by calculating removal percentages based on changes in concentration during treatment. Pseudo-first-order kinetics were analyzed using

plots of $\ln(C/C_0)$ versus reaction time, where C_0 is the initial antibiotic concentration, and C is the concentration at a given time. Linear regression was used to determine apparent degradation rate constants (k), with steeper slopes indicating faster degradation. Rate constants were compared among treatment conditions to evaluate the effects of hydrogen peroxide concentration and pH on degradation behavior. Data are presented as mean $\pm$ standard deviation from duplicate experiments, and error bars represent the standard deviation of replicate measurements.

3. Results and Discussion

3.1 Kinetic Analysis

Pseudo-first-order kinetic analysis was used to compare degradation rates under different treatment conditions. Linear relationships between $\ln(C/C_0)$ and reaction time indicated that degradation followed first-order behavior, where C_0 represents the initial concentration, and C represents the concentration at a given reaction time. The slope of each regression line corresponds to the apparent degradation rate constant (k), with steeper negative slopes indicating faster degradation. Increasing hydrogen peroxide concentration yielded larger rate constants because additional $HO^\bullet$ were generated during UV irradiation, thereby enhancing azithromycin oxidation

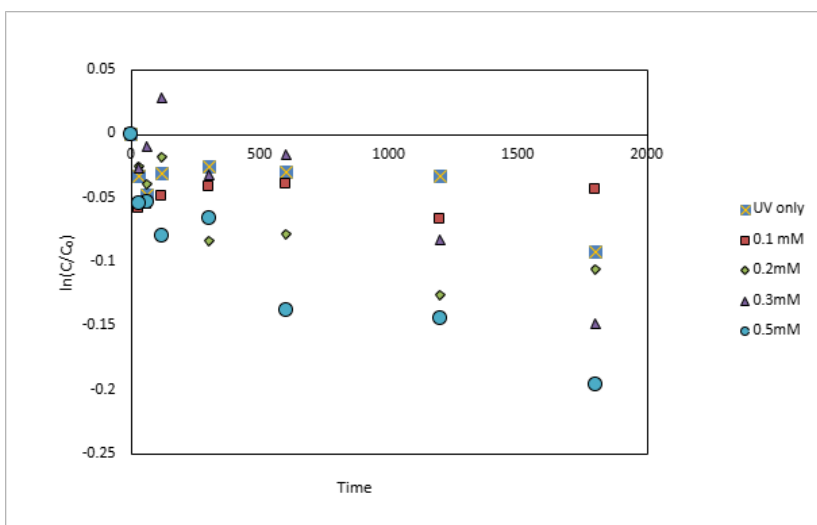


Figure 2. Pseudo-first-order degradation kinetics of azithromycin under UV-only and UV/H₂O₂ treatment conditions at pH 10. Linear relationships between $\ln(C/C_0)$ and reaction time indicate first-order degradation behavior, with steeper slopes corresponding to faster degradation rates.

3.2 Direct UV Photolysis of Azithromycin

To compare treatment effectiveness between direct photolysis and advanced oxidation, azithromycin and gamithromycin concentrations were monitored during UV-only and UV/H₂O₂ treatment. As expected, the addition of hydrogen peroxide enhanced degradation by generating $HO^\bullet$, a highly reactive oxidizing species capable of rapidly degrading

pharmaceutical contaminants. Increased degradation was observed with increasing H_2O_2 concentration, indicating that greater $\text{HO}\cdot$ production improved oxidation efficiency. In contrast, UV-only treatment resulted in substantially lower removal, demonstrating that direct photolysis was less effective than the UV/ H_2O_2 process under the conditions tested.

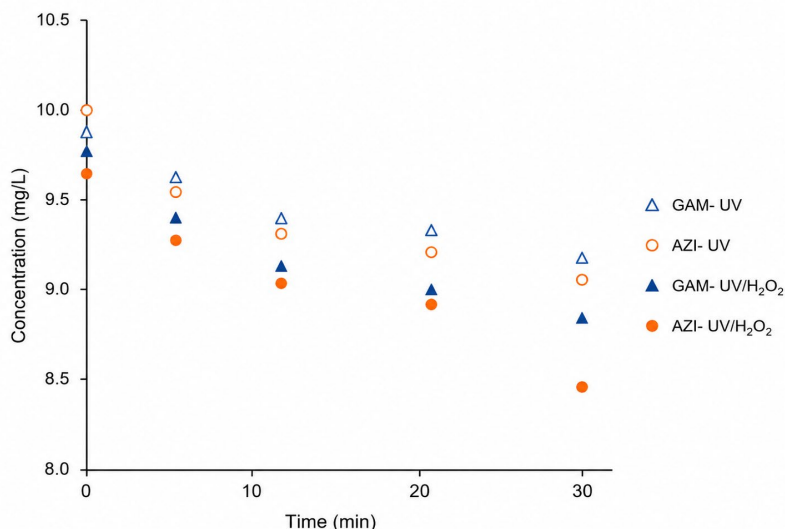


Figure 3. Comparison of azithromycin (AZI) and gamithromycin (GAM) concentrations during UV-only and UV/ H_2O_2 treatment. Greater concentration reductions were observed under UV/ H_2O_2 conditions for both antibiotics, indicating enhanced degradation through hydroxyl radical-mediated oxidation.

Direct UV photolysis produced limited degradation for both macrolides, indicating that azithromycin and gamithromycin were relatively resistant to direct UV irradiation at 254 nm. Azithromycin showed less than 10% removal under UV-only conditions, while gamithromycin showed only slightly different degradation behavior under the same treatment conditions. Overall, UV-only treatment was not sufficient for substantial removal of either compound. Based on organic chemistry principles, this limited degradation may be due to the macrolide structure, which contains a large lactone ring and saturated functional groups that do not strongly absorb at 254 nm. Any slight differences between azithromycin and gamithromycin may be related to differences in substituents, molecular size, and functional groups that affect UV absorbance and susceptibility to bond cleavage.

The addition of H_2O_2 generated $\text{HO}\cdot$, which increased macrolide degradation compared with UV-only treatment. For azithromycin, direct UV photolysis resulted in less than 10% removal, while the addition of 2 mM H_2O_2 increased degradation to 34.80% at pH 4, 36.08% at pH 7, and 58.76% at pH 10. This shows that azithromycin degradation increased by at least 24.80 percentage points and up to 48.76 percentage points compared with UV-only treatment. Gamithromycin showed a similar trend, with greater removal under UV/ H_2O_2 treatment than UV-only treatment, indicating that H_2O_2 addition also improved oxidation of

this compound. These results demonstrate that UV/H₂O₂ advanced oxidation is more effective than UV-only treatment for degrading macrolide antibiotics.

3.3 Effect of Hydrogen Peroxide Addition

To evaluate the influence of oxidant concentration on treatment performance, azithromycin degradation was compared across multiple hydrogen peroxide concentrations under UV/H₂O₂ treatment conditions. Hydrogen peroxide serves as the precursor for hydroxyl radical generation during UV irradiation; therefore, increasing H₂O₂ concentration is expected to enhance degradation efficiency.

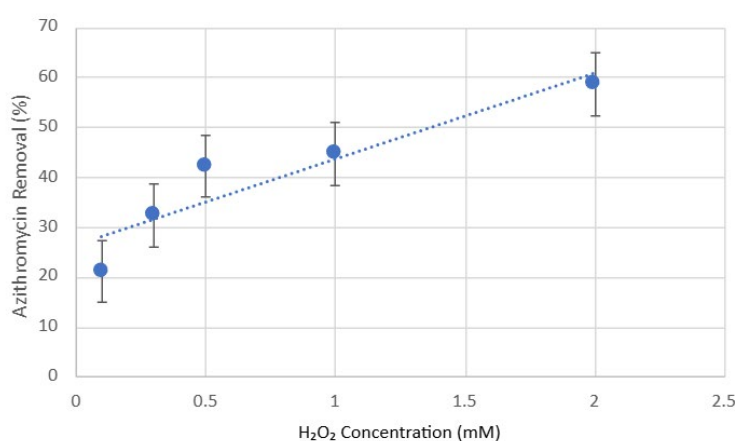


Figure 4. Effect of H₂O₂ concentration on azithromycin removal at pH 10 after 30 min of UV/H₂O₂ treatment. Removal increased from approximately 21% at 0.1 mM to 59% at 2.0 mM H₂O₂. Error bars represent the standard deviation of replicate analyses.

Hydrogen peroxide concentration significantly influenced the degradation efficiency of azithromycin. Although removal increased with increasing H₂O₂ concentration, the magnitude of improvement was greater at lower H₂O₂ doses than at higher doses. For example, increasing the H₂O₂ concentration from 0 to 0.3 mM increased azithromycin removal from 21.2% to 32.4%, representing a 1.53-fold increase in degradation efficiency. In contrast, further increases in H₂O₂ concentration produced smaller relative improvements in removal, suggesting diminishing returns at higher oxidant doses. This trend is consistent with the degradation rate constants, which increased with H₂O₂ concentration but at a decreasing rate. The reduced benefit observed at higher H₂O₂ concentrations may be attributed to radical scavenging reactions, in which excess H₂O₂ competes with azithromycin for HO•, thereby reducing the fraction of radicals available for contaminant degradation. Similar behavior has been reported in UV/H₂O₂ systems treating pharmaceutical contaminants, where an optimal oxidant concentration exists beyond which additional H₂O₂ provides limited improvements in treatment performance.

The results of this study are consistent with broader findings reported for advanced oxidation processes. Miklos et al. (2018) conducted a comprehensive review of advanced oxidation technologies. They concluded that UV/H₂O₂ systems are among the most effective approaches for degrading persistent organic contaminants, as they generate highly reactive hydroxyl radicals that rapidly oxidize complex molecules. The authors also reported that treatment performance is strongly influenced by operational parameters, including oxidant concentration, UV fluence, and water chemistry. In the present study, increasing hydrogen peroxide concentration resulted in greater azithromycin degradation, supporting the conclusion that hydroxyl radical availability is a key factor controlling treatment efficiency in UV/H₂O₂ systems.

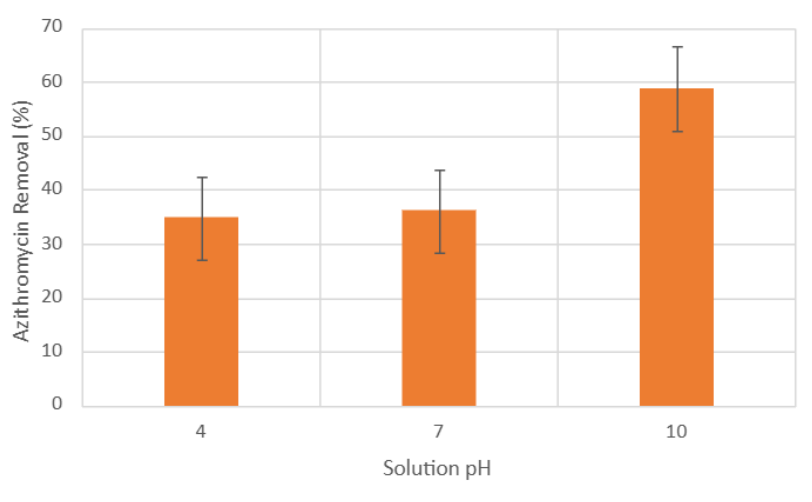


Figure 5. Effect of solution pH on azithromycin removal during UV/H₂O₂ treatment with 2 mM H₂O₂ after 30 min of irradiation. Removal efficiency increased from 34.80% at pH 4 to 58.76% at pH 10, indicating that alkaline conditions favored degradation.

3.4 Effect of pH on Azithromycin Degradation

Solution pH significantly influenced treatment efficiency, as shown in Figure 5. At pH 4, the highest removal efficiency achieved with 2 mM H₂O₂ was 34.80% after 30 minutes of treatment. At pH 7, removal increased slightly to 36.08%. The greatest degradation occurred at pH 10, where 58.76% removal was observed after 30 minutes using 2 mM hydrogen peroxide. These results indicate that alkaline conditions favored degradation within the UV/H₂O₂ system.

Solution pH affects UV/H₂O₂ degradation because it influences the chemistry of both the antibiotic and the oxidant system. The effectiveness of HO formation can vary under acidic, neutral, and alkaline conditions because pH influences radical formation, radical scavenging reactions, and the chemical form of the target compound.

Increased degradation under alkaline conditions may be attributed to enhanced oxidant activity and more favorable reaction pathways between hydroxyl radicals and azithromycin. Similar trends were reported by Zouli (2024), who observed improved azithromycin

degradation at higher pH values and increased hydrogen peroxide concentrations. These findings further support the conclusion that water chemistry plays an important role in determining the performance of UV/H₂O₂ treatment.

Miklos et al. (2018) also identified water chemistry and operational conditions as major factors controlling the performance of advanced oxidation processes. Their review emphasized that treatment efficiency can vary with changes in pH because solution chemistry influences radical generation, radical scavenging reactions, and contaminant reactivity. The agreement between the present study and previous literature highlights the importance of optimizing pH conditions when applying UV/H₂O₂ treatment for antibiotic removal.

4. Conclusions

UV/H₂O₂ treatment significantly improved azithromycin degradation compared to UV photolysis alone. The highest removal efficiency (58.76%) was observed at pH 10 with 2 mM H₂O₂, demonstrating the importance of both oxidant concentration and solution pH. The highest removal efficiency (58.76%) was observed at pH 10 with 2 mM H₂O₂, demonstrating the importance of both oxidant concentration and solution pH. Future work will focus on completing gamithromycin degradation experiments and evaluating the influence of pH on treatment performance for both macrolides. Additional studies will investigate the effects of environmentally relevant water matrix constituents, including bicarbonate, chloride, nitrate, salinity, and dissolved organic matter, on the efficiency of UV/H₂O₂ treatment. These experiments will provide a better understanding of how real water quality conditions affect antibiotic degradation and will support the development of advanced oxidation processes for practical wastewater treatment applications.

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6b. Papers/ posters Published

Tolle, C., Nejad, A. S., and Mangalgiri, K., 2025. UV-Treatment of Contaminants of Emerging Concern in Wastewater. Undergraduate Research Symposium, Oklahoma State University, Stillwater, OK. Summer 2025. Poster Presentation.

Tolle, C., Nejad, A. S., and Mangalgiri, K., 2025. UV-Treatment of Contaminants of Emerging Concern in Wastewater. Undergraduate Research Symposium, Oklahoma State University, Stillwater, OK. 2025. Poster Presentation.

Tolle, C., Nejad, A. S., and Mangalgiri, K., 2026. UV-Treatment of Contaminants of Emerging Concern in Wastewater. Undergraduate Research Symposium, Oklahoma State University, Stillwater, OK. 2026. Poster Presentation.

Tolle, C., Nejad, A. S., and Mangalgiri, K., 2025. UV-Treatment of Contaminants of Emerging Concern in Wastewater. Oklahoma Research Day, Northeastern State University, Tahlequah, OK. Poster Presentation.

Tolle, C., Nejad, A. S., and Mangalgiri, K., 2026. UV-Treatment of Contaminants of Emerging Concern in Wastewater. National Conference on Undergraduate Research, Richmond, VA. Poster Presentation.

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Advanced Oxidation of Azithromycin in Water Using UV/H₂O₂

Chole Tolle | Niblack Research Scholar

Oklahoma State University

PI: Dr. Kiranmayi Mangalgiri • Graduate Mentor: Ali Shahidi Nejad

Where Do Antibiotics in Water Come From?

Antibiotics can reach wastewater and surface water through several pathways

People & households

After antibiotics are taken, some of the drug or its metabolites can leave the body in urine and feces and enter sewage.

Hospitals & healthcare

High antibiotic use can contribute pharmaceutical residues to wastewater leaving healthcare facilities.

Livestock & agriculture

Veterinary antibiotics can reach soil and water through manure, runoff, and animal-waste management.

Medication disposal

Unused antibiotics that are flushed or poured down drains can enter the wastewater system directly.

Wastewater treatment

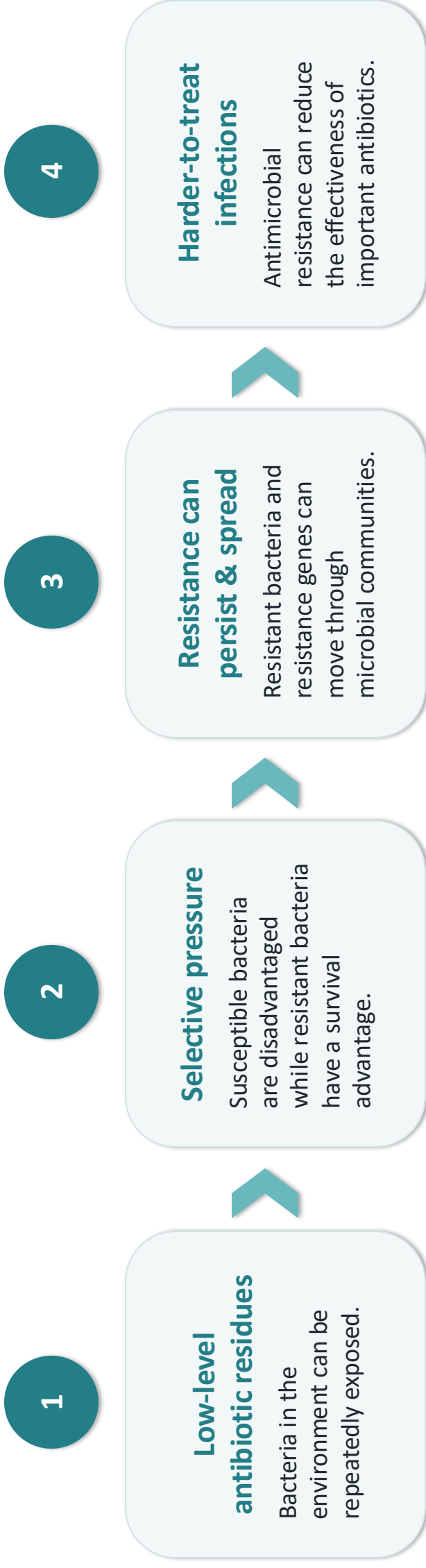
Treatment plants remove many contaminants, but they were not designed specifically to eliminate every pharmaceutical compound.



Antibiotic concentrations can be especially high in animal waste streams, with levels up to 871.7 mg/kg reported in animal manure Bolujoko et al., 2025

Why Are Antibiotic Residues in Water a Concern?

The major concern is not a prescription-sized dose in a glass of water—it is repeated environmental exposure and antimicrobial resistance

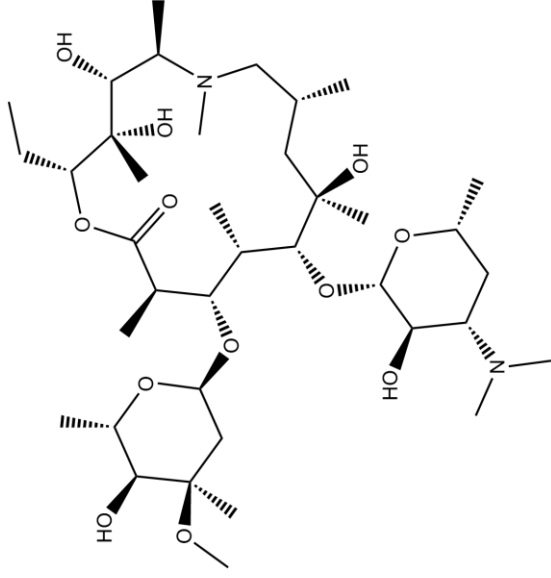


Why remove them? Preventing pharmaceutical residues from reaching water sources reduces unnecessary environmental exposure and supports safer, more effective water treatment.

Why Focus on Azithromycin?

A macrolide antibiotic used as the target pharmaceutical contaminant

AZITHROMYCIN Macrolide antibiotic



Azithromycin is a commonly used antibiotic that can enter wastewater after human use and excretion.

Its presence in water is important because antibiotics are biologically active compounds designed to affect bacteria.

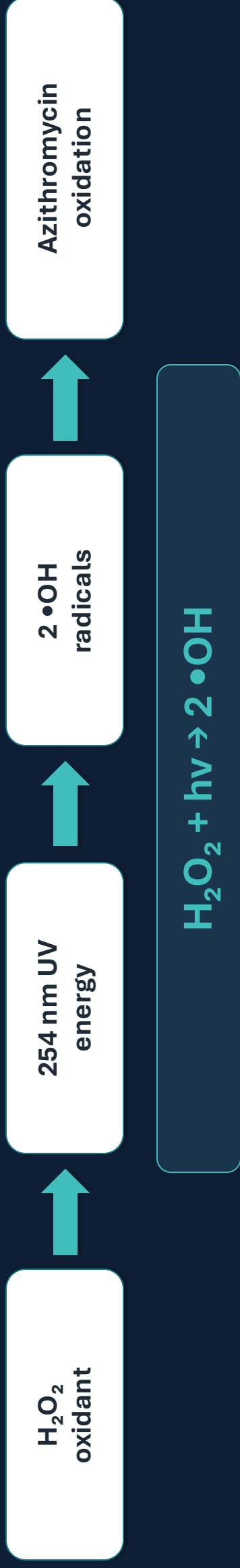
Environmental antibiotic exposure is studied partly because it can contribute to selective pressure that favors resistant bacteria.

Azithromycin therefore provides a useful target for testing whether advanced oxidation can improve pharmaceutical removal.

Research motivation: reduce the amount of biologically active antibiotic remaining in water before it reaches the wider environment.

How UV/H₂O₂ Advanced Oxidation Works

UV energy converts hydrogen peroxide into reactive hydroxyl radicals



Photolysis: H₂O₂ absorbs UV energy. The peroxide O–O bond can split and form two hydroxyl radicals (•OH).

Oxidation: •OH is highly reactive and attacks electron-rich sites in organic molecules, beginning the transformation of azithromycin.

Advanced oxidation combines physical irradiation with chemical oxidation, allowing greater degradation than UV photolysis alone under the tested conditions.

Oxidant dose must be optimized. More H₂O₂ can generate more •OH, but excessive H₂O₂ can compete for radicals (•OH + H₂O₂ → HO₂• + H₂O).

UV provides energy • H₂O₂ provides the radical precursor • •OH drives much of the contaminant oxidation

Research Question & Objectives

Which treatment conditions improve UV/H₂O₂ degradation of azithromycin?

How do H₂O₂ concentration, pH, and irradiation time influence azithromycin degradation?

UV vs. UV/H₂O₂

Determine whether adding an oxidant improves removal beyond UV alone.

H₂O₂ dose

Evaluate how oxidant availability changes degradation.

pH 4, 7, 10

Test acidic, near-neutral, and alkaline conditions to determine whether solution chemistry changes treatment performance.

Kinetics

Use concentration-versus-time data to calculate observed degradation rates.

Evaluate matrix effects

Matrix effects are reserved for future work because finalized water-quality data were not included in the report.

The core experiments isolate the effects of UV, oxidant dose, pH, and reaction time.

How the UV Reactor Works

Controlled 254 nm irradiation allows treatment conditions to be compared consistently



Reaction solution is prepared with azithromycin, phosphate buffer, and the selected H_2O_2 dose and pH.

Aliquots are transferred into quartz tubes. Quartz is used because it transmits the 254 nm UV wavelength used for irradiation.

The tubes are positioned in the carousel reactor around the low-pressure UV lamps so the irradiation geometry is controlled.

UV exposure begins the reaction. H_2O_2 photolysis generates $\bullet\text{OH}$ while direct UV effects may also contribute.

Tubes/samples are collected at predetermined irradiation times to build a concentration-versus-time profile.

The sample is quenched after collection so residual oxidant does not continue reacting before chemical analysis.

Keeping lamp wavelength, tube geometry, volume, and sampling procedure consistent helps make treatment conditions comparable.

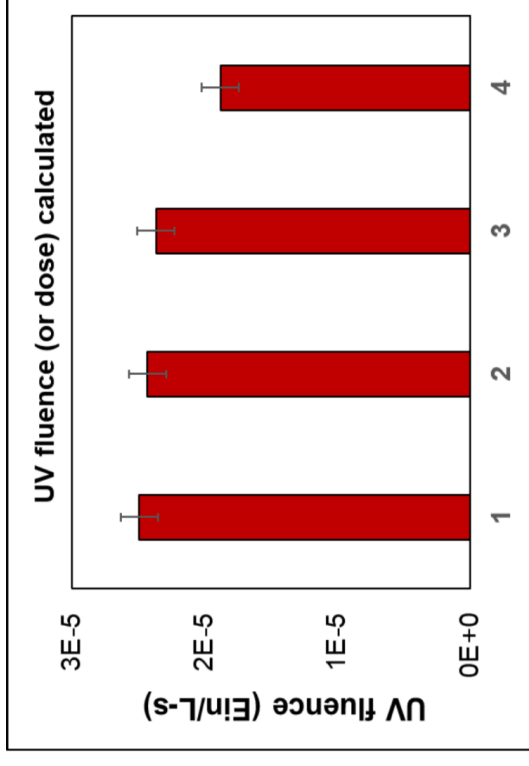
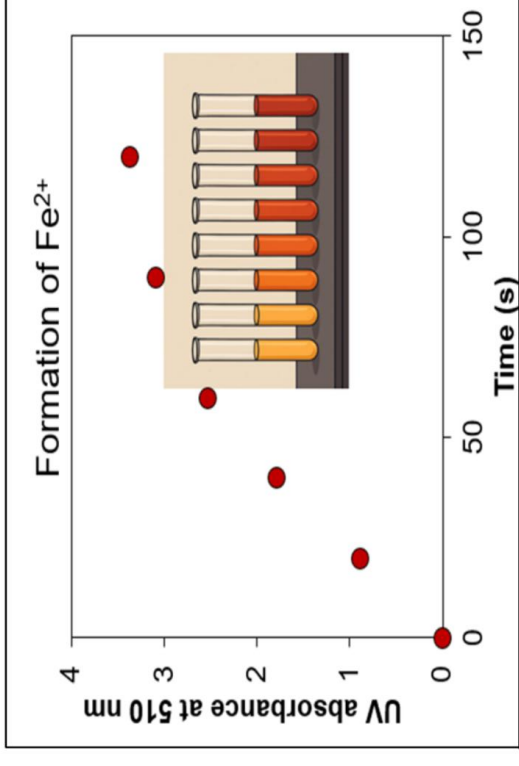
UV Reactor Characterization

Potassium ferrioxalate actinometry estimates how much usable light the reactor delivers

Photon flux
 1.87×10^{-5}
Einstein $L^{-1} s^{-1}$

Quantum yield (Φ)
1.25

- Phosphate ferrioxalate actinometry was used to quantify UV light delivery from the reactor.
- Fe^{2+} formation increased with irradiation time, as measured by absorbance at **510 nm**.
- Calculated UV fluence was compared across the four photoreactors to evaluate consistency in light delivery.



Why it matters for UV/ H_2O_2 : photon delivery affects H_2O_2 photolysis, which affects $\bullet OH$ generation and therefore contaminant degradation.

Experimental Design

Controlled variables make it possible to isolate the effects of pH and H₂O₂

Target compound	Azithromycin	The contaminant being degraded
Initial AZI	1 mg/L	Same starting concentration for comparison
UV wavelength	254 nm	Activates H ₂ O ₂ and provides consistent irradiation
pH	4, 7, 10	Represents acidic, near-neutral, and alkaline conditions
H ₂ O ₂	Multiple doses	Tests the effect of oxidant availability
Buffer	10 mM phosphate	Helps maintain the selected pH
Treatments	UV-only / UV + H ₂ O ₂	Separates UV effects from advanced oxidation

Why multiple pH values? pH can change antibiotic speciation and radical reaction pathways, so the same treatment may perform differently at different pH.

Pseudo-First-Order Kinetics

Turning concentration measurements into a rate that can be compared across experiments

$$\ln(C/C_0) = -k_{\text{obs}}t$$

At each sampling time, the remaining azithromycin concentration (C) is divided by its initial concentration (C_0).

Taking the natural log of C/C_0 converts an ideal first-order exponential decay into a straight-line relationship with time.

When $\ln(C/C_0)$ versus time is approximately linear, the negative slope is the observed rate constant, k_{obs} .

A larger k_{obs} means the parent azithromycin concentration is decreasing more quickly.

“Pseudo” first order means the complicated $\text{UV}/\text{H}_2\text{O}_2$ chemistry is represented by one apparent rate constant under a fixed set of experimental conditions.

Why calculate k_{obs} ?

Condition A
smaller |slope|

Condition B
larger |slope|

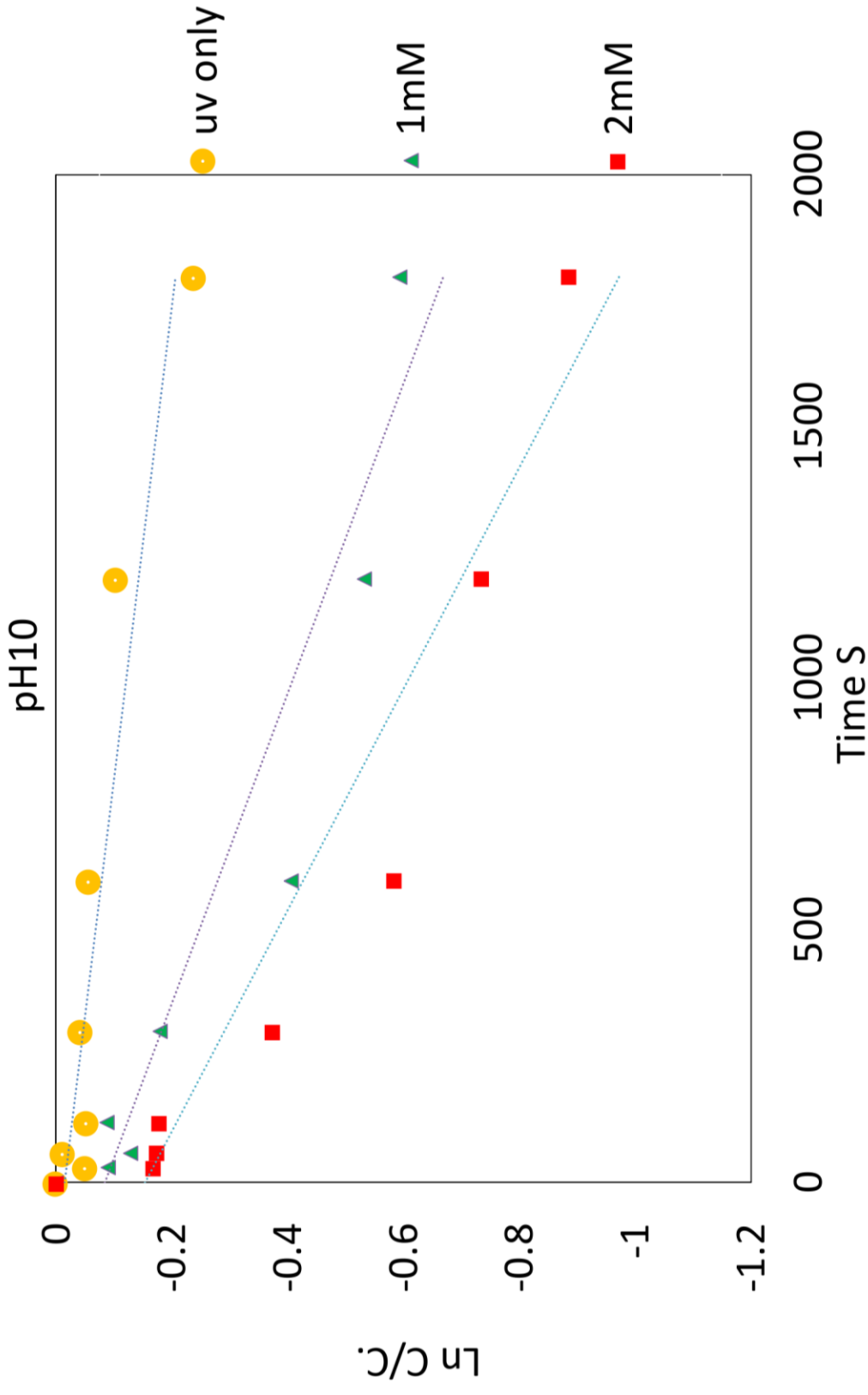
It provides a single number for comparing treatments.

You can compare UV-only vs. $\text{UV}/\text{H}_2\text{O}_2$, different H_2O_2 doses, or different pH values.

The linear fit also helps show whether the pseudo-first-order model reasonably describes the measured data.

Steeper negative slope $\rightarrow$ larger k_{obs} $\rightarrow$ faster apparent degradation

Pseudo-First-Order Degradation Kinetics at pH 10



Key Takeaway

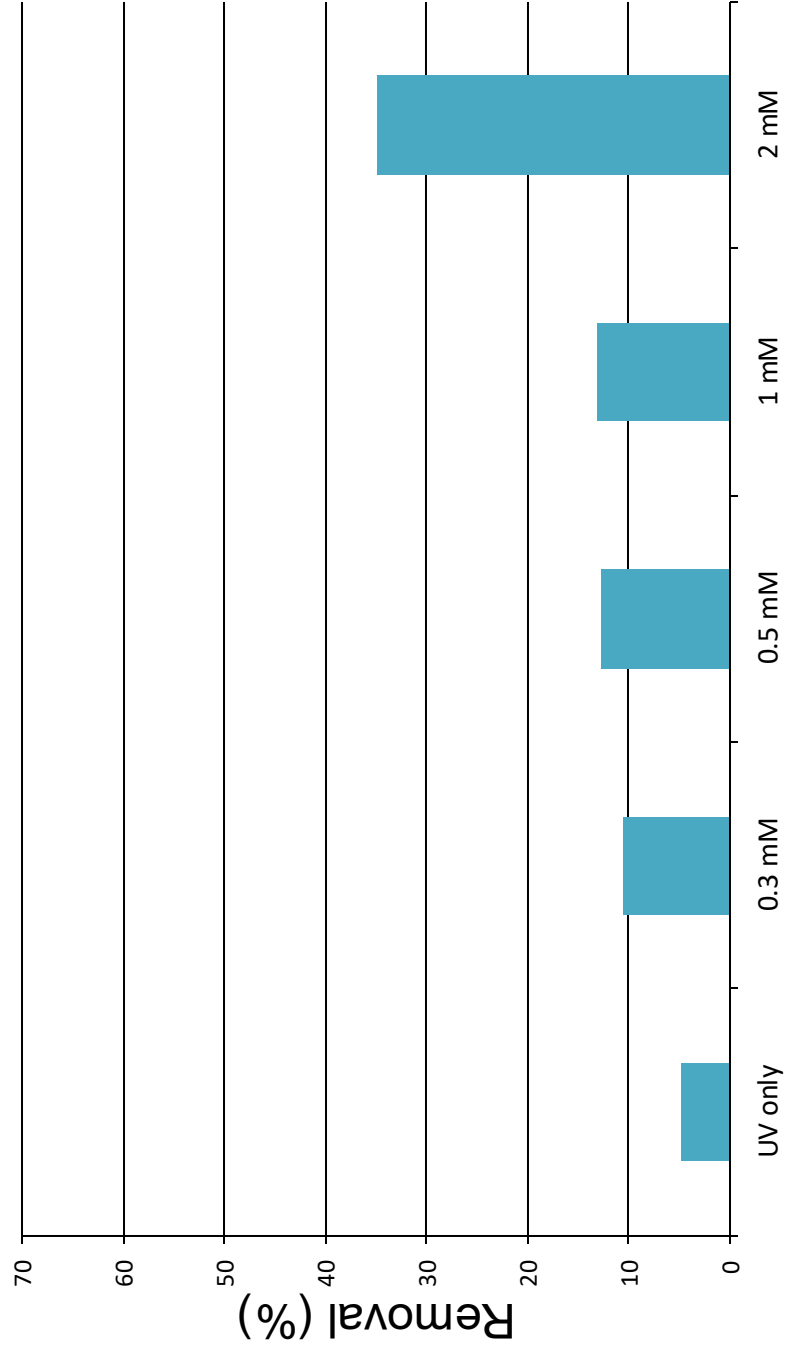
- Increasing H_2O_2 increased the degradation rate.
- **2 mM H_2O_2 had the steepest negative slope,** indicating the fastest azithromycin degradation.
- UV alone showed the slowest degradation.

Steeper negative slope =
larger k_{obs} = faster
degradation

Effect of H₂O₂ Concentration at pH 4

1 mg/L azithromycin • 10 mM phosphate buffer • 30 min irradiation • 254 nm UV

Removal (%)



Key result

UV-only removal: 4.70%

Removal increased to 34.80% at 2 mM H₂O₂.

Increasing H₂O₂ generally increased azithromycin removal.

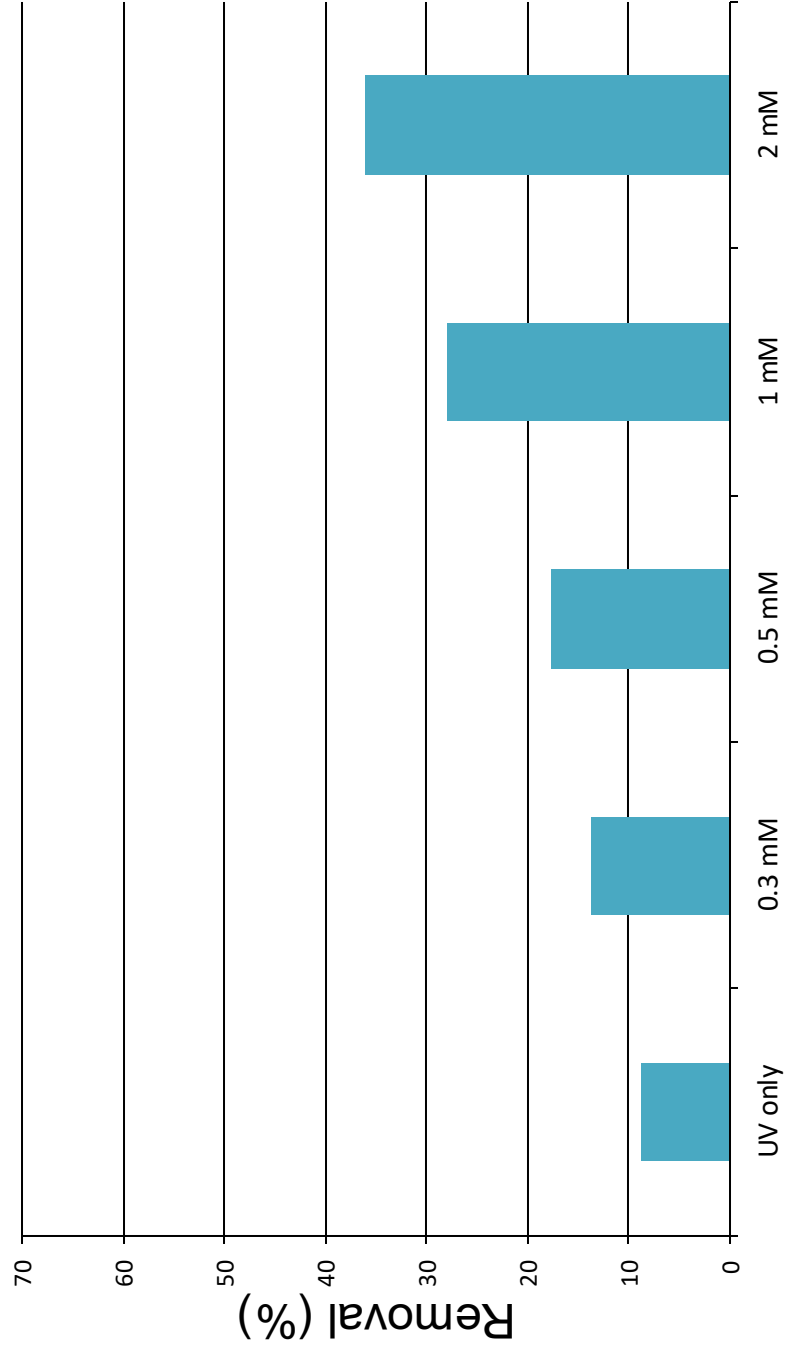
UV/H₂O₂ outperformed UV alone at this pH.

Takeaway: At pH 4, adding H₂O₂ substantially improved removal compared with UV alone.

Effect of H₂O₂ Concentration at pH 7

1 mg/L azithromycin • 10 mM phosphate buffer • 30 min irradiation • 254 nm UV

Removal (%)



Key result

UV-only removal: 8.72%

Removal increased to 36.08% at 2 mM H₂O₂.

Increasing H₂O₂ generally increased azithromycin removal.

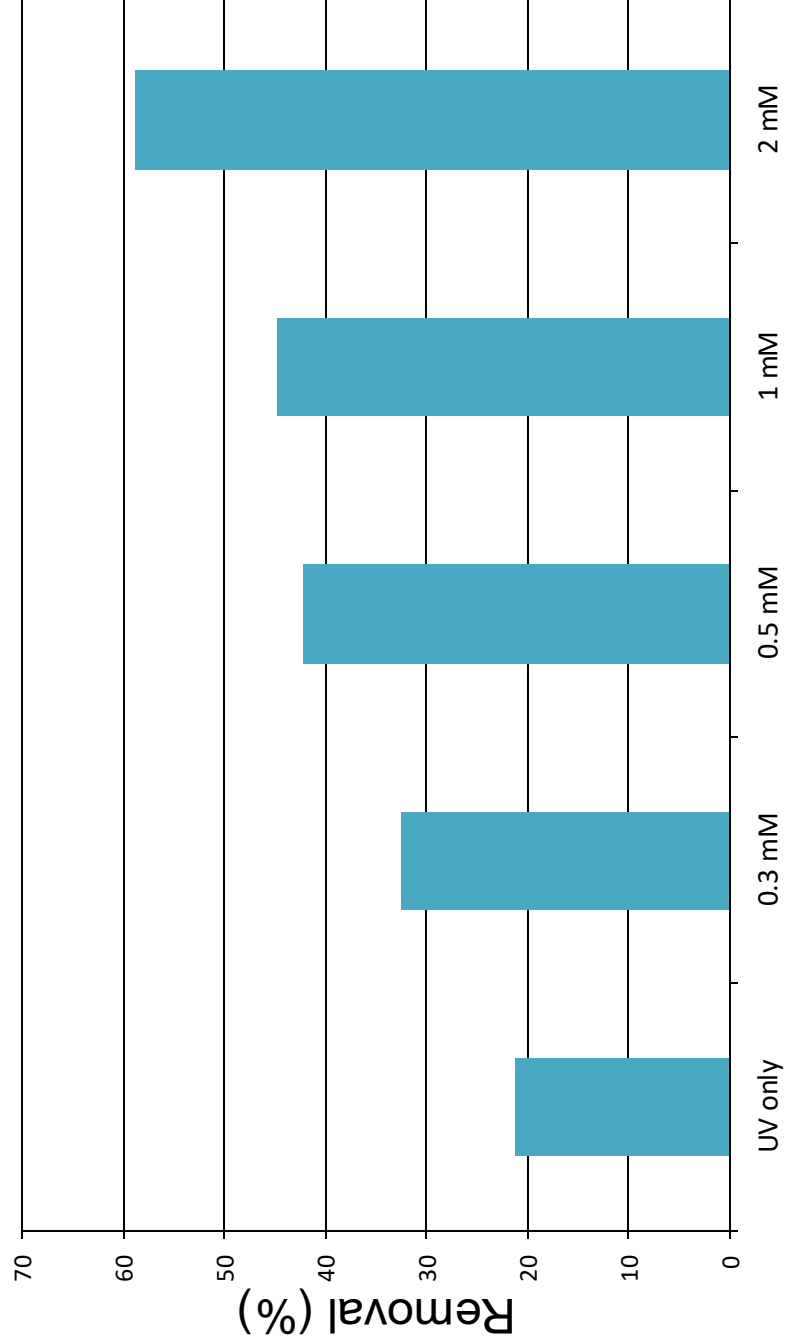
UV/H₂O₂ outperformed UV alone at this pH.

Takeaway: At pH 7, adding H₂O₂ substantially improved removal compared with UV alone.

Effect of H₂O₂ Concentration at pH 10

1 mg/L azithromycin • 10 mM phosphate buffer • 30 min irradiation • 254 nm UV

Removal (%)



Takeaway: pH 10 + 2 mM H₂O₂ gave the highest observed removal: 58.76%.

Key result

UV-only removal: 21.20%

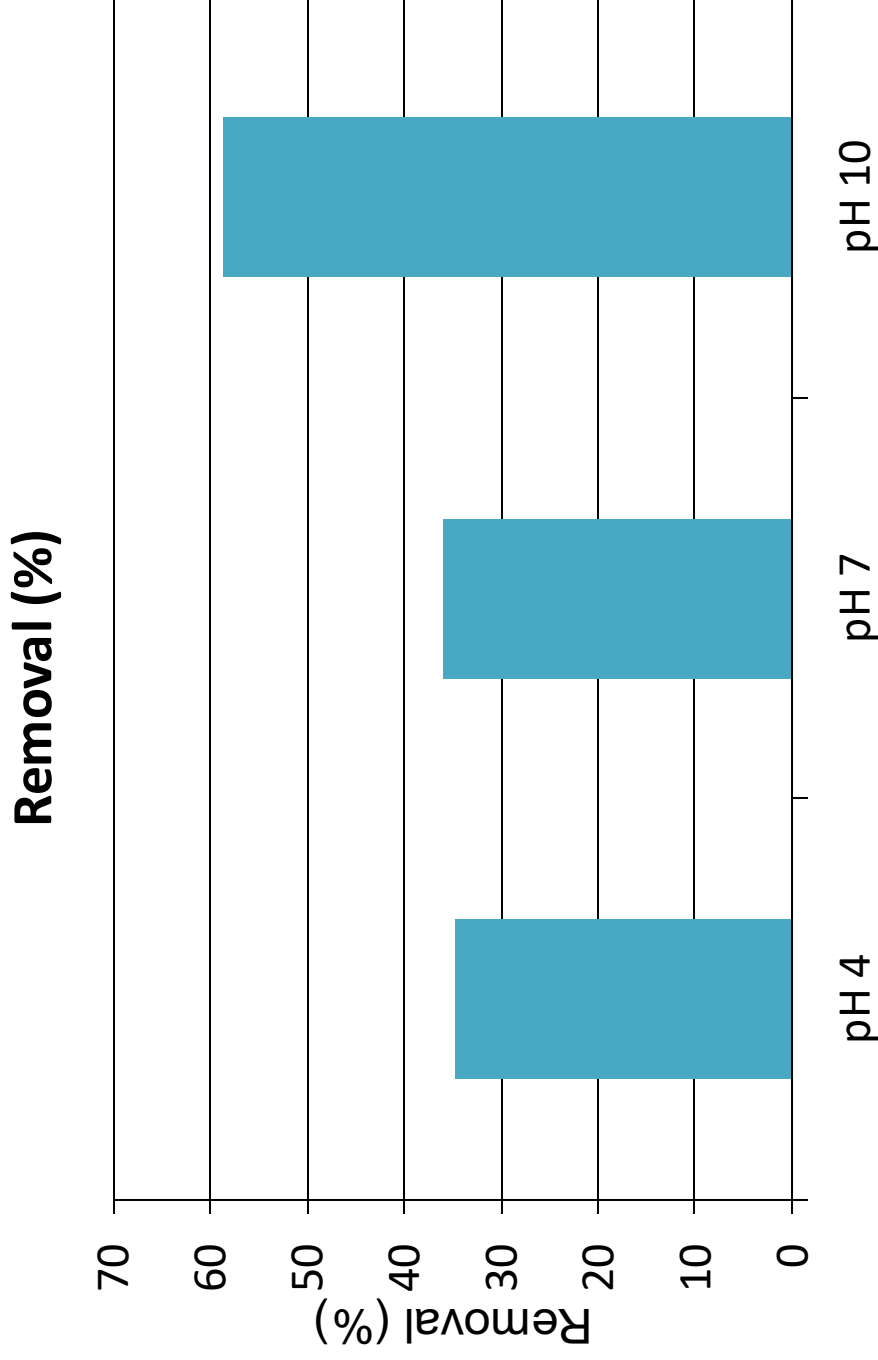
Removal increased to 58.76% at 2 mM H₂O₂.

Increasing H₂O₂ generally increased azithromycin removal.

This was the strongest pH condition tested.

Effect of pH on Azithromycin Degradation

Comparison of 30-minute removal at 2 mM H₂O₂



What the data show

pH 4 and pH 7 produced similar removal (~35–36%).

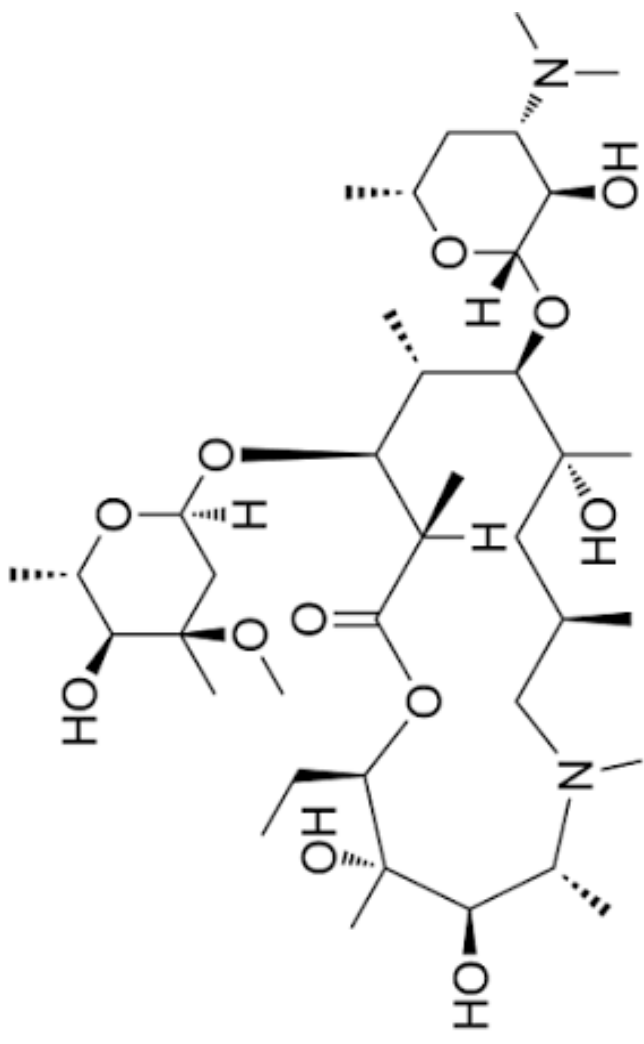
pH 10 increased removal to 58.76%.

Alkaline conditions gave the best treatment performance under these conditions.

Why Did Higher pH Improve Degradation?

Why does this matter when it comes to water treatment?

- Azithromycin contains **ionizable amine groups**, so its chemical form changes with pH.
- At **lower pH**, azithromycin is more **protonated and positively charged**.
- As pH increases, azithromycin becomes **less protonated**.
- This change in **chemical speciation** can affect how azithromycin reacts during UV/H₂O₂ treatment.
- At **pH 10**, azithromycin was likely **more susceptible to oxidation**.
- **pH 10 produced the highest removal: 58.8%.**



Key Findings

What the experiments show about azithromycin degradation

UV only

4.7–21.2% removal

UV-only removal remained below the best UV/H₂O₂ condition and increased with pH.

UV/H₂O₂

Enhanced degradation

Adding H₂O₂ introduced radical-driven oxidation.

pH effect

58.8% at pH 10

Alkaline conditions gave the highest reported 30-min removal at 2 mM H₂O₂.

Kinetics

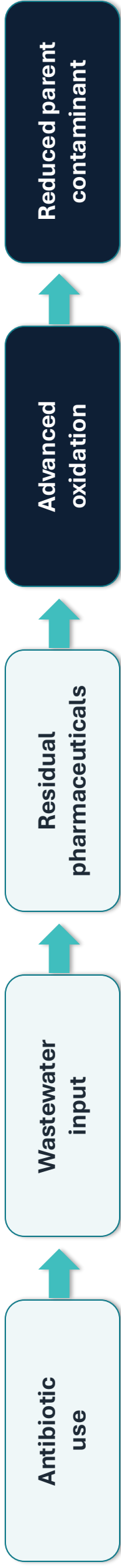
Pseudo-first-order
analysis

Rate constants provide a way to compare treatment conditions beyond final removal.

Overall: treatment performance depended on both the oxidation process and the chemical conditions of the water.

Environmental Significance

Why optimizing UV/H₂O₂ treatment matters beyond the laboratory



Advanced oxidation can provide an additional treatment barrier for organic contaminants that are difficult to remove completely by conventional processes.

Understanding pH, H₂O₂ dose, UV exposure, and matrix chemistry helps identify conditions that use light and chemicals more effectively.

For antibiotic contaminants, reducing the concentration of the biologically active parent compound may help reduce environmental exposure.

Practical treatment design must also consider energy use, chemical cost, transformation products, toxicity, and performance in real water.

Limitations & Future Research

Next steps after the current azithromycin experiments



Evaluate nitrate, chloride, bicarbonate, and real-water matrix effects as future work.



Repeat key treatment conditions and strengthen replicate statistics.

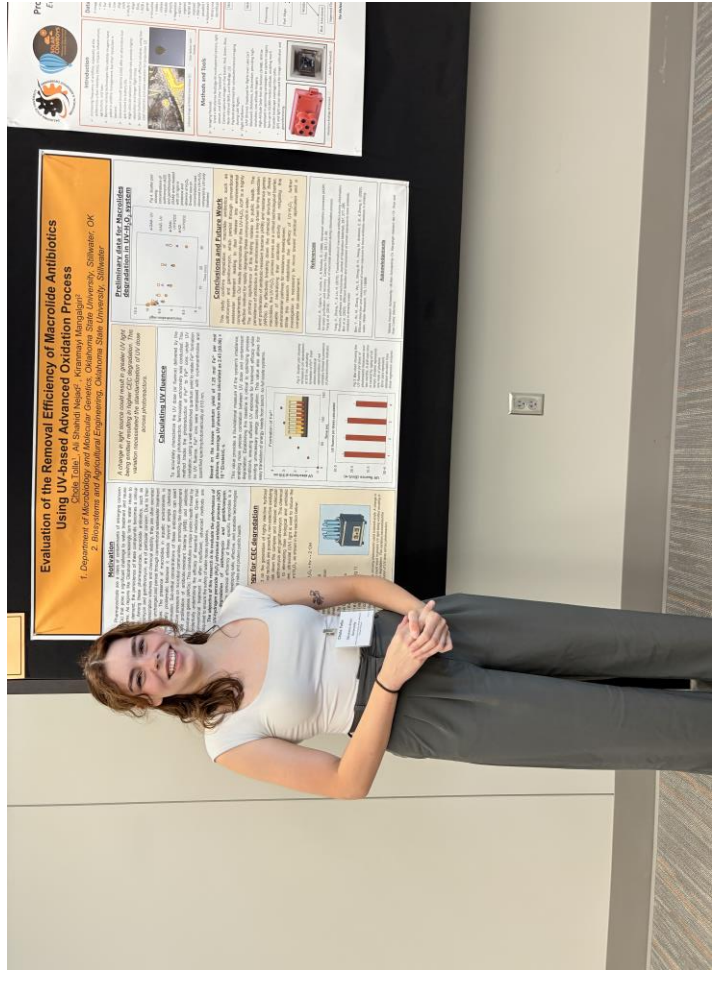
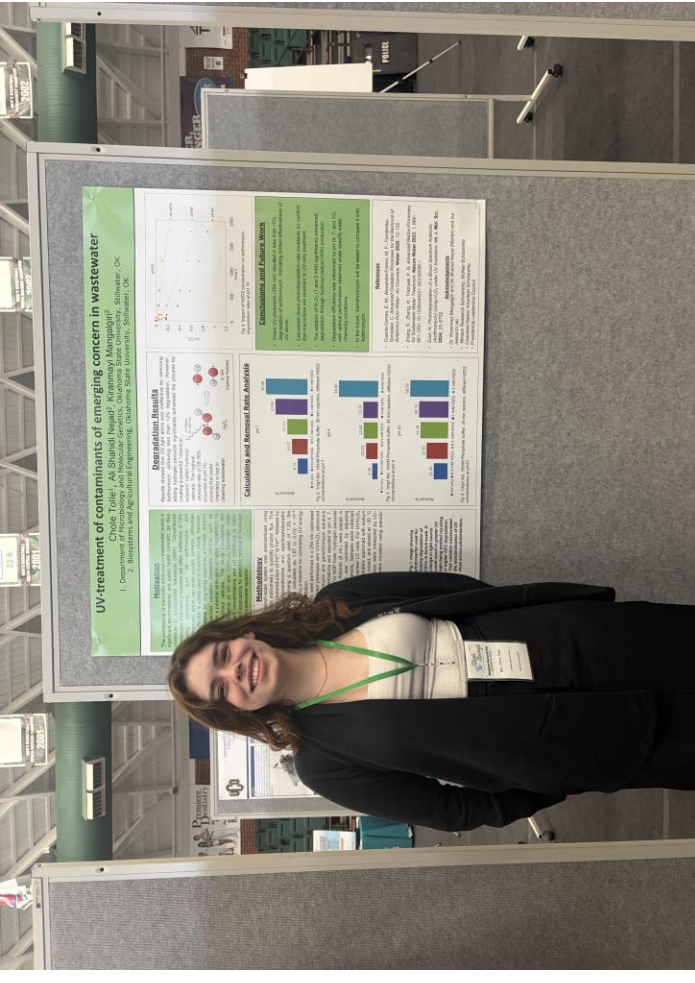
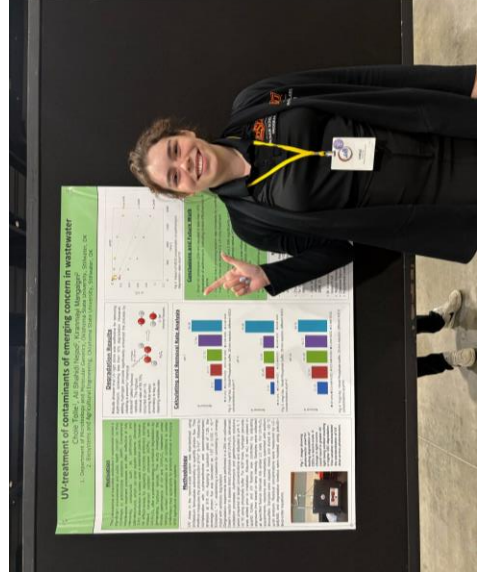
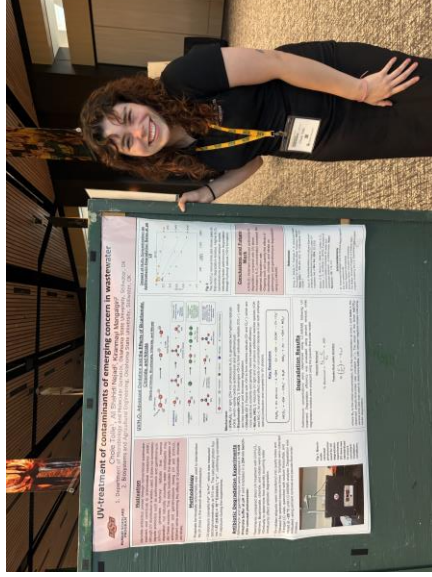
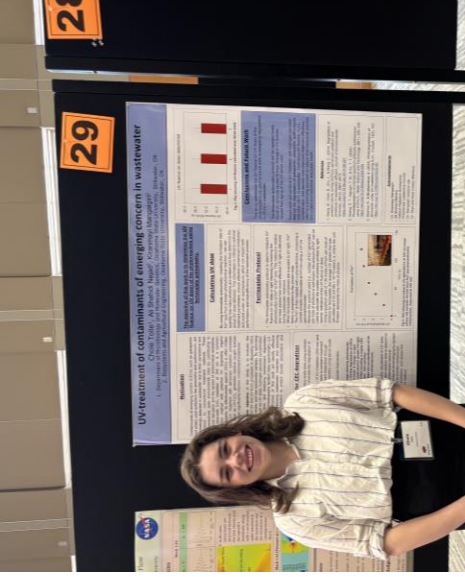


Identify transformation products and assess toxicity/biological activity.



Optimize UV energy and H₂O₂ dose together for treatment efficiency.

Conferences



Thank You

Dr. Kiranmayi Mangalgiri

Ali Shahidi Nejad

Niblack Research Scholars Program

McNair Scholars Program

Oklahoma State University

Questions?

Catalytic Conversion of Cyclopentanone to Sustainable Aviation Fuel Precursors

Noor Atiyeh

School of Chemical Engineering

Faculty Sponsor: Hong Je Cho

Graduate Student Mentor: Mohd Tauhid Khan

ABSTRACT

Sustainable aviation fuel (SAF) is needed to reduce the aviation industry's reliance on fossil fuels. In this study, the self-aldol condensation of biomass-derived cyclopentanone (CPO) to form sustainable aviation fuel precursor (dimer of CPO; CPO₂) was investigated using zeolite catalysts. The effects of the zeolite structure, pore size, Brønsted acidity, and metal oxide loading on catalytic performance under solvent and solventless conditions were evaluated. Zeolite Y, ZSM-5, and Al-BEA were studied. Among the parent catalysts tested, zeolite Y exhibited the highest CPO₂ yield due to its larger pore size and volume, which improved reactant diffusion and access to active sites. Lower Brønsted acid site density of zeolites promoted higher selectivity towards the desired product. Loading CaO onto the parent zeolite drastically reduced reactant conversion, while MgO loading moderately reduced reactant conversion while improving selectivity towards CPO₂. These results demonstrate that zeolite pore structure and acidity influence catalytic performance in the aldol condensation of CPO.

1. Introduction

The depletion of petroleum resources to meet global oil demands has led to a growing shift towards renewable energy sources. While the auto industry is addressing this challenge through the partial adoption of electric vehicles, fully electrifying airplanes is not currently feasible, leaving a critical need for alternative energy sources. In the United States alone, over 26 billion gallons of jet fuel are consumed annually, a figure that is expected to double within the next twenty years (Holladay, Abdullah and Heyne, 2020). Additionally, the aviation sector is responsible for approximately 2.4% of global CO₂ emissions, significantly contributing to the greenhouse effect and global warming (Abrantes et al., 2021). This underscores the critical need for sustainable aviation fuel (SAF) to address the industry's dependence on fossil fuels and contribution to climate change. According to earlier research, meeting 100% of aviation fuel demand with SAF by 2050 could cut emissions by 63% (ICAO, 2019). In this regard, biomass has garnered significant attention for SAF production because it is abundant, renewable, low-cost, carbon-neutral, and compatible with existing infrastructure (Holladay, Abdullah and Heyne, 2020). Specifically, biomass-derived

oxygenates such as cyclopentanone (CPO) can undergo aldol condensation over zeolite catalysts to produce jet fuel precursors (i.e., 10-carbon CPO₂ and 15-carbon CPO₃) due to zeolites' porosity

and Brønsted

acidity (Figure 1)

(Holladay,

Abdullah and

Heyne, 2020;

Abrantes et al.,

2021; Cueto et al.,

2024). However, it

is not yet clearly

understood how zeolite porosity and Brønsted acidity, in combination with solvents, influence the overall catalytic performance in this reaction. Moreover, integrating basic metal oxides such as CaO and MgO with zeolites show promise in the reaction due to the synergy of acid-base catalytic active sites, but its effectiveness remains elusive.

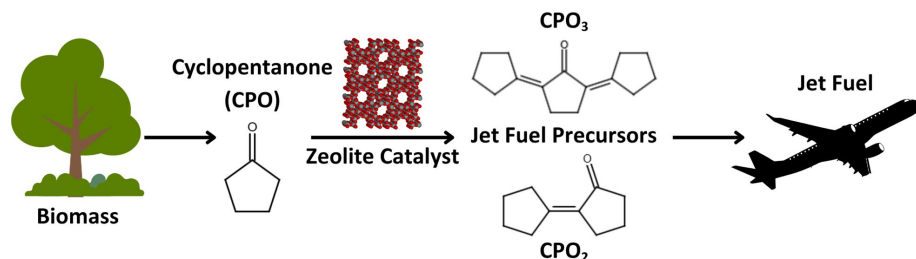


Figure 1 Sustainable Routes for Producing Aviation Fuels from Biomass

The overarching goal of this project is to improve catalyst performance in converting biomass-derived compounds (CPO) into SAF precursors. This requires improving understanding of how 1) zeolite porosity and Brønsted acid density combined with solvents and 2) zeolite-supported basic metal oxides impact catalytic performance in jet fuel precursor synthesis via the aldol condensation reactions.

2. Experimental Details

2.1 Catalyst Preparation

The parent zeolite catalysts tested were zeolite Y (Si/Al=15), zeolite Y (Si/Al=40), Al-BEA (Si/Al=12.5; CP814E), and ZSM-5 (Si/Al=15; CBV3024E). The two zeolite Y samples were obtained from Thermo Fisher Scientific, and Al-BEA and ZSM-5 were obtained from Zeolyst. CaCl₂ was obtained from Fisher Bioreagents and MgCl₂ was obtained from Sigma Aldrich, which were used as precursor salts for the loading of CaO and MgO onto the parent zeolite catalysts. To prepare the metal oxide loaded zeolites, the desired amount of metal salt precursor was dissolved in DI water. Half of the solution was added to the zeolite catalyst,

and the resulting mixture was stirred. The remaining metal chloride solution was then added, and the mixture was stirred. The catalyst was dried at 80°C for 18 h. Finally, the loaded zeolites were calcined in a tube furnace under air flow at 550°C for 4 h with a heating ramp of 2°C/min.

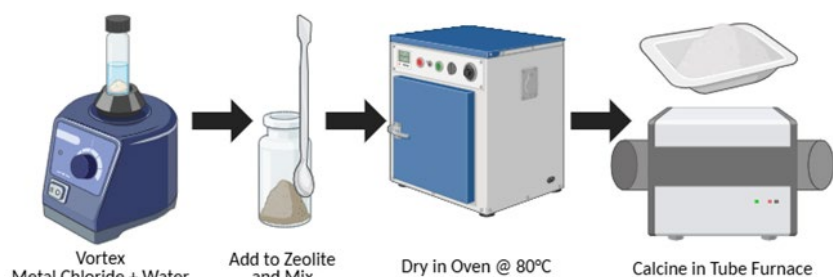


Figure 2 Catalyst Preparation Procedure

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2.2 Catalyst Characterization

Crystallinity and framework structure of the catalysts were confirmed using X-ray diffraction (XRD) on a Philips diffractometer with $\text{CuK}\alpha$ radiation ($\lambda_1 = 1.5406 \text{ \AA}$, $\lambda_2 = 1.5444 \text{ \AA}$, $K\alpha_2/K\alpha_1 = 0.5$) operated at 45 kV and 40 mA. Diffraction patterns were collected in continuous scan mode over a 2θ range of 4° to 40° with a 0.06° step size and 0.5 s per step. The textural properties of the zeolites, including BET surface area, micropore volume, and total pore volume, were determined via nitrogen physisorption at 77 K on a Quantachrome Autosorb IQ-C-XR (3-station) analyzer. Prior to measurement, 400-600 mg of each sample was degassed at 300 °C for 4 h to remove any adsorbed impurities and moisture. Subsequently, nitrogen adsorption-desorption isotherms were recorded across a relative pressure range (P/P_0) of 0.005-0.995 with a 30 s equilibration time. The Brunauer-Emmett-Teller (BET) method ($P/P_0 = 0.05$ -0.25) was used to calculate BET surface area. Micropore volume was obtained from the t -plot method. The total pore volume was calculated from the amount of nitrogen adsorbed at $P/P_0 = 0.995$.

2.3 Reaction Performance Testing

To evaluate catalytic performance, aldol condensation reactions were carried out in a thick-walled glass vial used as a batch reactor. Catalytic performance was studied under two reaction conditions:

- I. 0.0875 mL CPO + 1.5 mL solvent + 120 mg catalyst, 130°C, 24 h
- II. 2.285 mL CPO + 100 mg catalyst, 130°C, 24 h

Reaction temperature was controlled using an aluminum heating block, and the reaction mixture was continuously stirred by a magnetic stir bar. Samples were then filtered using a syringe filter. Gas chromatography (GC) and GC-mass spectrometry (MS) were used for product quantification and identification, as well as for calculating reactant conversion and product selectivity.

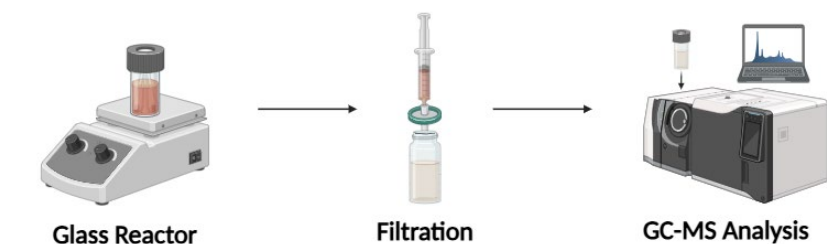


Figure 3 Experimental Procedure

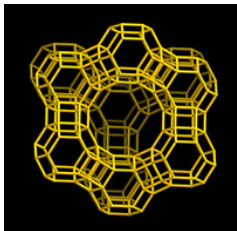
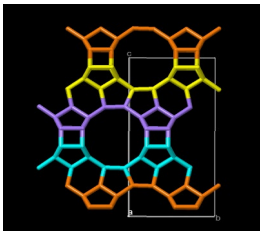
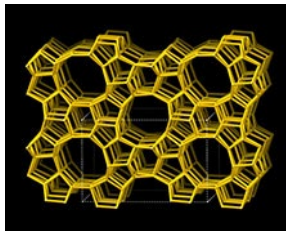
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3. Results

3.1 Evaluating Catalyst Properties

Zeolite Y possesses 12-membered aluminosilicate rings and wide supercages (Hattori and Ono, 2018). Zeolite Y exhibits both larger pore size (0.75 nm and 1.1 nm) and larger pore volume than Al-BEA and ZSM-5 (Table 1). Additionally, zeolite Y exhibits a higher BET surface area than the other zeolites (Table 2). Al-BEA also possesses 12-membered rings and is considered a large pore zeolite due to its pore size of 0.75 nm (Hattori and Ono, 2018). ZSM-5 contains 10-membered ring channels and is considered a medium pore zeolite (Hattori and Ono, 2018). ZSM-5 exhibits the smallest pore size and micropore volume among the tested zeolites.

Table 1 Structural Properties of Catalysts

	Zeolite Y	Al-BEA	ZSM-5
Framework Code	FAU	BEA	MFI
Pore Size	0.75 nm & 1.1 nm	0.75 nm	0.56 nm
Structure			

Zeolite framework structures adapted from the International Zeolite Association Structure Database (Baerlocher et al.).

Table 2 Textural Properties of Catalysts

Catalyst	Micropore Volume (cm ³ /g)	BET Surface Area (m ² /g)	Total Pore Volume (cm ³ /g)
Zeolite Y (Si/Al=15)	0.20	716	0.33
Zeolite Y (Si/Al=40)	0.20	789	0.53
Al-BEA (Si/Al=12.5)	0.17	674	1.13
ZSM-5 (Si/Al=15)	0.11	420	0.27

XRD and nitrogen physisorption data were used to confirm the structural and textural properties of the commercially obtained zeolite catalysts. The XRD data in Figure 4 confirm that the zeolites show FAU, BEA, and MFI frameworks, respectively, without having phase impurity.

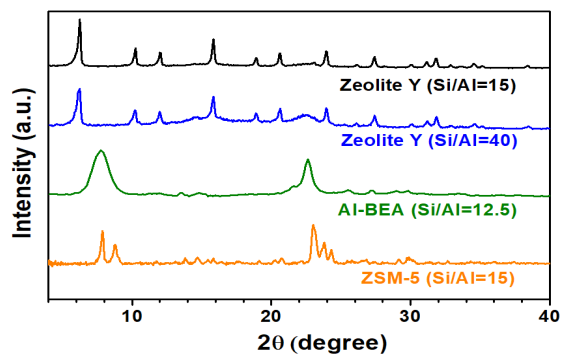


Figure 4 XRD Patterns of Zeolites Used in the Reaction

The silica-to-alumina ratio (Si/Al) of a zeolite influences the Brønsted acid site density of the catalyst. Within the tetrahedral zeolite framework, substitution of Si⁴⁺ by Al³⁺ introduces a negative charge that must be balanced by a cation (Hattori and Ono, 2018). In H-form zeolites, this charge is balanced by H⁺, creating Brønsted acid sites. Therefore, increasing the aluminum content increases the number of Brønsted acid sites, causing zeolites with lower Si/Al ratios to exhibit higher Brønsted acidity. Zeolite Y (Si/Al=40) exhibits the lowest Brønsted acidity among the parent catalysts.

Aldol condensation reactions can be catalyzed by either acid sites or base sites. Loading basic metal oxides such as CaO or MgO onto the zeolite introduces basic sites that can promote aldol condensation through enolate formation mechanisms (Kovacheva et al., 2002; Ngo et al., 2019). These newly introduced basic sites alter the overall acid-base properties of the catalyst, which can affect reactant conversion and product selectivity.

3.2 Aldol Condensation Tests

The performance of the zeolite catalysts was evaluated under varying reaction conditions, including the self-aldol condensation of CPO with and without solvent. The reactions were carried out in a batch reactor for 22 h or 24 h. The temperature of the reaction was held constant at 130°C.

Moderate reactant conversion was achieved in the self-aldol condensation of CPO in ethanol (EtOH) solvent. Reactant conversion was highest using zeolite Y (Si/Al=15) at approximately 50%, while the lowest observed conversion was obtained with zeolite Y (Si/Al=40) at approximately 35%. CPO conversion with Al-BEA and ZSM-5 fell within the range of 35-50%. The highest yield of the desired product, CPO₂, was obtained with zeolite Y (Si/Al=40) at 32%. This zeolite exhibited high selectivity toward the desired product under these reaction conditions. Zeolite Y (Si/Al=15) displayed lower selectivity toward the desired product, and most of the yield was oligomerized product. No CPO₂ formation was observed with Al-BEA or ZSM-5. These results are likely attributed to limited pore sizes of BEA and MFI, compared with CPO (kinetic diameter=0.58 nm) and CPO₂ (kinetic diameter=0.72 nm). ZSM-5 catalyst promoted the formation of ECPO (2-ethyl-2-cyclopenten-1-one). Reaction performance with zeolite Y was also evaluated using cyclohexane solvent. The CPO conversion in cyclohexane solvent is higher than in EtOH solvent for both zeolite Y (Si/Al=15) and zeolite Y (Si/Al=40). However, the selectivity toward CPO₂ was lower. Product yield using zeolite Y (Si/Al=15) was 19% and 22% with zeolite Y (Si/Al=40).

Due to the low selectivity toward the desired product in solvent, the self-aldol condensation of CPO was further evaluated under solventless conditions. The catalyst to reactant ratio was lowered from 1.37g catalyst/mL CPO to 0.04 g catalyst/mL CPO. The conversion of CPO with zeolite Y (Si/Al=15) and zeolite Y (Si/Al=40) was 16.1% and 15.8%, respectively. CPO₂ yield was higher with zeolite Y (Si/Al=40) at 11.2%, compared to 6.0% with zeolite Y (Si/Al=15).

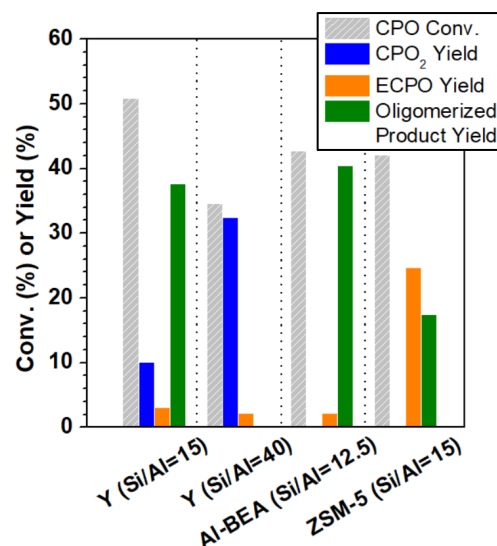


Figure 6 Product Distribution for 0.0875 mL CPO + 1.5 mL EtOH + 120 mg Catalyst, 130°C, 22 h

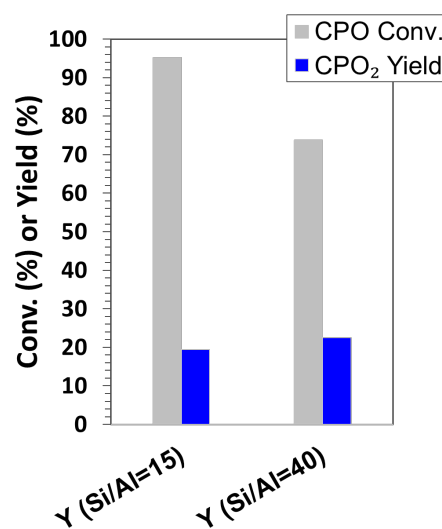


Figure 5 Product Distribution for 0.0875 mL CPO + 1.5 mL Cyclohexane + 120 mg Catalyst, 130°C, 22 h

To improve selectivity, the zeolite catalysts were loaded with either 10 wt% CaO (CaOY) or MgO (MgOY). Reactant conversion decreased substantially with CaO-loaded zeolites, with CPO conversion ranging from 1-2%. A more moderate decrease in reactant conversion was observed with MgO-loaded zeolite Y compared to the parent zeolite. CPO conversion with MgOY (Si/Al=15) was 12.4%, and 13.2% with MgOY (Si/Al=40). CPO₂ yields of 9.6% and 11.2% were obtained using MgOY (Si/Al=15) and MgOY (Si/Al=40), respectively.

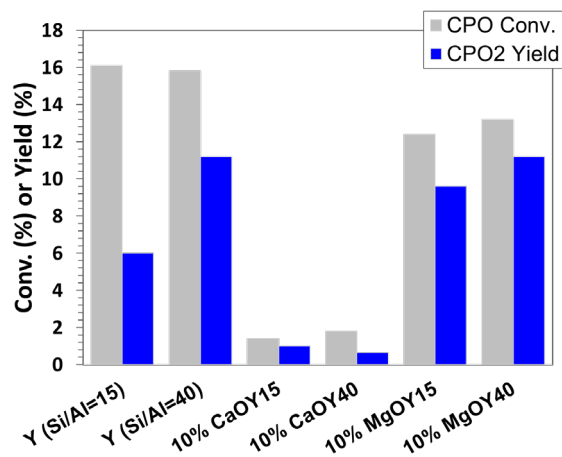


Figure 7 Product Distribution for 2.285 mL CPO + 100 mg Catalyst, 130°C, 24 h

4. Discussion and Conclusions

Of the tested parent zeolite catalysts, only zeolite Y (Si/Al=15 and Si/Al=40) promoted CPO₂ formation. Zeolites Al-BEA (Si/Al=12.5) and ZSM-5 (Si/Al=15) primarily promoted the formation of oligomerized products and ECPO in ethanol solvent. The larger pore sizes of zeolite Y allow reactant molecules to diffuse more easily through the zeolite and access the acid sites required to catalyze the aldol condensation reaction. Additionally, the higher acid site density of zeolite Y (Si/Al=15) compared to zeolite Y (Si/Al=40) promoted increased oligomerization and coke formation. In ethanol solvent, zeolite Y (Si/Al=40) exhibited a CPO₂ yield of 32% and the highest selectivity toward the desired product.

Reaction testing under solventless conditions revealed similar conversions between zeolite Y (Si/Al=15) and zeolite Y (Si/Al=40), but higher CPO₂ yields with zeolite Y (Si/Al=40). Overall, selectivity toward CPO₂ was higher with the less acidic zeolite. Loading CaO onto Zeolite Y reduced conversion to approximately 1-2%, while loading MgO caused only a moderate decrease in reactant conversion. Higher selectivity was achieved with the introduction of basic sites through MgO loading compared to the parent zeolite. The addition of basic sites promotes the base-catalyzed aldol condensation pathway of CPO. No trimer products of CPO (CPO₃) formation were observed, likely due to steric hindrance caused by the micropores of zeolite catalysts.

Future work includes increasing the catalyst-to-reactant ratio under solventless conditions for the aldol condensation reaction. Additionally, hierarchical zeolites with dual

micropores and mesopores (i.e., pores between 2 nm and 50 nm) may be evaluated as catalysts to alleviate mass transfer of reactants and products.

5. Summary

The self-aldol condensation of CPO proceeds over zeolite catalysts to form the sustainable aviation fuel precursor CPO₂. Catalyst properties such as zeolite structure and acidity affect the performance of the reaction. Overall, zeolite Y showed the highest CPO₂ yield due to its larger pore size. Reduced acid site density promoted greater selectivity towards CPO₂. Introducing basic sites via CaO loading reduced reactant conversion, while MgO loading increased selectivity toward CPO₂.

6. Appendices

6a. Acknowledgements

I would like to sincerely thank Dr. and Mrs. Niblack for making this opportunity possible through the Niblack Research Scholars program. Additionally, I would like to thank Dr. Cho for his mentorship, guidance, and support throughout this project. I am grateful for my graduate student mentors, Mohd Tauhid Khan, Md Razaul Karim and Vinayak Srivastava for their guidance and always encouraging me to ask questions.

6b. Papers Published

Atiyeh, N., Srivastava, V., Khan, M.T., Cho, H.J. (2025) Catalytic Conversion of Cyclopentanone and Furfural to Sustainable Aviation Fuel Precursors [Poster presentation]. OSU Undergraduate Summer Research Expo, Stillwater, OK, 24 July.

Atiyeh, N., Karim, M.R., Srivastava, V., Cho, H.J. (2026) Catalytic Conversion of Cyclopentanone to Sustainable Aviation Fuel Precursors [Poster presentation]. OSU Undergraduate Research Symposium, Stillwater, OK, 21 April.

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Catalytic Conversion of Cyclopentanone to Sustainable Aviation Fuel Precursors

Noor Atiyeh

School of Chemical Engineering,
Oklahoma State University

Niblack Research Scholars Day

Oct. 2nd, 2026



Cho
Research
Group

Introduction

Why Sustainable Aviation Fuel?

- Growing demand for jet fuel and depletion of petroleum resources
 - Need for alternative fuel sources
- Auto industry: electric cars
- Not feasible to fully electrify airplanes
- Sustainable aviation fuel (SAF) produced from biomass
 - Abundant, renewable, compatible with existing infrastructure
 - Reduces CO₂ emissions



Introduction

Aldol Condensation Reaction

- Biomass derived oxygenates such as cyclopentanone (CPO) can undergo carbon-carbon coupling reactions to form jet fuel precursors
- Reaction can be catalyzed by zeolites

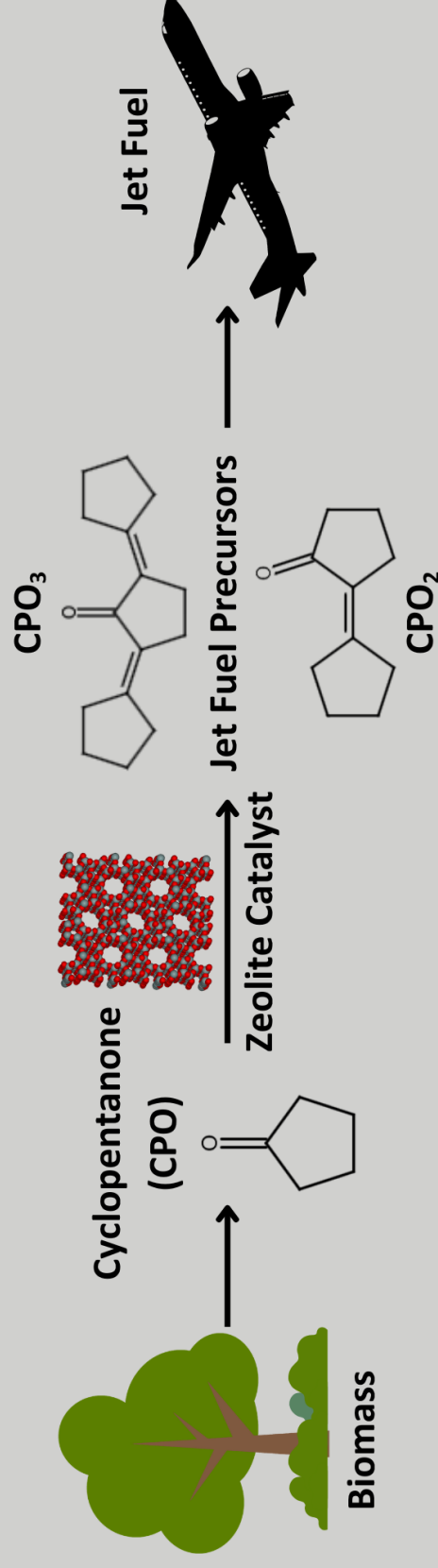


Figure 1 Sustainable Routes for Producing Aviation Fuels from Biomass



Introduction

Research Gaps

- It is not yet clearly understood how zeolite porosity and Brønsted acidity, in combination with solvents, influence the overall catalytic performance in this reaction

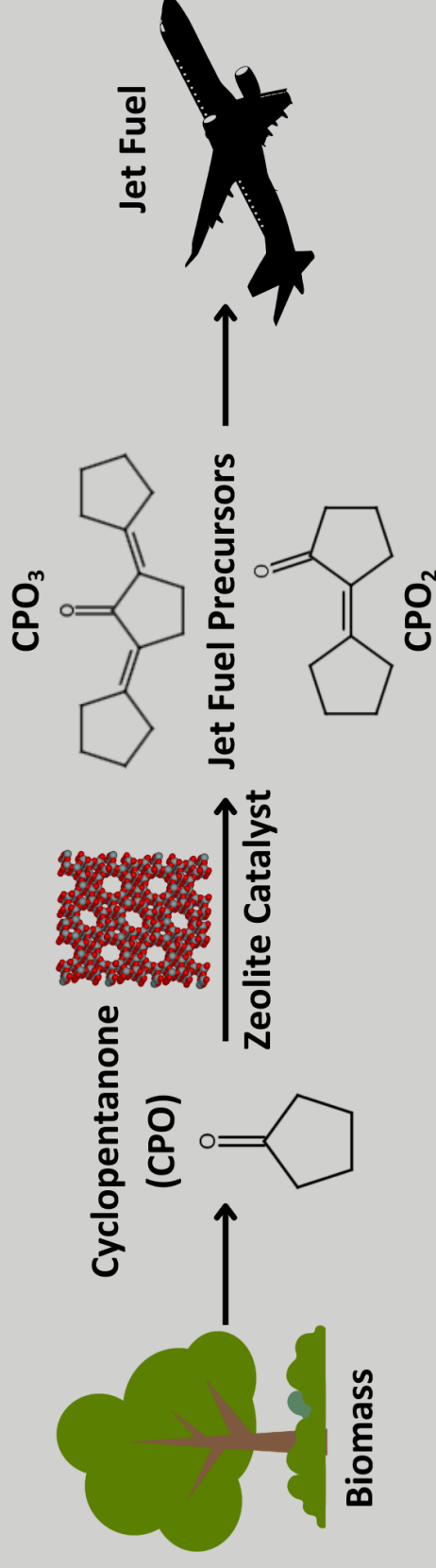


Figure 1 Sustainable Routes for Producing Aviation Fuels from Biomass



Objectives

- Investigate the influence of zeolite structure on catalytic performance in the production of SAF precursors
 - Zeolite porosity
 - Brønsted acid density
 - Solvents
 - Zeolite-supported basic metal oxides (CaO, MgO)



What are Zeolites?

Why use them as catalysts?

- Zeolites are porous, inorganic, crystalline aluminosilicates
- Solids, heterogeneous catalysts
- High surface areas, defined pore structures, tunable acidity, and reusability

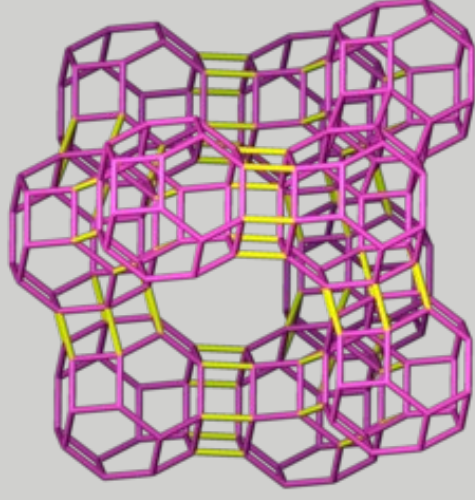
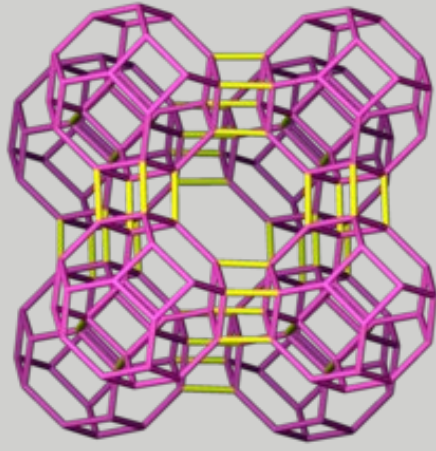


Figure 2 Zeolite Structure

By Nao1958 - Own work, CC BY-SA 4.0,
<https://commons.wikimedia.org/w/index.php?curid=75387204>



What are Zeolites?

Acid Qualities

- Acid qualities can be manipulated by changing the silica-to-alumina (Si/Al) ratio
- Increasing the Si/Al ratio decreases the acid site density
 - Due to the tetrahedral structure of zeolites, Al atoms must be balanced by a proton, creating a Brønsted acid site

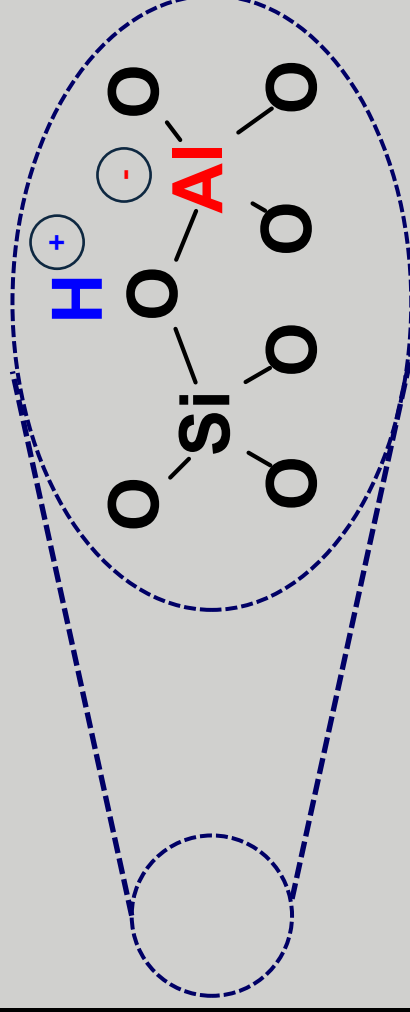
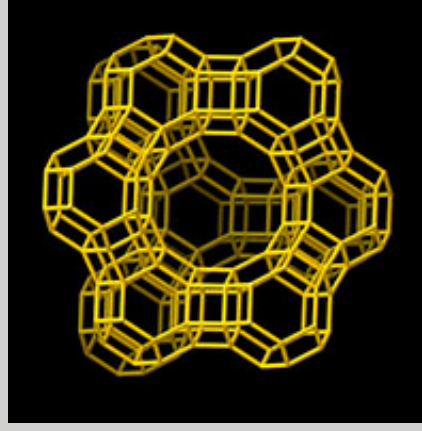


Figure 3 Zeolite Structure and Acid Qualities



Methods

Catalyst Preparation

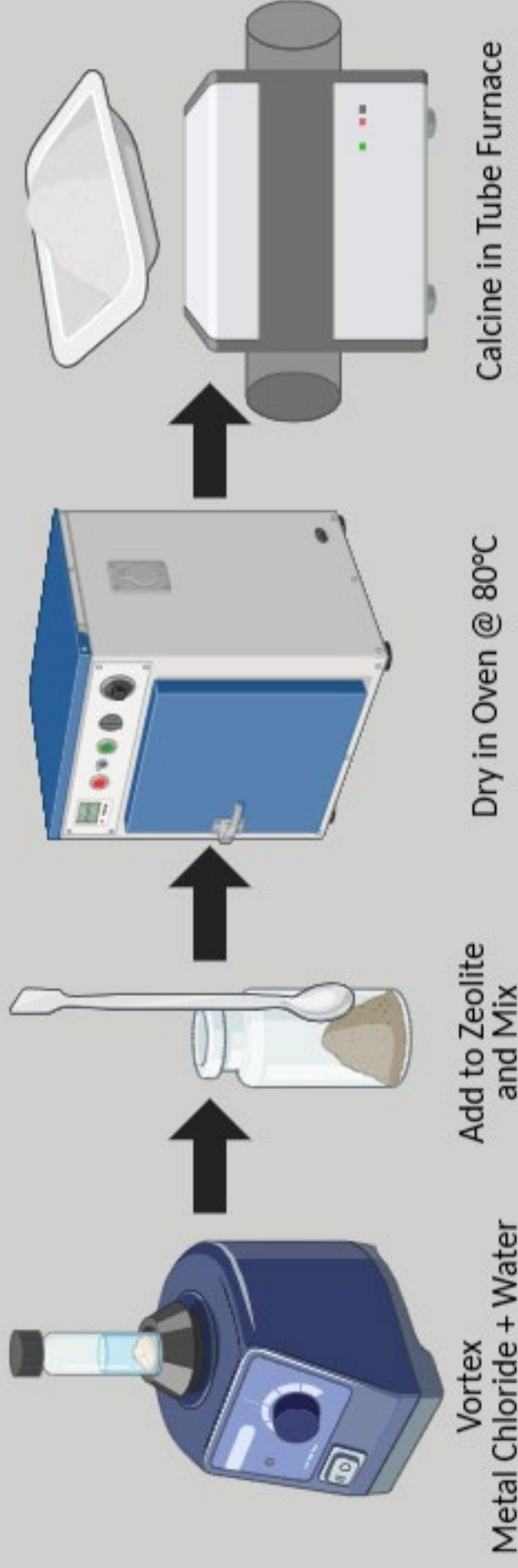


Figure 4 Catalyst Preparation Procedure

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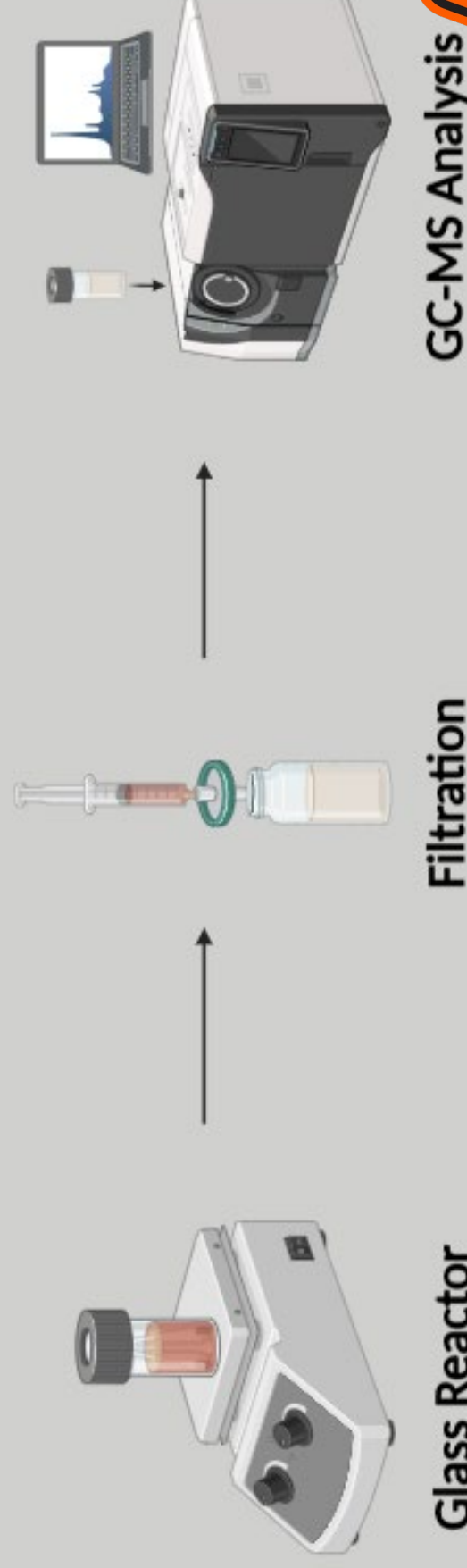


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Methods

Reaction Testing

- Reaction Conditions:
 - I. 0.0875 mL CPO + 1.5 mL solvent + 120 mg catalyst, 130°C, 22 h
 - II. 2.285 mL CPO + 100 mg catalyst, 130°C, 24 h



Glass Reactor

Filtration

GC-MS Analysis

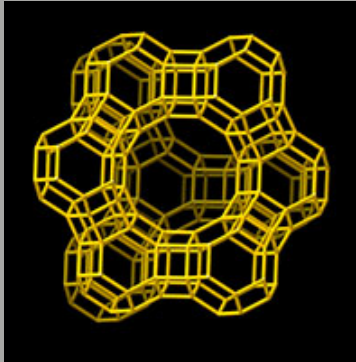
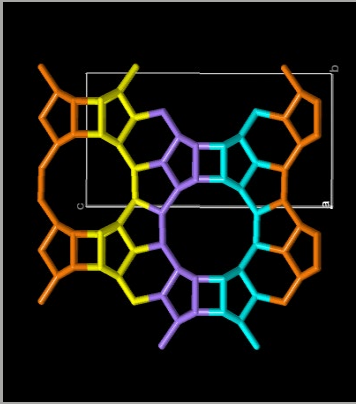
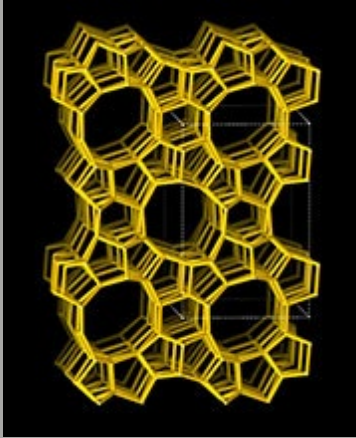


Figure 5 Experimental Procedure

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Zeolite Catalysts and Properties

Table 1 Structural Properties of Catalysts

	Zeolite Y	Al-BEA	ZSM-5
Framework Code	FAU	BEA	MFI
Pore Size	0.75 nm & 1.1 nm	0.75 nm	0.56 nm
Structure			

Zeolite framework structures adapted from the International Zeolite Association Structure Database (Baerlocher et al.).



Results: Zeolite Characterization

Table 2 Textural Properties of Catalysts

Catalyst	Micropore Volume (cm ³ /g)	BET Surface Area (m ² /g)	Total Pore Volume (cm ³ /g)
Zeolite Y (Si/Al=15)	0.20	716	0.33
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Al-BEA (Si/Al=12.5)	0.17	674	1.13
ZSM-5 (Si/Al=15)	0.11	420	0.27

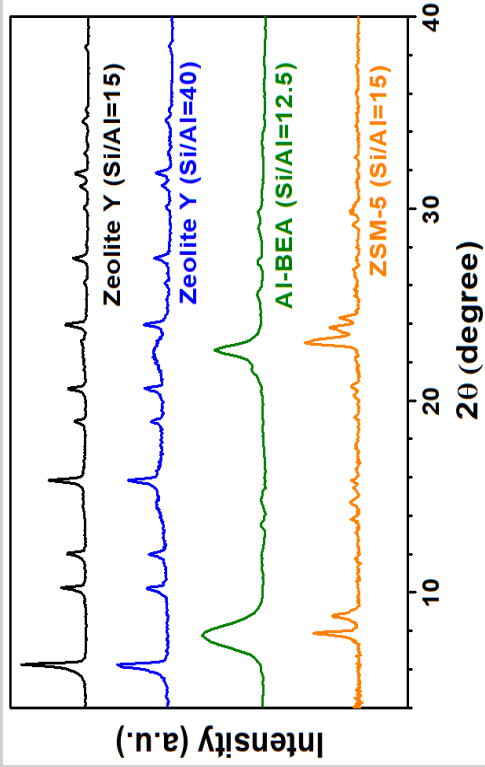


Figure 6 XRD Patterns of Zeolites Used



Figure 7 Zeolite Y Si/Al=40

Results: Reaction Testing

0.0875 mL CPO + 1.5 mL solvent + 120 mg catalyst, 130°C, 22 h

In Ethanol Solvent

- Only Zeolite Y promotes CPO₂ formation
- Larger pore sizes, access to acid active sites
- Diameter of CPO is 0.58 nm
- Diameter of CPO₂ is 0.72 nm

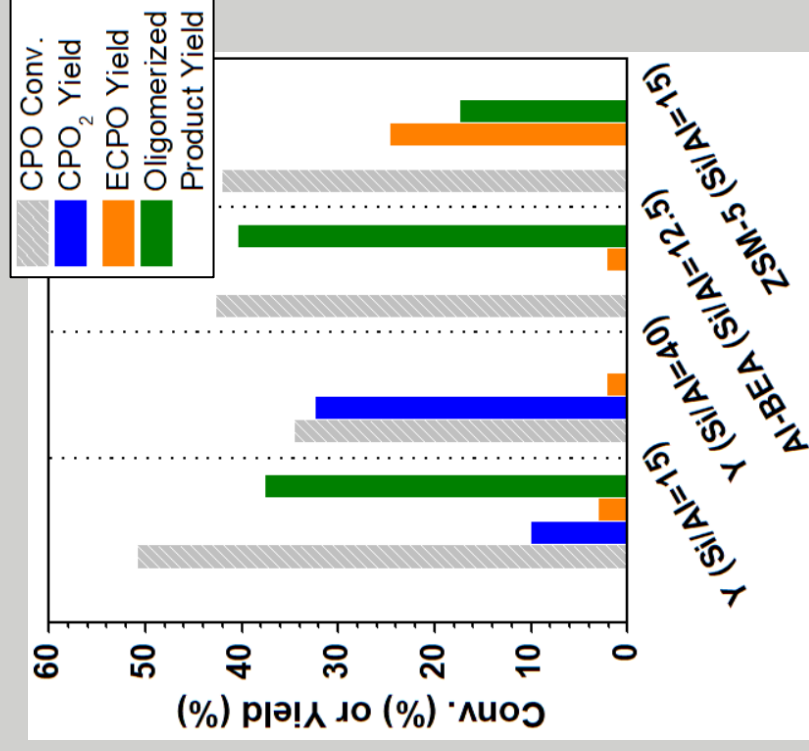


Figure 8 Product Distribution for reaction in ethanol



$$Conv. (\%) = \frac{CPO \text{ Consumed}}{CPO \text{ In}} \times 100\%$$

$$Yield (\%) = \frac{2 \cdot CPO_2 \text{ Formed}}{CPO \text{ In}} \times 100\%$$



Results: Reaction Testing

0.0875 mL CPO + 1.5 mL solvent + 120 mg catalyst, 130°C, 22 h

In Ethanol Solvent

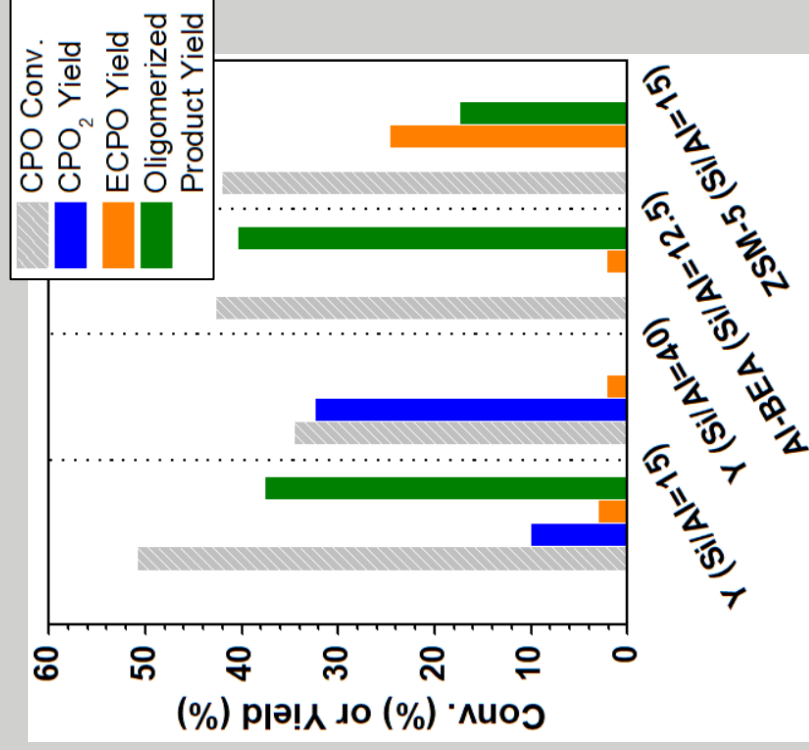


Figure 8 Product Distribution for reaction in ethanol

In Cyclohexane Solvent

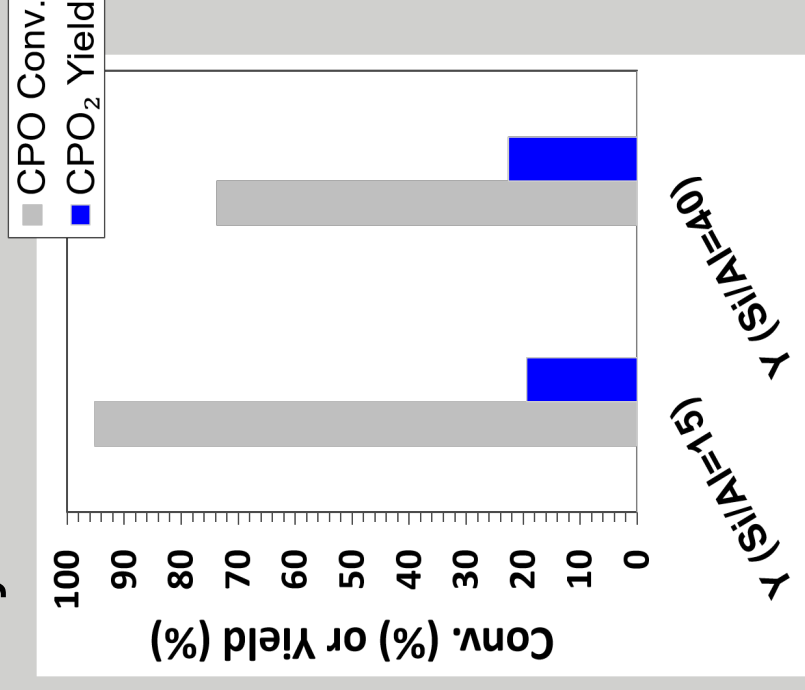


Figure 9 Product Distribution reaction in cyclohexane



Results: Reaction Testing

2.285 mL CPO + 100 mg catalyst, 130°C, 24 h

Solventless Conditions

- Loading CaO reduces product yield
- Loading MgO increases product selectivity

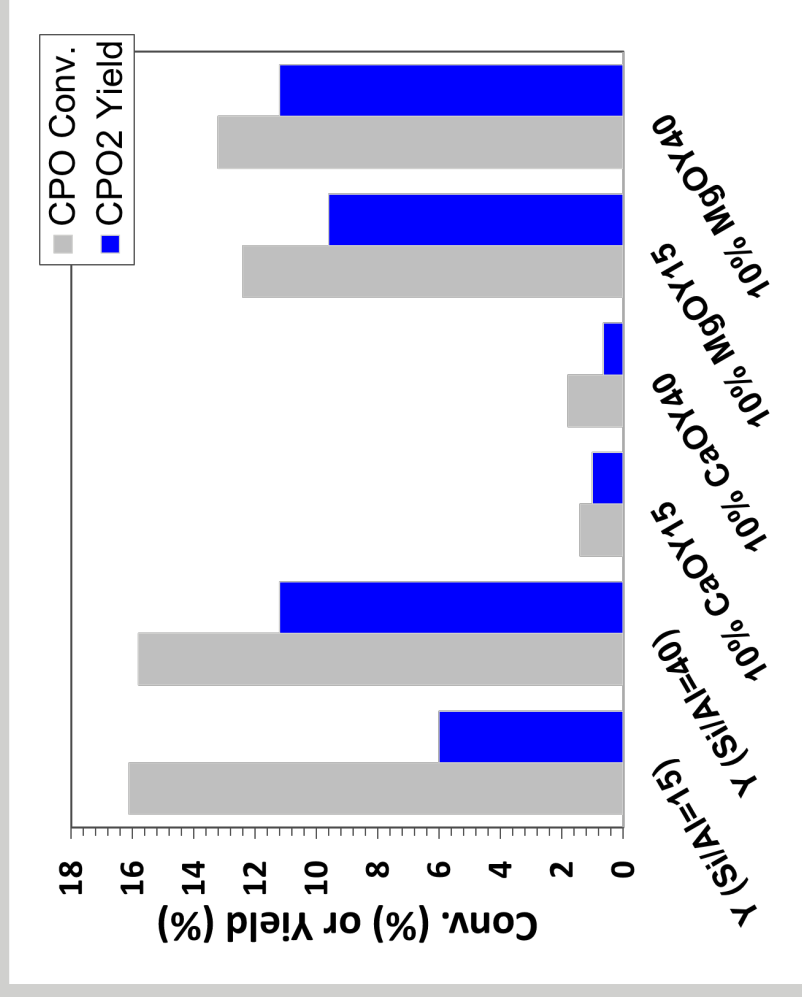


Figure 10 Product Distribution for 2.285 mL CPO + 100 mg Catalyst, 130°C, 24 h



Discussion and Conclusions

- Only Zeolite Y promotes CPO_2 formation
 - Larger pore sizes, access to acid active sites
- Zeolites Al-BEA and ZSM-5 promotes side product formation
- Higher acid site density promotes formation of side products
 - Oligomerization and coke formation
- In ethanol solvent, zeolite Y (Si/Al=40) exhibited a CPO_2 yield of 32% and the highest selectivity toward the desired product
- Loading CaO reduces conversion
- Loading MgO increases selectivity



Acknowledgements

I am deeply grateful to Dr. and Mrs. Niblack for supporting my research and providing me with this valuable opportunity to grow as a researcher!

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Adam Meringolo

Dr. Christine Johnson



Multifactorial Evaluation of Structural Stability of Sodium Alginate-Gelatin Hydrogels for Dental Applications: Effects of pH, Crosslinking, and Fabrication Techniques

Rachel Davis

Chemical Engineering

Faculty Sponsor: Dr. Sundar Madihally

Graduate Student Mentor: Finn Poling

ABSTRACT

Hydrogels are a dynamic class of materials with significant potential in biomedical and dental applications due to their viscoelasticity, porosity, biocompatibility, and high-water content. Hydrogels made of natural materials such as alginates, chitosan, and gelatin have been explored for various regenerative applications. However, the structural stability of sodium alginate hydrogels under physiologically relevant oral conditions of fluctuating pH at body temperature remains insufficiently characterized. These conditions may influence long-term dimensional stability, swelling behavior, and degradation. Additionally, crosslinking conditions and fabrication techniques on hydrogel performance require further investigation. In this study, 3% sodium alginate hydrogels containing 6% gelatin were fabricated using casting protocol and custom-built 3D printers. Hydrogels were crosslinked with either 1% or 4% calcium chloride, incubated in potassium phosphate buffers at pH 5.8, 6.6, and 7.2 under temperatures of 18 °C or 37°C and monitored for 96 hours. The pH was measured over time. Also, structural changes were quantified using ImageJ and DinoCapture 3.0 analysis. Buffer-only conditions were included as controls. Results demonstrated that hydrogel behavior was strongly influenced by pH, crosslinking concentration, casted gel diameter, and fabrication method. Acidic conditions (pH 5.8) resulted in the greatest structural stability and retention, while neutral conditions (pH 7.2) led to increased swelling and degradation. Higher crosslinking concentration (4% CaCl_2) enhanced stability, while 1% crosslinking required longer soak times but enabled successful 3D printing with maintained fiber geometry over 96 hours. These findings highlight the multifactorial nature of hydrogel stability and suggest that stability is highly dependent on the environment in addition to preparation techniques. This work establishes a framework for understanding structural behavior of sodium alginate hydrogels under varying pH conditions and supports the development of innovative biomaterials for improved long-term treatment outcomes in restorative dentistry.

1. Introduction

The oral environment is a dynamic, complex ecosystem, characterized by continuous microbial activity and fluctuations in saliva composition. Salivary pH typically ranges from 6.5 to 7.8 under normal conditions but can decrease significantly due to dietary intake and bacterial metabolism, reaching values as low as 4.5, when saliva buffering conditions cannot effectively neutralize erosive acids. Critically, enamel demineralization occurs below a pH of approximately 5.5, making pH fluctuations a key factor in the development of dental caries. Dental caries and periodontal disease remain among the most prevalent chronic conditions worldwide, often requiring restorative intervention. Carbohydrate intake feeds bacteria, which secrete organic acids and lead to the dissolution of the minerals in the tooth. Cavities are typically treated by removing the decayed tissue and filling the structure with restorative material.

Current restorative materials (amalgam, resin-based composite, glass ionomer, and resin-modified glass ionomer) have demonstrated clinical success but present several limitations, including susceptibility to degradation in acidic environments, limited long-term durability, aesthetic concerns, high cost, and potential biocompatibility issues. Over time, exposure to fluctuating oral conditions may contribute to material failure, secondary decay, and the need for replacement. As a result, there is a growing need for innovative biomaterials that can better withstand the chemical and physical challenges of the oral environment while maintaining functionality and aesthetics. Materials must ideally be less susceptible to decay and degradation, as they are expected to last for many years, potentially decades, withstand mechanical stress and fluctuations in oral pH, demonstrate longevity and affordability, and resist microleakage. Cosmetics are also at play, with dentists and patients valuing biocompatible materials that are aesthetically similar to the natural coloration of teeth.

Hydrogels are a cutting-edge biomaterial with vast potential for dental applications, with attractive properties like biocompatibility, porosity, and viscoelasticity. Hydrogels consist of crosslinked hydrophilic polymers which can mimic aspects of natural tissue and have been widely investigated for applications in drug delivery, tissue engineering, and regenerative dentistry, including periodontal/pulp diseases, craniomaxillofacial defects, oral cancer, orthodontic tooth moving, and peri-implantitis. Though hydrogels have been extensively studied as effective scaffolding for the regeneration of dental tissues, there is little evaluation of their effectiveness of potential for use in restorative procedures. Additionally, previous work has shown the potential for 3D printing hydrogels, especially for soft tissue regeneration. Sodium alginate is an attractive hydrogel-forming polymer due to its ease of gelation, low toxicity, and ability to be ionically crosslinked using calcium ions. Alginate (AG) is a naturally derived polysaccharide composed of β -D-mannuronic acid and α -L-guluronic acid residues arranged in block structures. Gelation occurs through ionic crosslinking with divalent cations

such as Ca^{2+} , forming an egg-box structure. The structural stability of alginate-based hydrogels under fluctuating oral pH and temperature conditions is not well understood, limiting their translation into clinical use. Furthermore, emerging fabrication techniques such as 3D printing introduce additional variables, including printability and shape fidelity, which must be evaluated alongside traditional material properties.

This study addresses these gaps by systematically investigating the effects of key variables relevant to the oral environment and hydrogel fabrication methods. Specifically, pH (5.8–7.2) was selected to represent acidic to near-neutral intraoral conditions, temperature (18°C vs. 37°C) to compare ambient and physiological conditions, crosslinking concentration (1% vs. 4% CaCl_2) to evaluate structural integrity, and gel size to assess dimensional effects on stability and measurement reliability. Additionally, both casting and 3D printing fabrication methods were examined to evaluate their influence on hydrogel performance. The objective of this work is to evaluate how these factors influence the structural stability, swelling behavior, and degradation of sodium alginate–gelatin (AG-G) hydrogels in simulated oral conditions, while also assessing their feasibility for 3D printing applications, building a framework to inform the design of hydrogel-based materials for dental applications.

2. Experimental Details

Hydrogel Preparation

Hydrogels were prepared using a formulation of 3% (w/v) sodium alginate and 6% (w/v) gelatin (Type B, bovine skin). A 3% alginate concentration was selected due to its established use in biomedical applications and its increased stiffness compared to lower concentrations (e.g., 2%), providing improved structural integrity. The polymer solution was heated in a convection oven (1000 W) at 120°C for 1–1.25 hours and subsequently mixed under continuous heat using magnetic stirrer until homogeneous. Prepared hydrogels were stored under refrigeration and reheated to approximately 65°C, or until fully liquefied, prior to sample preparation. Hydrogels were crosslinked using calcium chloride (CaCl_2) solutions at concentrations of either 1% or 4% (w/v), depending on experimental condition.

Buffer Preparation and Experimental Conditions

To simulate intraoral conditions, hydrogels were incubated in phosphate buffer solutions prepared using potassium phosphate dibasic and potassium phosphate monobasic. Buffer solutions were prepared at a concentration of 9 mM, consistent with reported salivary potassium levels (8.9 ± 4.2 mmol/L) and adjusted to target pH values of 5.8, 6.6, and 7.2 (selected to represent intraoral pH range) using hydrochloric acid (HCl) or sodium hydroxide (NaOH). Phosphate buffers were chosen due to their ability to reliably maintain pH within this physiologically relevant range and prepared according to AAT Bioquest Online Calculator.

Hydrogel samples were submerged in buffer solutions and incubated at either 18°C (room temperature) or 37°C (physiological temperature) for up to 96 hours. Buffer-only controls were included to distinguish solution-driven pH changes from those caused by hydrogel degradation.

Hydrogel Casting and Sample Preparation

For casted samples, the heated hydrogel solution was poured into casting trays and subjected to sonication for 20–25 minutes to remove air bubbles. Crosslinking solution (1% or 4% CaCl₂) was then applied to induce gelation. Biopsy punch of diameter of approximately 8 mm and 15 mm were used to prepare uniform circular discs.

3D Printing of Hydrogels

Hydrogels were also fabricated using a custom-built 3D printing system to evaluate printability and structural integrity at smaller scales. Printing was conducted using a coaxial extrusion setup (PBS core, gel solution entering from side) with a core flow rate of 0.0542 mL/min and shell flow rate of 2 mL/min. Crosslinking occurred during printing within a 10 mL bath of 1% CaCl₂ maintained at 37°C. Printed hydrogel fibers were then transferred to 1 mL phosphate buffer solutions at pH 5.8 and 7.2 and incubated at 37°C for up to 96 hours.

Data Collection and Analysis

Hydrogel samples were monitored and photographed at defined time intervals over a 96-hour period. Buffer pH was measured using a calibrated Fisher Scientific Accumet Basic AB15 pH meter. Structural changes, including swelling, shrinkage, and degradation, were assessed through visual inspection and quantitative image analysis using ImageJ software, while DinoLite microscopy with DinoCapture 3.0 software was used for printed fibers.

3. Results

Buffer Control Validation and Dimensional Changes of Hydrogel

Buffer-only controls exhibited minimal variation in pH over the 96-hour incubation period under both 18°C and 37°C conditions. Across all tested pH levels (5.8, 6.6, and 7.2), pH fluctuations remained below 0.6 units, confirming that the phosphate buffer systems effectively resisted external perturbations, and no differences were found between temperature. These findings indicate that subsequent pH changes observed in hydrogel-containing samples can be attributed primarily to gel–environment interactions rather than buffer instability. Diameters of 15 mm gels increased significantly at neutral pH (7.2) whereas at acidic pH (5.8) stability was maintained, with intermediate changes observed in pH 6.6.

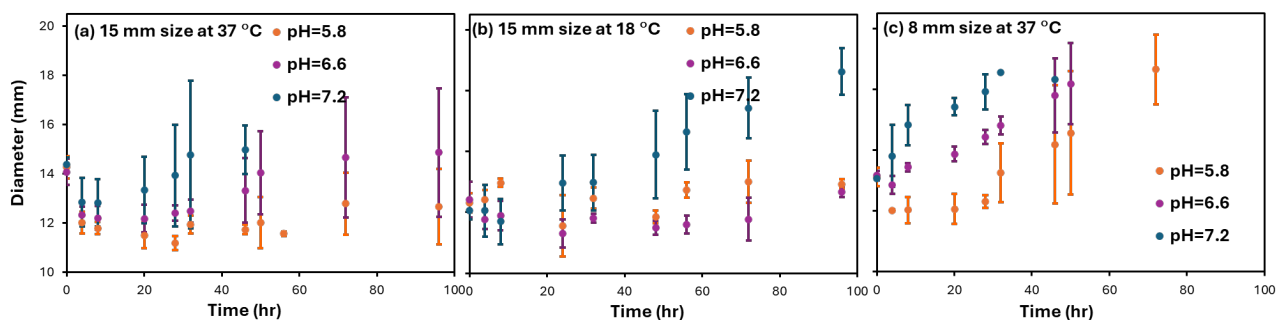


Figure 1. Effect of incubation pH, temperature, and hydrogel size on stability of the hydrogel.

Effect of pH on Hydrogel Properties

Hydrogel stability was strongly influenced by environmental pH. Samples incubated at pH 5.8 demonstrated the highest structural integrity, maintaining shape and exhibiting minimal swelling over the 96-hour period. In contrast, gels at pH 7.2 showed swelling and progressive degradation earlier, while pH 6.6 exhibited intermediate behavior. All conditions demonstrated gradual increases in surrounding pH over time. No significant differences were observed in diameter changes based on starting gel diameter or with gels incubated at room temperature vs. body temperature. 15 mm gels provided more reliable quantification, and 37 °C represents physiological conditions thus these were selected to proceed with testing.

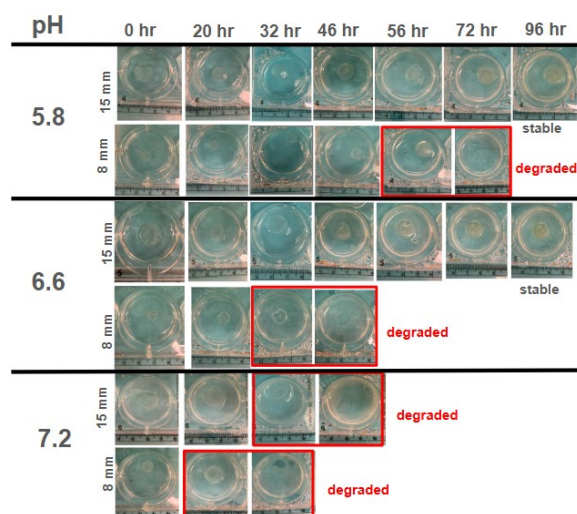


Figure 2. Stability of 8 mm and 15 mm AG-G casted hydrogel discs incubated at 37°C under varying pH conditions.

Crosslinking Variations and Effect of Gelatin

Crosslinking duration (0.5, 2, and 24 hours) did not significantly affect hydrogel diameter or swelling behavior. However, crosslinking concentration significantly influenced hydrogel stability. Gels crosslinked with 4% CaCl_2 exhibited minimal diameter changes compared to swelling observed with 1% crosslinked gels. Additionally, at pH 5.8, 1% crosslinked gels showed increasing pH over time, whereas 4% gels remained stable or slightly decreased in pH but at pH 7.2, no differences were found with both conditions exhibiting similar pH increase. The presence of gelatin did not affect diameter changes but led to more variation in pH behavior.

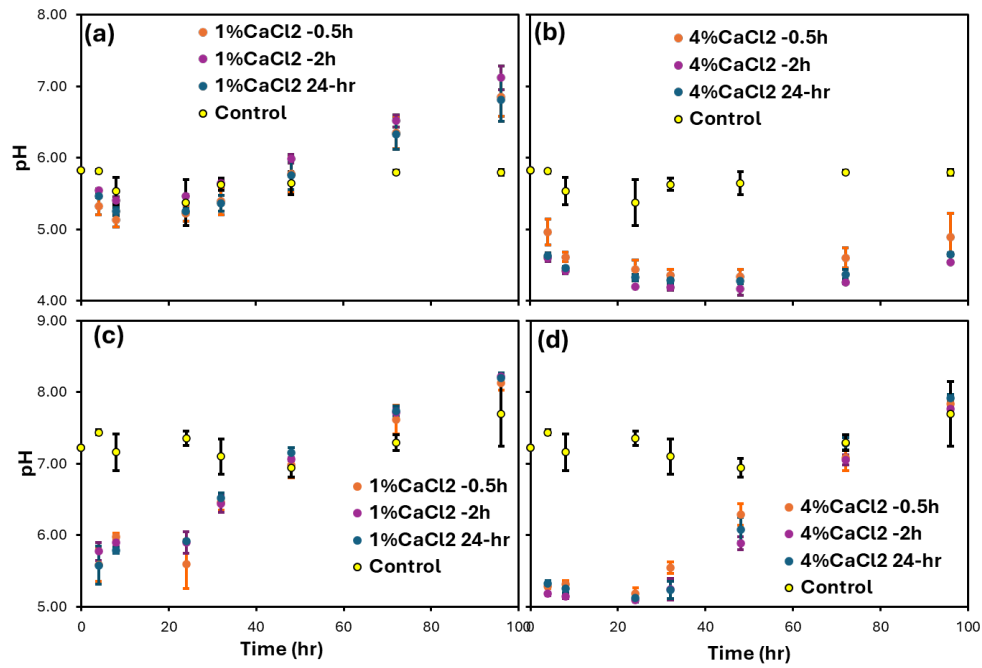


Figure 3. 15 mm size hydrogels were formed in CaCl₂ bath for different times and incubated at 37 °C and pH was measured at intervals. (a –b) initial pH = 5.8 and (c-d) initial pH =7.2

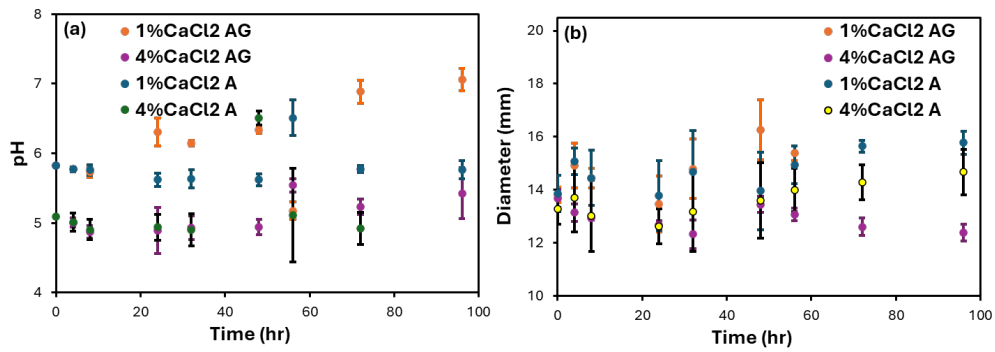


Figure 4. 3% AG with and without 6% G were formed in CaCl₂ bath and then 15 mm size samples were incubated at 37 °C at 5.8 pH. (a) pH changes and (b) diameter changes.

3D Printed Hydrogels

3D printed hydrogel fibers crosslinked with 1% CaCl₂ were evaluated to assess hydrogel behavior at smaller scale. These fibers maintained stable diameter over 96 hours under acidic conditions while neutral conditions resulted in shrinkage. Gelatin inclusion improved fiber stability and reduced diameter variability in acidic conditions. Overall, 1% printed fibers demonstrated enhanced stability trends to 1% casted gels.

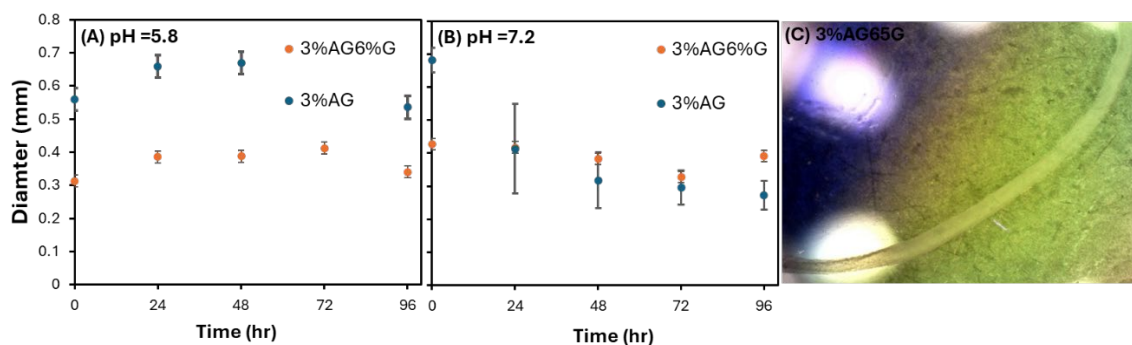


Figure 5. 3% AG with and without 6% G formed in 1% CaCl_2 bath and then incubated at 37 °C. (a) 5.8 pH and (b) 7.2 pH. (c) Example microscope image of 3D-printed gel fiber.

4. Discussion

The results of this study demonstrate that the structural stability of AG-G hydrogels is strongly dependent on pH, crosslinking, and fabrication techniques. pH emerged as a dominant factor governing hydrogel behavior, with acidic conditions (pH 5.8) promoting structural integrity and neutral conditions (pH 7.2) accelerating swelling and degradation. These findings are consistent with previous work with AG hydrogels and the egg-box model of alginate crosslinking. The observed pH-dependent behavior may be attributed to ion exchange mechanisms, where in higher pH environments divalent calcium ions that stabilize the gel matrix are displaced, weakening the crosslinked network, resulting in increased swelling, loss of structural integrity, and degradation. The gradual increase in pH observed across conditions may be due to the release of residual ions and polymer relaxation processes. This behavior highlights the importance of considering not only how the environment affects the material, but also how the material alters the surroundings.

Crosslinking concentration was identified as another factor influencing hydrogel performance. Hydrogels crosslinked with 4% CaCl_2 consistently demonstrated greater dimensional stability and resistance to pH fluctuations compared to those crosslinked with 1% CaCl_2 . This is attributed to the increased crosslink density, which enhances network rigidity and reduces susceptibility to ion exchange and mechanical deformation. In contrast, lower crosslinking concentrations resulted in more swelling and pH variation, indicating a less stable polymer network. Other variables, including temperature, sample size, and crosslinking time, exhibited minimal influence on hydrogel behavior, suggesting that hydrogel stability is primarily governed by chemical interactions. Results from 3D printed hydrogels showed similar trends to casted gels, although improved structural retention was observed under certain conditions, suggesting fabrication-dependent differences in stability. Printed fibers with 1% CaCl_2 crosslinking exhibited better stability, retaining structural integrity in conditions that

were shown to degrade in prior experiments. Future study is needed to optimize material formulation and fabrication parameters for successful biomaterial design.

Most previous work on hydrogels has focused on regenerative applications rather than evaluating performance under conditions relevant to the oral environment. This study adds to existing knowledge by systematically examining hydrogel behavior under simulated intraoral conditions and by incorporating fabrication methods relevant to clinical translation, including 3D printing, which is now widely used in custom-fit restoration. Results suggest that alginate-based hydrogels are currently more suitable for temporary or short-term applications. Their stability under acidic conditions and tunable properties make them promising candidates for applications such as drug delivery systems, provisional restorations, and regenerative scaffolds. However, their susceptibility to degradation under neutral conditions limits their use in load-bearing or long-term restorative applications without further modification.

Future work should consider evaluating the mechanical properties for use in dental applications such as strength and fracture resistance. Also, the experimental duration should be extended beyond 96 hours for evaluating long-term behavior. Further, using more complex buffers mimicking proteins, enzymes, and microbial components present in saliva would help understand their role in hydrogel stability. Additionally, investigation of microbial interactions and other clinically relevant oral conditions is needed. Additionally, assessment of biocompatibility, adhesion, and integration with natural tooth structure will be essential for determining clinical feasibility.

5. Summary

- pH: Acidic conditions (pH 5.8) produced the greatest stability, while neutral pH (7.2) led to increased swelling and degradation.
- Crosslinking concentration: Higher crosslinking (4% CaCl₂) significantly improved structural stability compared to 1%.
- Temperature: No significant effect on hydrogel stability between 18°C and 37°C.
- Sample size: Gel diameter (8 mm vs. 15 mm) did not affect behavior, though 15 mm improved measurement reliability.
- Crosslinking time: No impact on structural stability across 0.5–24 hours.
- Gelatin inclusion minimized diameter changes resulting in more stable gels.
- 3D printing: Printed hydrogels remained stable in acidic conditions but exhibited shrinkage in neutral pH
- 4% CaCl₂ crosslinked, 3%AG6%G hydrogel is most stable and best for resisting pH and diameter changes

6. Appendices

6a. Acknowledgements

I would like to thank Dr. and Mrs. Niblack for the Niblack Research Scholars Program, as well as for providing the opportunity and funding for this research project. I also gratefully acknowledge Dr. Johnson and the Division of the Vice President for Research for their support during my time as a scholar. I am especially grateful to my faculty mentor, Dr. Sundar Madihally, for his guidance, support, and encouragement throughout this project and during my time as an undergraduate researcher. I would also like to thank my graduate mentor, Finn Poling, for teaching me new 3D printing techniques and providing valuable support. I would like to acknowledge Dr. Bikkina for providing access to microscopy equipment. I also extend thanks to Dr. Florez and Dr. Khajotia at the University of Oklahoma College of Dentistry for the opportunity to be involved in clinically relevant dental research on a project involving bleaching hydrogels.

6b. Papers Published

Poster Presentations:

1. Multifactorial Evaluation of pH Stability and 3D Printability of Sodium Alginate Hydrogels for Dental Applications (Spring 2026)
2. Effects of pH and Temperature on the Stability and Structural Characteristics of Hydrogels (Summer 2025)
3. Evaluating Structural Properties of Hydrogels for Use in Dental Applications (Spring 2025)

Publications:

Two publications are in preparation at the time of writing this report.

6c. Literature Cited

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SCHOOL OF
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College of Engineering, Architecture and Technology



Multifactorial Evaluation of Structural Stability of Sodium Alginate-Gelatin Hydrogels for Dental Applications

Rachel Davis

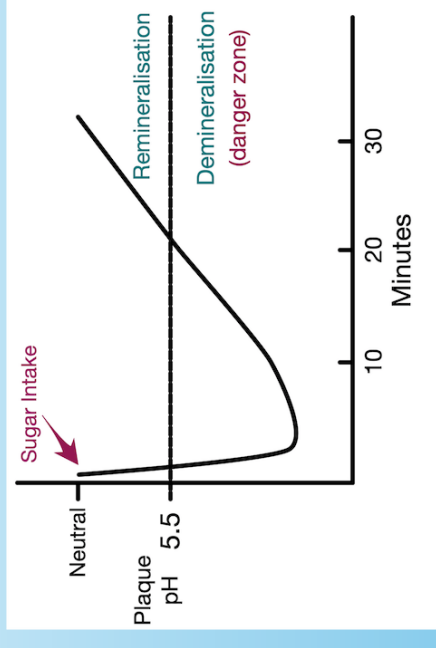
Faculty Sponsor: Dr. Sundar Madihally
Graduate Student Mentor: Finn Poling

NIBLACK RESEARCH SCHOLARS PROGRAM

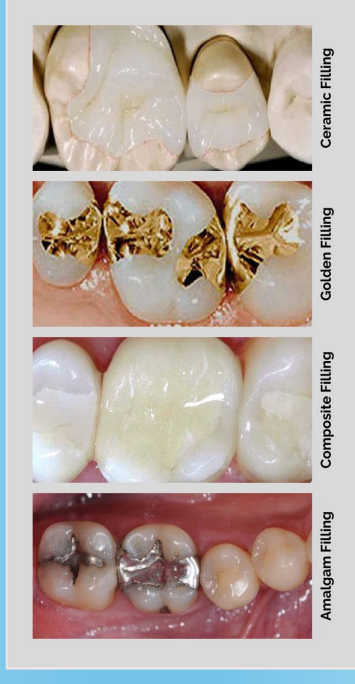
Motivation

- Dental caries (cavities) are highly prevalent
- Oral environment is a dynamic ecosystem
- Salivary pH is normally 6.5-7.8, can drop to ~4.5
- Enamel demineralizes < pH 5.5
- Downsides of Current Materials
 - Limited long-term biocompatibility & fracture resistance
 - Premature failure (esp. in acidic conditions)
 - High Cost
 - Staining/discoloration
- Dental materials must withstand pH fluctuation, mechanical stress, and resist degradation

Stephan Curve



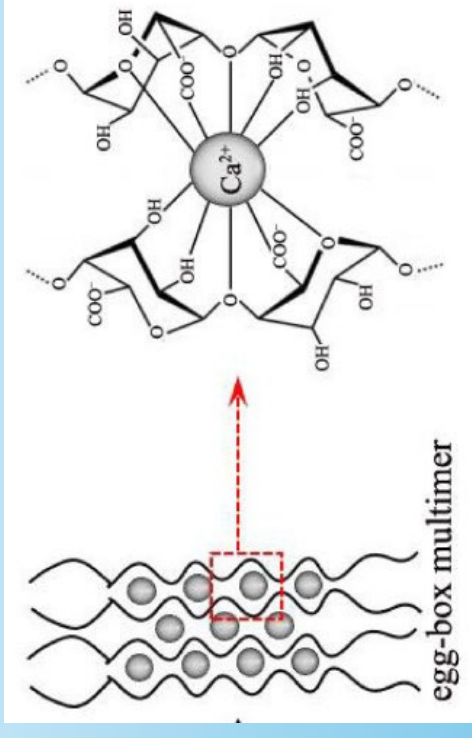
Current Restorative Materials



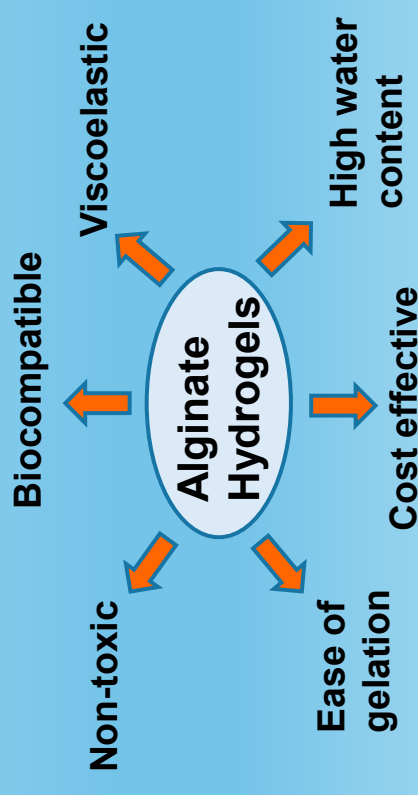
Hydrogels as Biomaterials

- Crosslinked hydrophilic polymer networks
- Alginate composed of β -D-mannuronic acid and α -L-guluronic acid residues
- Ionic crosslinking with Ca^{2+}
- Gap:
 - Gel stability in realistic intraoral conditions is currently uncharacterized
 - Effect of fabrication techniques
 - Limited hydrogel work applied to restoratives

Egg-Box Model



Adapted from Figure 2. (Zhang, 2022)



Objectives

- Evaluate how these key variables affect structural stability, swelling, and degradation of AG-G hydrogels

Polymer Composition

Crosslinking

CaCl₂
Concentration
(1% vs. 4%) &
various soak
durations

Fabrication Method

Casted discs vs.
3D-Printed fibers

Environment

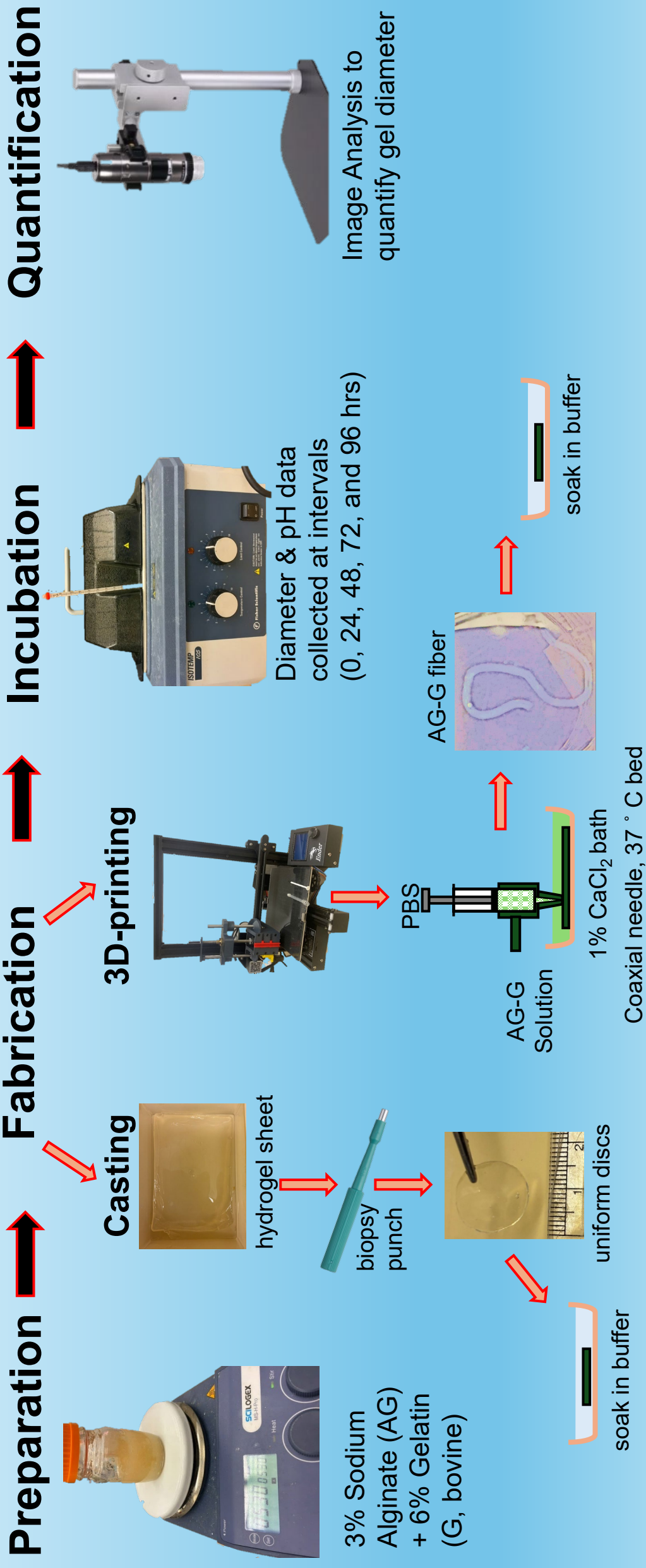
pH Effects

Gels evaluated
in pH 5.8, 6.6, &
7.2 phosphate
buffers

Temperature

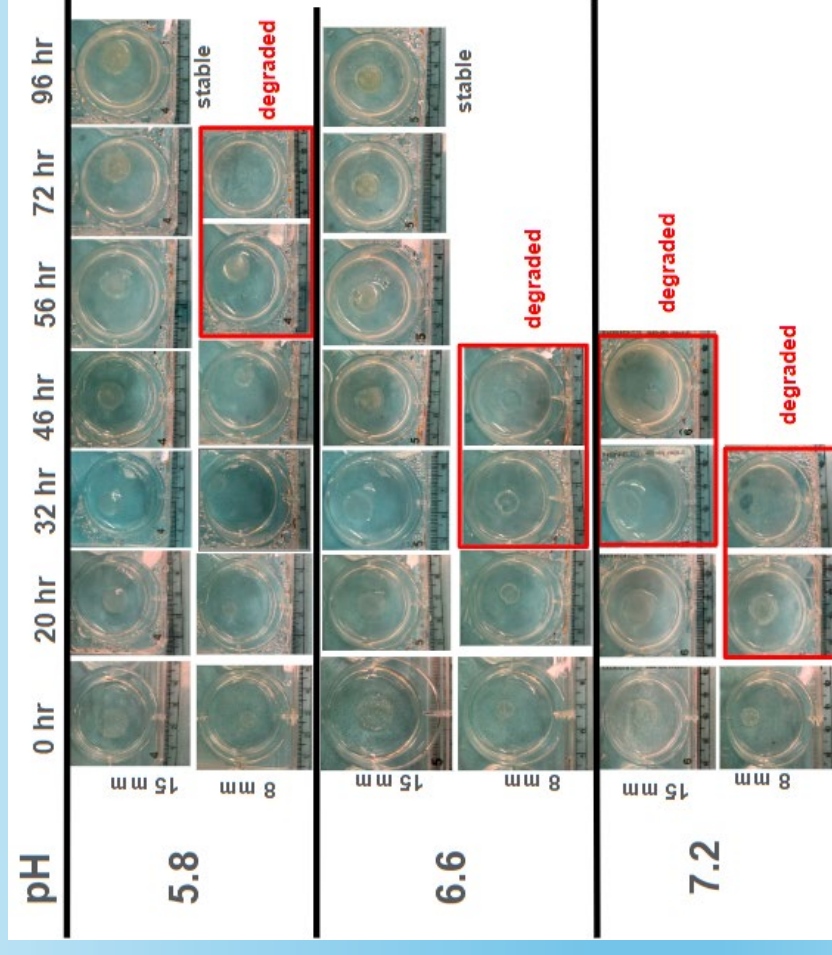
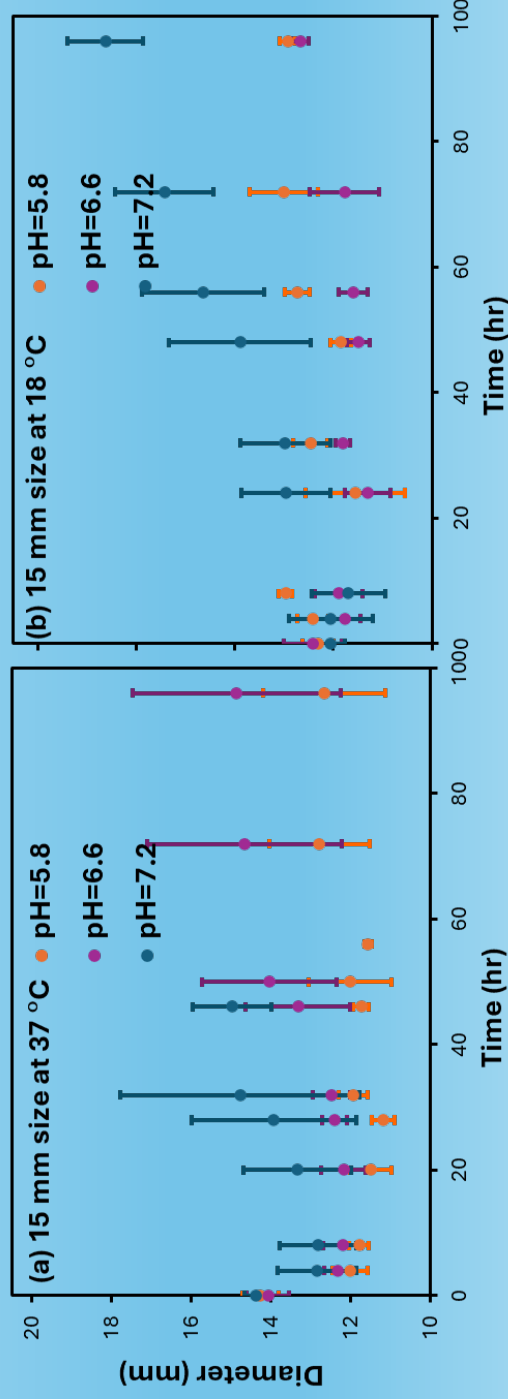
18°C vs. 37°C

Protocol



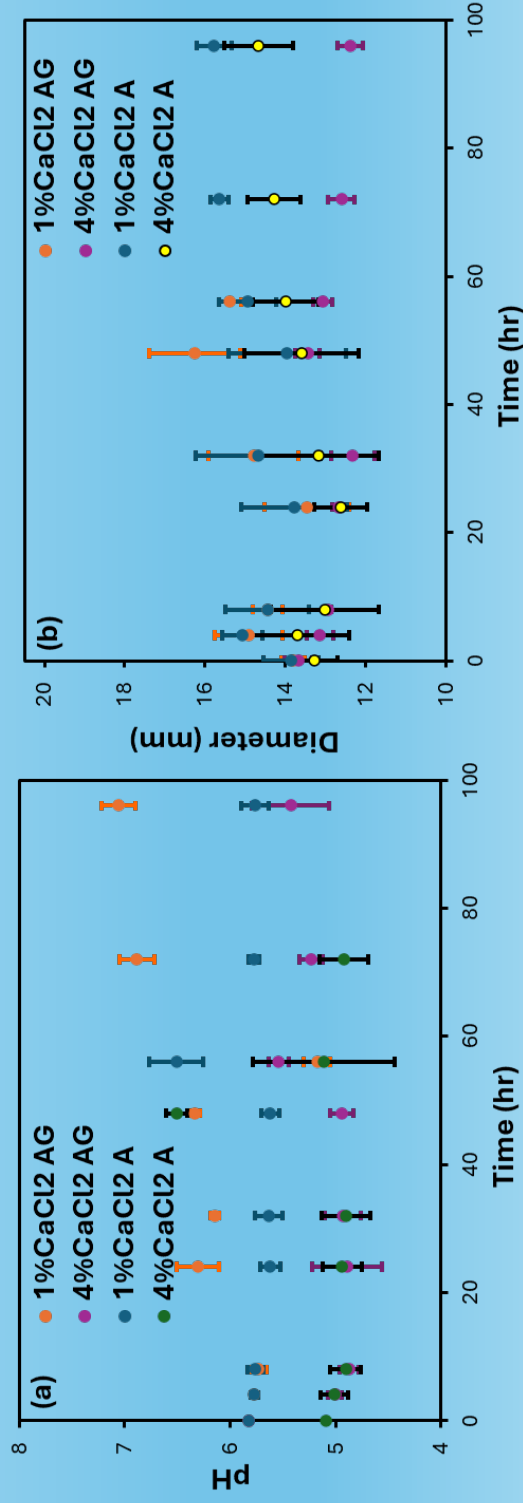
pH is the Dominant Driver

- Gels are most suited to acidic environment
- Neutral conditions exhibited swelling and early degradation
- Higher pH displaces Ca^{2+} via ion exchange
- No effect of temperature



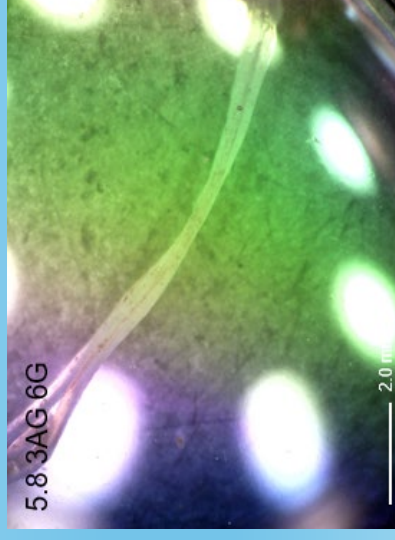
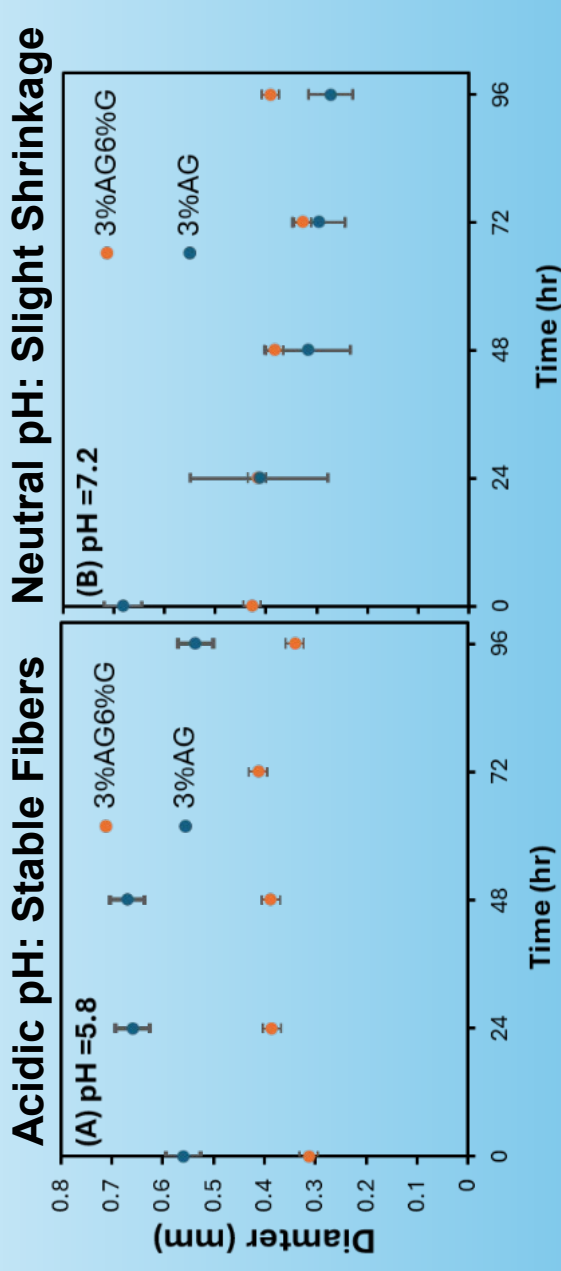
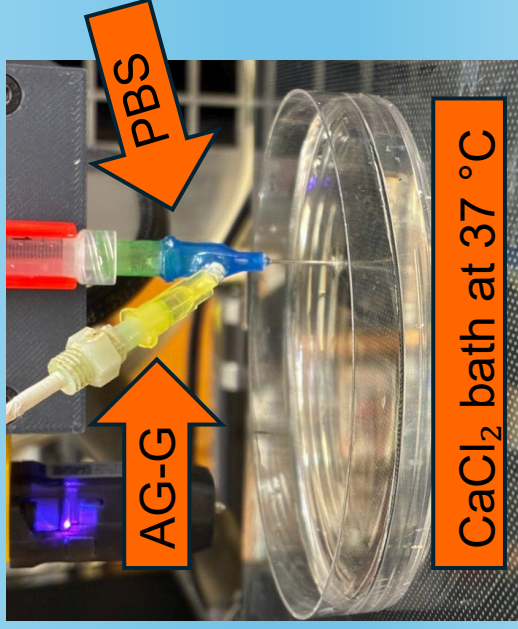
Crosslinking Concentration Matters

- 4% CaCl_2 crosslink conditions are most optimal for stability
 - Minimal swelling & diameter change
 - Better resistance to pH changes
 - Higher $[\text{Ca}^{2+}]$ results in stronger network
- Gelatin (G) inclusion reduced diameter variability & improved fiber stability



3D-Printing Enables Successful Fabrication

- Printed 1% crosslinked fibers outperformed cast 1% gels
- Gelatin improved fiber stability and reduced diameter variability
- All combinations stable at 96 hrs



Summary

- Overall, AG-G stability is governed by pH and crosslinking concentration
 - Acidic pH (5.8) produced the greatest stability
 - Neutral pH (7.2) led to increased swelling and degradation
- Higher crosslinking concentration (4%) significantly improved stability
- 3D-Printing is feasible with lower crosslinking conditions (1%)
- 3% AG 6% G, 4% CaCl₂ hydrogel is most stable, resisting pH and diameter changes most
- Further evaluation needed to determine efficacy for clinical use

Collaboration Highlight

- Studied dissolved oxygen release characteristics in bleaching hydrogels used clinically
- Details limited under MTA

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Department of Dental Biomaterials

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THANK YOU

SESSION FOUR:

Molly McGaughy

“Defining the Antifungal Mechanism of Myeloperoxidase Against *Cryptococcus neoformans*”

Research Area: Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Karen Wozniak

Graduate Student Mentor: Ayesha Nair

Taylor Mills

“Diversity and Evolutionary Significance of Anaerobic Gut Fungi in Testudines”

Research Area: Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Mostafa Elshahed

Graduate Student Mentor: Sakalya De Silva

Ty Young

“Comparative Metagenomic Analysis of the Gut Community of Ratites”

Research Area: Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Mostafa Elshahed

Graduate Student Mentor: Md. Mehedi Hasan

Defining the Antifungal Mechanism of Myeloperoxidase Against *Cryptococcus neoformans*

Molly McGaughy

Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Karen Wozniak

Graduate Student Mentor: Ayesha Nair

Abstract:

Cryptococcus neoformans is an opportunistic fungal pathogen that primarily affects those who are immunocompromised and causes the disease cryptococcal meningitis. *C. neoformans* infections have become increasingly important in many communities due to the higher prevalence of HIV/AIDS, killing approximately 112,000 individuals with AIDS annually. The current antifungal drugs are extremely toxic, and combined with the rise of antifungal resistance, new therapies are needed. Previous work by our lab showed that dendritic cells (DCs) can kill *Cryptococcus*, and components of the DC lysosome are important in antifungal activity. DCs are phagocytic cells that engulf *Cryptococcus* for antigen presentation to T cells, which then mount an immune response. Our lab identified five DC lysosomal proteins with antifungal activity, one of which is myeloperoxidase (MPO). We hypothesized that MPO kills *C. neoformans* by damaging the cell wall and membrane of the fungus. To test this, we conducted MIC assays to determine the concentration needed for antifungal activity for future experiments using fluorescent and electron microscopy. I have also used *Cryptococcus* mutant libraries specific to cryptococcal phosphatases and kinases to identify mutants affected by treatment with MPO to identify the mechanism of action. I also used scanning and transmission electron microscopy (SEM and TEM) to examine physical effects of MPO treatment. Results of mutant library analyses after MPO treatment showed increased resistance in mutants with genes involved in inositol phosphate metabolism. SEM and TEM studies of MPO-treated cryptococcal cells showed release of intracellular contents and misshapen fungal cells. This phenotype was rescued by the addition of sorbitol for osmotic stability, indicating potential cell wall/membrane damage followed by osmotic lysis *Cryptococcus* due to MPO treatment. Future studies will examine the resistant mutants to determine their susceptibility to MPO in antifungal assays. With these studies, we hope to gain a better understanding of the effects that MPO has on *C. neoformans*, which could potentially lead to more effective therapies for cryptococcosis.

1. Introduction:

Cryptococcus neoformans is an opportunistic fungal pathogen that primarily affects immunocompromised individuals and is the causative agent of cryptococcal meningitis, a life-threatening fungal infection of the central nervous system. The disease is especially prevalent in patients with HIV/AIDS and remains a major global health concern, causing

approximately 112,000 AIDS-related deaths annually [1]. Infection typically begins through inhalation of fungal spores into the lungs, where the organism can survive host immune defenses and disseminate to the brain. Current treatment options for cryptococcosis include amphotericin B, flucytosine and azole antifungals [2]. These are associated with significant toxicity, prolonged treatment durations, limited accessibility in developing regions and the growing emergence of antifungal resistance [2,3]. As a result, there is a critical need to identify alternative antifungal mechanisms and develop novel therapeutic strategies against *C. neoformans*, such as immune-based therapies.

Dendritic cells (DCs) are professional antigen-presenting cells that play an essential role in both innate and adaptive immunity. These immune cells engulf fungal pathogens and process antigens for presentation to T cells, ultimately initiating antifungal immune responses [4]. They also possess lysosomal compartments containing antimicrobial proteins and enzymes capable of directly damaging pathogens [4]. Previous work from our laboratory identified DC lysosomal proteins with anti-cryptococcal activity including Coronin, matrix metalloproteinase-25 (MMP25), human neutrophil elastase (HNE), NOSTRIN and myeloperoxidase (MPO) [5].

Myeloperoxidase is a heme-containing enzyme most commonly associated with neutrophils and other phagocytic cells, where it contributes to microbial killing through the production of reactive oxygen species and hypochlorous acid [6]. MPO-mediated oxidative stress has been shown to damage proteins, lipids, membranes and nucleic acids in a variety of microbial organisms [6]. However, the precise antifungal mechanism of MPO against *C. neoformans* remains poorly understood. Preliminary findings from our laboratory suggested that MPO may disrupt fungal cell wall or membrane integrity, leading to fungal death.

The fungal cell wall and membrane are critical for maintaining structural integrity, osmotic stability, and survival within hostile host environments. Alterations in these structures can lead to membrane collapse, leakage of intracellular contents, and eventual osmotic lysis. In addition, pathways involved in phospholipid signaling, membrane organization and stress responses are known to contribute to fungal resistance against environmental and oxidative stresses [7]. Therefore, understanding how MPO interacts with these cellular systems may provide insight into novel antifungal targets.

In this study, we investigated the mechanism of MPO-mediated anti-cryptococcal activity. Minimum inhibitory concentration (MIC) assays were used to determine the antifungal activity of MPO at different fungal inoculum densities. Mutant library screening was performed to identify genes and pathways associated with resistance or susceptibility to MPO treatment. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to visualize structural damage following MPO exposure. Finally, growth assays using sorbitol as an osmotic stabilizer were conducted to evaluate whether MPO-induced killing involved disruption of osmotic stability. These studies aimed to determine whether MPO targets the fungal cell wall/membrane and to characterize the mechanisms.

2. Materials and Methods:

Strains and Media: *C. neoformans* strain mNeon Green H99 (gift of Damian Krysan, University of Iowa) was grown on Yeast Peptone Dextrose (BD Biosciences, San Diego, CA) agar plates (YPD plates) for 48 hours. For minimum concentration assays (MIC) and modified growth assays, they were grown in shaking YPD broth at 30 °C for 18 hours. They were then washed with sterile phosphate buffered saline (PBS) three times. The concentration of the cells was then counted on a hemocytometer with trypan blue to exclude dead cells. The concentrations were then determined by these counts. For growth assays, phosphate buffer at pH 5.5 and phosphate buffer containing sorbitol were used. For mutant libraries, we acquired cryptococcal mutants from the Fungal Genetics Stock Center (Kansas State University, deposited by Yong-Sun Bahn, Yonsei University, Korea). These are cryptococcal mutants with mutations in kinase and phosphatase signaling pathways. For studies using the mutant libraries, the same growth steps were taken, but they were aliquoted into a 96 well plate for storage in the freezer.

Minimum Concentration Assay (MIC): *C. neoformans* was grown as described above and washed. Following quantification, a media control RPMI-MOPS, pH 7.0, was placed in the final column of a 96 well plate. The cells were placed in all wells at 0.5×10^3 cells/mL. MPO (Novus Biotechnique, Minneapolis, MN) was diluted to 100 µg/mL in the first column of wells in the plate and then serially diluted. They were then incubated for 48 hours at 35°C and then analyzed by optical density using a BioTek platereader (Aligent, Santa Clara, CA). The study was repeated with 1×10^6 cells/mL. This was done twice at each concentration because the MIC at a lower concentration needed to be verified for mutant library studies and a higher is needed for electron microscopy.

Mutant Libraries: Mutant library studies were based on published flow cytometry studies [8]. The mNeonGreen H99 stock and a mutant library stock were removed from the -80°C freezer and then allowed to thaw. Once thawed, using a frogger, the cells were transferred into 96 well plates of YPD to incubate for 18h at 30°C. Cells were read in the platereader, and if optical densities (ODs) were similar, then cells were transferred to new 96-well plates at a 1:1 ratio in phosphate buffer at pH 5.5. These were then either treated with MPO at the MIC concentration or untreated and incubated for 1.5 hours at 30°C. The 1.5-hour time frame is the half killing time for MPO. After incubating, controls for the flow cytometer were made and then samples from the plates were evaluated on a flow cytometer (Novocyte 3000, Aligent) using NovoExpress software (Agilent).

Growth Assays: *C. neoformans* were grown and washed as above, and then were either grown with/without sorbitol and treated with/without MPO at the MIC concentration. The initial inoculum was diluted and plated on YPD plates for 48 hours at 30°C, and then CFUs were counted. Following incubation of cryptococcal cells under the various conditions for 24 hours at 30°C, cells from those wells were diluted and plated on YPD plates for 48 hours at 30°C, and then CFUs were counted. Then using the software Prism (GraphPad, San Diego, CA), the CFUs were graphed with error bars and the statistics were analyzed.

Electron Microscopy (EM): *C. neoformans* cells were grown in YPD broth in a shaking incubator at 30°C for 18h. Following washing and counting, cells were diluted to 1×10^6 cells/mL in phosphate buffer or phosphate buffer with sorbitol. Cells were treated with the MIC of MPO for 1.5h at 30°C. Control cells were incubated in phosphate buffer

alone. Following incubation, cells were fixed for 2 hr. in 2.0% glutaraldehyde in 0.1M cacodylate buffer at room temperature. Following fixation, samples were rinsed 3 times in buffered wash for 15 minutes each. Samples were then taken to the Microscopy center for remaining steps. Cells were fixed for 1 hr in 1% OSO_4 (aqueous) at room temperature, then rinsed 3 times in buffered wash for 15 minutes each. Samples were then dehydrated in increasing concentrations of ethanol for 15 minutes each (50%, 70%, 90%, 95% and 100% three times). Following dehydration, for Scanning EM (SEM) cells were washed 2 times for 5 minutes with hexamethyldisilazane. Cell pellets were mounted on stubs with silver paint or double-sticky tape. Then samples were coated with Au-Pd. Cells were viewed on the scanning electron microscope and images were taken. Following dehydration for Transmission EM (TEM), cells were washed 2 times for 5 minutes each with propylene oxide. Cells were then put into a mix of propylene oxide and Poly/Bed overnight. The vials were then uncapped in a fume hood until most of the solvent evaporated. Cells were then embedded in 100% embedding medium and placed in a 60°C oven to polymerize for 48h. Embedded cells were sliced using a diamond blade, and cells were imaged using a transmission electron microscope.

3. Results

Minimum inhibitory concentration (MIC) was determined for MPO. In these studies we evaluated the MIC for two different concentrations of *C. neoformans* – one for mutant library studies and one for microscopy studies. Results in Figure 1A, B show that the

A.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.050	0.065	0.057	0.059	0.061	0.064	0.080	0.077	0.084	0.092	0.068	0.041
B	0.056	0.071	0.060	0.063	0.066	0.114	0.087	0.089	0.095	0.095	0.103	0.039
C	0.062	0.063	0.071	0.062	0.067	0.081	0.081	0.076	0.076	0.076	0.079	0.040
D	0.056	0.050	0.063	0.066	0.072	0.082	0.082	0.080	0.074	0.080	0.080	0.041

B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.057	0.053	0.053	0.055	0.053	0.048	0.059	0.076	0.072	0.071	0.078	0.041
B	0.060	0.070	0.060	0.062	0.057	0.058	0.073	0.077	0.068	0.072	0.075	0.041
C	0.063	0.061	0.060	0.069	0.070	0.059	0.073	0.079	0.080	0.081	0.087	0.042
D	0.067	0.058	0.067	0.060	0.060	0.053	0.067	0.073	0.075	0.068	0.086	0.042

Figure 1: Minimum inhibitory concentration (MIC) assay of MPO against *C. neoformans*. Heatmap representation of optical density (OD) measurements from a broth microdilution MIC assay evaluating the antifungal activity of myeloperoxidase (MPO) against *C. neoformans* at an inoculum density of (A) 0.5×10^3 cells/ml or (B) 1×10^6 cells/mL. The MIC for MPO was determined to be 6.25 $\mu\text{g}/\text{ml}$, corresponding to column 5 (A) or 6 (B) of the assay plate. Lower OD values indicate decreased fungal growth and greater antifungal activity, while higher OD values indicate increases fungal proliferation.

MIC for MPO against *C. neoformans* was 6.25 $\mu\text{g}/\text{ml}$, regardless of inoculum.

Cryptococcal mutant library analyses show MPO affects fungal signaling pathways.

We next asked whether cryptococcal phosphatase and kinase mutants were more susceptible to MPO than WT cells. Using

flow cytometry to measure populations, we found that several cryptococcal mutants were either more or less susceptible to MPO treatment (Table 1). Pathways affected were the inositol phosphate metabolism pathway, the nicotinate and nicotinamide metabolism pathway, the glycerol lipid metabolism pathway, and the Glycosylphosphatidylinositol (GPI)-anchor bio-synthesis pathway. Genes involved in the Inositol phosphate metabolism pathway included *INM1*, *INM101*, and *SAC101*, which are associated with phosphatidylinositol signaling, osmotic regulation, and maintenance of cell wall integrity. In the nicotinate and nicotinamide metabolism pathway, *NYPI* encodes a NAD

diphosphatase involved in NAD metabolism and cellular stress responses. In the glycerolipid metabolism pathway, *DPP101* encodes a hypothetical protein associated with lipid metabolic processes and membrane-associated pathways. In involved in the GPI-anchor synthesis pathway, *CWH43* encodes a hypersensitive response-related membrane protein involved in membrane organization and cell wall-associated processes. Resistance of these mutants to MPO suggests that alterations in inositol metabolism, NAD metabolism, glycolipid metabolism, and GPI-anchor biosynthesis and membrane integrity contribute to cryptococcal resistance to MPO-induced cell wall/membrane damage.

Gene Name	Gene protein product	Ratio mutant/neon Green no MPO	Ratio mutant/Neon Green with MPO	Percent Change
INM1	Myo-inositol-1 (or 4) - monophosphatase	3.89:1	30.54:1	784.85%
INM101*	Myo-inositol-1 (or 4) - monophosphatase	4.75:1	20.28:1	314.92%
URKI	Nucleotide metabolism and pyrimidine metabolism	1.23:1	0.95:1	-24.16%
SAC101*	Inositol polyphosphate-5-phosphatase	3.78:1	12.83:1	214.96%
CWH43	Calcofluor white hypersensitive protein	7.50:1	12.33:1	69.63%
SSU72	RNA polymerase II subunit A domain phosphatase SSU72	17.85:1	10.98:1	-28.91%
DPP101	Hypothetical protein	11.99:1	23.10:1	86.20%
PKA1	Hormone signaling	1.22:1	0.97:1	-20.55%
PKA2	Hormone signaling	1.34:1	1.11:1	-15.88%
NYP1	NAD diphosphatase	16.82:1	28.99:1	73.02%
CCR4	Glucose-repressible alcohol dehydrogenase	10.08:1	24.78:1	115.13%
AKG2*	Inositol phosphate metabolism	0.94:1	1.08:1	15.34%
DAK101	Carbon metabolism	1.12:1	0.95:1	-23.98%
GUT1	Glycerolipid metabolism	1.02:1	0.72:1	-30.08%
IGI2	Pyrimidine metabolism	1.18:1	0.71:1	-38.59%

Table 1: Differential representation of *C. neoformans* mutant strains following MPO treatment in mutant library screening assays. Mutant library screening following myeloperoxidase (MPO) on *C. neoformans* mutant strains compared to wild-type cells. Ratios of mutant to wild-type (mNeonGreen) cells were determined, and percent change was calculated. Positive percent change values indicate increased growth of mutant strains after MPO treatment, and negative values indicate decreased growth of mutants.

Electron microscopy reveals cell wall and membrane changes following MPO treatment. We next wanted to visualize the cryptococcal cells following treatment with MPO. Scanning electron microscopy (SEM) was used to evaluate surface changes in *C. neoformans* following treatment with MPO in phosphate buffer or phosphate buffer with

sorbitol. Results (Figure 2) showed that untreated cells displayed normal spherical morphology with smooth and intact cell surfaces. In contrast, MPO-treated cells had structural abnormalities, including cell surface collapse, deformation, and aggregation,

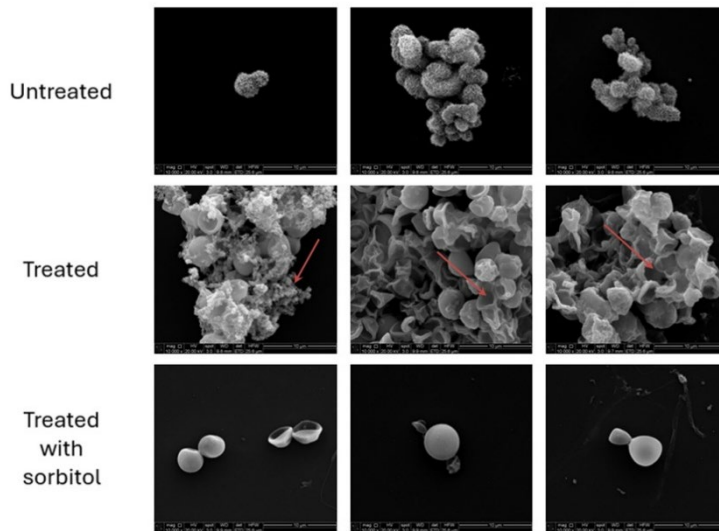


Figure 2: Scanning electron microscopy (SEM) analysis of *C. neoformans* morphology following MPO treatment in phosphate buffer. SEM was used to evaluate structural changes in *C. neoformans* cells following treatment with myeloperoxidase (MPO) in phosphate buffer. Untreated cells displayed normal spherical morphology with smooth and intact cell surfaces. MPO-treated cells exhibited pronounced structural abnormalities (red arrows). MPO treatment in phosphate buffer supplemented with sorbitol, an osmotic stabilizer, shows a more intact and rounded morphology.

consistent with extensive cellular damage. When sorbitol, an osmotic stabilizer, was added, cells retained a more intact and rounded morphology compared to MPO treatment alone. These data suggest that osmotic stabilization protects *C. neoformans* from MPO-induced damage, supporting a role for cell wall/membrane stress in MPO antifungal activity. Transmission electron microscopy (TEM) was used to examine structural changes in the cell wall and membrane of *C. neoformans* cells following treatment with MPO in phosphate buffer (Figure 3).

Untreated cells exhibited intact cellular architecture, including well-defined cell walls and membranes. In contrast MPO-treated cells had structural damage characterized by disrupted cell membranes, loss of cellular integrity, and cellular collapse. When sorbitol was added, cells retained more intact structural features with reduced membrane and cell wall damage compared to MPO treatment alone. These observations support the hypothesis that MPO-mediated antifungal activity involves disruption of cell wall/membrane integrity.

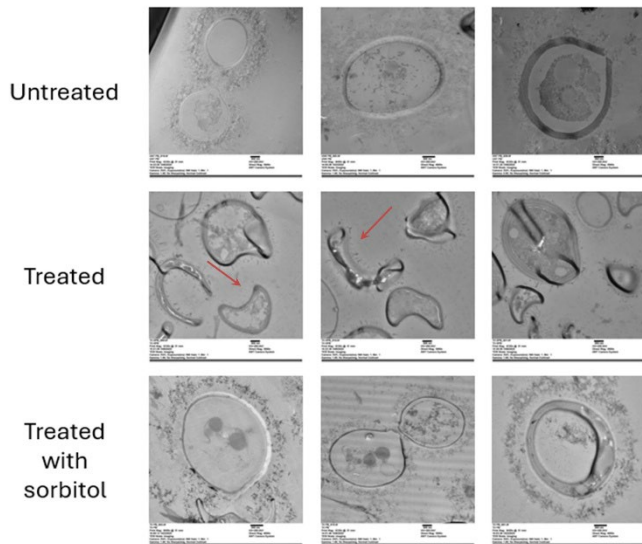


Figure 3: Transmission electron microscopy (TEM) analysis of *C. neoformans* structure following MPO treatment in phosphate buffer. Transmission electron microscopy (TEM) was used to examine structural changes in *C. neoformans* cells following treatment with myeloperoxidase (MPO) in phosphate buffer. Untreated cells exhibited intact cellular architecture. MPO-treated cells displayed severe structural damage (red arrows). MPO treatment in phosphate buffer supplemented with sorbitol, an osmotic stabilizer, showed reduced membrane damage.

Sorbitol rescued MPO killing of *C. neoformans*. We next wanted to verify that incubation in an osmotically stable buffer, such as sorbitol, could also prevent the antifungal activity of MPO. Results (Figure 4) showed that cells treated with MPO in phosphate buffer alone exhibited a significant reduction in CFUs ($p < 0.05$), whereas MPO treatment in sorbitol buffer resulted in comparable survival to untreated controls. These findings suggest that osmotic stress of cell wall/membrane disruption may contribute to the antifungal effects observed during treatment.

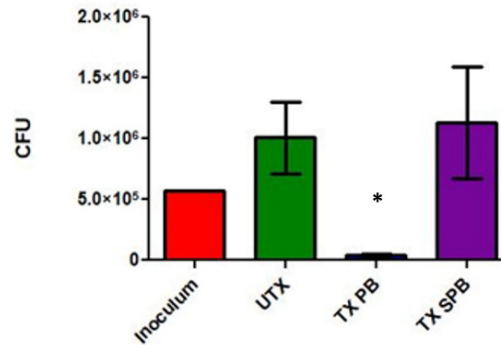


Figure 4: Effect of buffer conditions on *C. neoformans* survival following treatment. Colony-forming units (CFUs) of *C. neoformans* were quantified in the untreated inoculum control, treatment in phosphate buffer (TX PB), and treatment in sorbitol-containing phosphate buffer (TX SPB), with untreated cells in phosphate buffer (UTX) included as a comparison control.

4. Discussion:

The results of this study support the hypothesis that the dendritic cell lysosomal protein myeloperoxidase (MPO) exhibits antifungal activity against *C. neoformans* through mechanisms involving disruption of the fungal cell wall/membrane and osmotic instability. The MIC assays consistently demonstrated growth inhibition at both high and low inoculum concentrations. Mutant library analyses showed that several mutants were associated with inositol phosphate metabolism. *C. neoformans* relies on inositol metabolism for survival and virulence within the host environment, particularly within the central nervous system where inositol concentrations are elevated [9].

Additional pathway analyses identified genes involved in membrane-associated and metabolic processes (Table 1). The enrichment of the *NYPI* mutant suggests that NAD metabolism and oxidative stress responses may influence fungal susceptibility to MPO. Because MPO generates oxidative products, altered NAD metabolism may enhance resistance to oxidative damage by affecting redox balance within the fungal cell. Similarly, increased growth of *DPP101* and *CWH43* mutants implicates glycerolipid metabolism and glycosylphosphatidylinositol (GPI)-anchor biosynthesis in resistance mechanisms. GPI-anchor synthesis and membrane organization are critical for maintaining fungal cell wall structure and membrane integrity. Alterations in these pathways may modify membrane composition or reduce the effectiveness of MPO-mediated oxidative damage.

The growth assays and microscopy further support a mechanism involving osmotic stress and cell wall/membrane damage. Treatment with MPO in phosphate buffer alone resulted in a significant reduction in fungal survival, whereas supplementation with sorbitol significantly restored viability. Electron microscopy findings provided direct visual evidence of MPO-induced structural damage. SEM demonstrated pronounced morphological abnormalities in MPO-treated cells, including cell collapse, membrane disruption, deformation and aggregation. TEM further revealed extensive intracellular damage, including disorganized cytoplasmic contents, disrupted membranes and loss of cellular integrity. Importantly, cells treated in sorbitol-containing buffer retained more normal structural features, further supporting the conclusion that osmotic stabilization

partially protects against MPO-mediated damage. The protective effect of sorbitol strongly suggests that MPO treatment compromises the integrity of the fungal cell wall or membrane, resulting in osmotic instability and cellular death. Collectively, these observations strongly support the hypothesis that MPO damages the fungal cell wall and membrane, leading to osmotic imbalance and eventual cellular collapse.

One limitation of this study is that the precise targets of MPO within *C. neoformans* remain undefined. While the mutant analyses and osmotic rescue experiments strongly suggest membrane and cell wall involvement, additional studies are needed to determine whether MPO directly oxidizes membrane lipids, cell wall polysaccharides or associated proteins. Furthermore, MPO activity in vivo likely occurs in combination with other lysosomal enzymes and immune effectors with dendritic cells and macrophages. Future studies should examine synergistic interactions between MPO and other antifungal lysosomal proteins identified in this study. Additional characterization of resistant mutants may also provide greater insight into pathways involved in fungal stress responses and survival during MPO exposure. Overall, this study demonstrates that MPO possesses antifungal activity against *C. neoformans* and provides evidence that its mechanism involves disruption of fungal cell wall/membrane integrity and osmotic stability. These findings contribute to the growing understanding of how innate immune effector molecules target fungal pathogens and may ultimately aid in the development of novel antifungal therapies for cryptococcosis.

5. Summary:

This study investigated the antifungal effects and potential mechanism of action of myeloperoxidase (MPO), a dendritic cell lysosomal protein, against *Cryptococcus neoformans*. MIC assays demonstrated that MPO inhibited fungal growth in a concentration-dependent manner at both high and low inoculum concentrations. Mutant library analyses identified several enriched mutants associated with inositol phosphate metabolism, glycerolipid metabolism, NAD metabolism and GPI-anchor biosynthesis pathways, suggesting that membrane organization, osmotic regulation and stress response pathways may contribute to resistance against MPO treatment.

Growth assays showed that supplementation with sorbitol, an osmotic stabilizer, \ rescued fungal morphology and survival following MPO treatment, indicating that osmotic stress and disruption of cell envelope integrity likely contribute to MPO-mediated killing. Structural analyses using SEM and TEM further supported this conclusion by revealing severe morphological and intracellular damage in MPO-treated cells, including membrane disruption, cellular collapse, cytoplasmic disorganization and leakage of intracellular contents. Sorbitol-treated cells retained more intact morphology and cellular organization, further supporting a role for osmotic stabilization in protection against MPO-induced damage.

Overall, these findings suggest that MPO exerts antifungal activity against *C. neoformans* by disrupting fungal cell wall and membrane integrity, leading to osmotic instability and cellular damage. These results improve our understanding of dendritic cell-mediated antifungal defense mechanisms and may contribute to the development of novel therapeutic strategies targeting fungal cell envelope integrity in cryptococcosis.

6. Appendices

Acknowledgements:

I would like to thank Damin Krysan for providing the mNeonGreen H99 strain of *Cryptococcus neoformans* and the Fungal Genetics Stock Center for providing the cryptococcal mutant libraries used in this study. I also thank the microscopy facility staff for their assistance with scanning and transmission electron microscopy imaging. Special thanks are extended to Dr. and Mrs. Niblack for supporting this work through the Niblack Research Scholarship. I would also like to sincerely thank Dr. Karen Wozniak for her mentorship, guidance and support throughout this project, as well as Ayesha Nair for her mentorship, training and assistance during the course of this research.

Presentations:

- Presented a poster at Missouri Valley Branch Meeting of the American Society for Microbiology (ASM) (March 2025)
- Presented a poster at the OCRID Research Symposium (April 2026)
- Presented a poster at the Oklahoma State Undergraduate Symposium (April 2026)
- Presented a poster at the Oklahoma State Microbiology and Molecular Genetics Symposium and was awarded third place in the undergraduate section (April 2026)

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Defining the Antifungal Mechanism of Myeloperoxidase Against *Cryptococcus* *neoformans*

Molly McGaughy

Microbiology and Molecular Genetics

Faculty Sponsor: Dr. Karen Wozniak

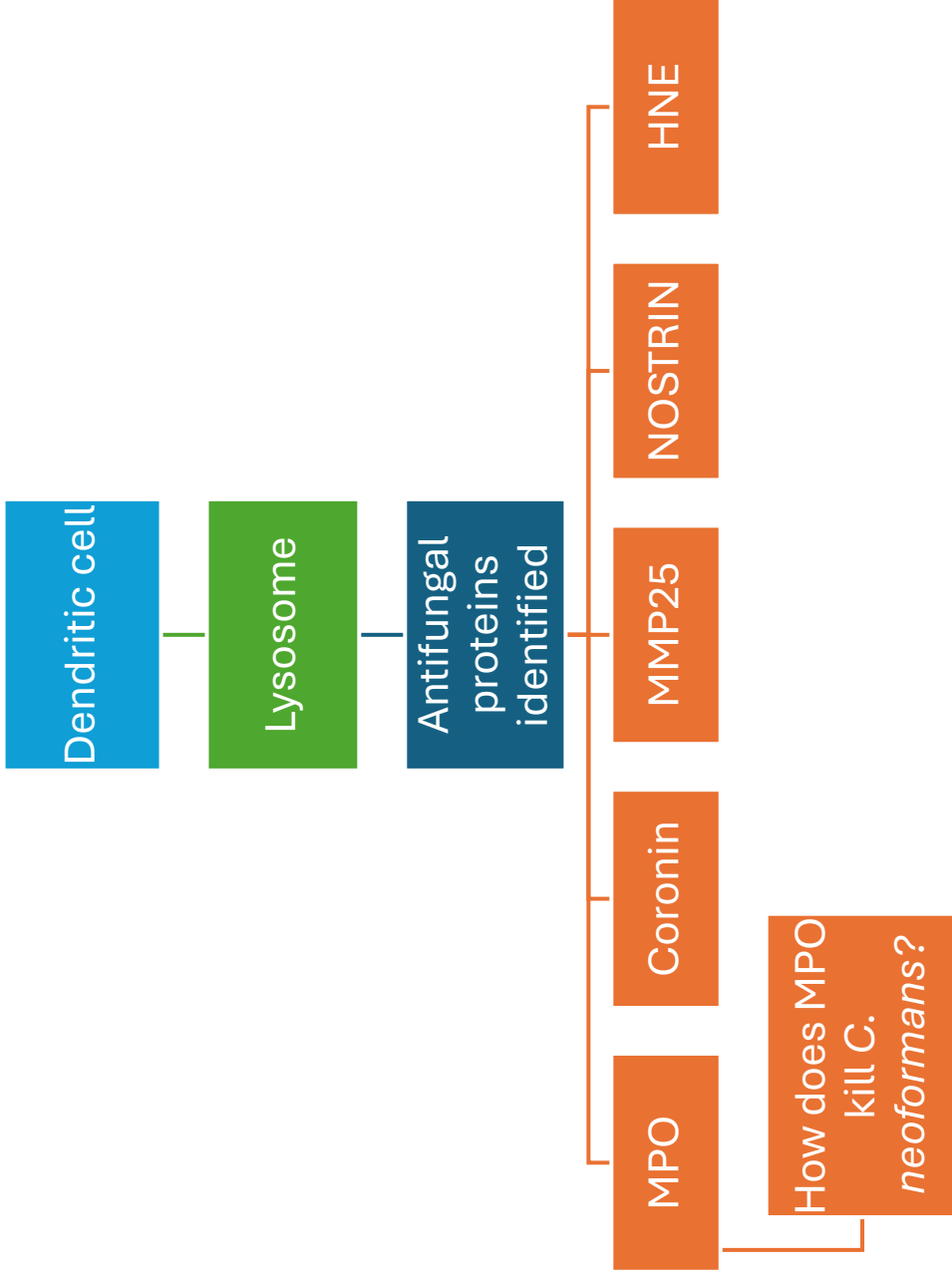
Graduate Student Mentor: Ayesha Nair

Background

- *Cryptococcus neoformans* is an opportunistic fungal pathogen that primarily affects immunocompromised individuals.
- Infection can begin in the lungs and disseminate to the brain, causing cryptococcal meningitis.
- Cryptococcal infections cause approximately 112,000 AIDS-related deaths annually.
- Current antifungal treatments have limitations including toxicity, prolonged treatment, limited accessibility and increasing antifungal resistance.
- These limitations highlight the need for new antifungal therapies and mechanisms.

Introduction

- Dendritic cells (DCs) are antigen-presenting immune cells that can engulf and kill *C. neoformans*.
- Previous studies from our laboratory identified five dendritic cell lysosomal proteins with anti-cryptococcal activity.
 - Coronin, MMP25, HNE, NOSTRIN, and **Myeloperoxidase (MPO)***
- MPO is a heme-containing enzyme capable of producing reactive oxygen species and hypochlorous acid.
- MPO-mediated oxidative stress can damage proteins, lipids, membranes and nucleic acids.
- However, the mechanism by which MPO kills *C. neoformans* is not fully understood.



Hypothesis

- **We hypothesized that MPO kills *C. neoformans* by damaging the fungal cell wall and membrane, resulting in osmotic instability and fungal cell death.**
- To investigate this mechanism, we used:
 - Minimum inhibitory concentration (MIC) assays
 - Cryptococcal mutant library screening
 - Scanning electron microscopy (SEM)
 - Transmission electron microscopy (TEM)
 - Sorbitol osmotic rescue/growth assays

Minimum Inhibitory Concentration for MPO

- MIC assays were performed to determine the concentration of MPO required to inhibit *C. neoformans* growth.
- Two inoculum densities were evaluated:
 - 0.5x10³ cells/mL for mutant library studies
 - 1x10⁶ cells/mL for electron microscopy studies
- The MIC of MPO was 6.25µg/mL at both inoculum densities.
- This concentration was used for subsequent experiments.
- Lower optical density (OD) values represent decreased fungal growth and greater antifungal activity.

A.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.050	0.065	0.057	0.059	0.061	0.064	0.080	0.077	0.084	0.092	0.068	0.041
B	0.056	0.071	0.060	0.063	0.066	0.114	0.087	0.089	0.095	0.095	0.103	0.039
C	0.062	0.063	0.071	0.062	0.067	0.081	0.081	0.076	0.076	0.076	0.079	0.040
D	0.056	0.050	0.063	0.066	0.072	0.082	0.082	0.080	0.074	0.080	0.080	0.041

B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.057	0.053	0.053	0.055	0.053	0.048	0.059	0.076	0.072	0.071	0.078	0.041
B	0.060	0.070	0.060	0.062	0.057	0.058	0.073	0.077	0.068	0.072	0.075	0.041
C	0.063	0.061	0.060	0.069	0.070	0.059	0.073	0.079	0.080	0.081	0.087	0.042
D	0.067	0.058	0.067	0.060	0.060	0.053	0.067	0.073	0.075	0.068	0.086	0.042

Cryptococcal Mutant Library Studies

- Kinase and phosphatase mutant libraries were screened following MPO treatment.
- Mutant growth was compared with wild-type mNeonGreen H99 cells.
- Several mutants demonstrated altered susceptibility to MPO.
- Pathways associated with MPO resistance included:
 - **Inositol phosphate metabolism**, NAD metabolism, glycerolipid metabolism, GPI-anchor biosynthesis
- INM1, INM101 and SAC101 were associated with phosphatidylinositol signaling, osmotic regulation and maintenance of cell wall integrity.
- These results suggest that pathways involving membrane organization and osmotic regulation contribute to resistance against MPO.

Gene Name	Gene protein product	Ratio mutant/Neon Green no MPO	Ratio mutant/Neon Green with MPO	Percent Change
INM1	Myo-inositol-1 (or 4) - monophosphatase	3.89:1	30.54:1	784.85%
INM101*	Myo-inositol-1 (or 4) - monophosphatase	4.75:1	20.28:1	314.92%
URKI	Nucleotide metabolism and pyrimidine metabolism	1.23:1	0.95:1	-24.16%
SAC101*	Inositol polyphosphate-5-phosphatase	3.78:1	12.83:1	214.96%
CWH43	Calcofluor white hypersensitive protein	7.50:1	12.33:1	69.63%
SSU72	RNA polymerase II subunit A domain phosphatase SSU72	17.85:1	10.98:1	-28.91%
DPP101	Hypothetical protein	11.99:1	23.10:1	86.20%
PKA1	Hormone signaling	1.22:1	0.97:1	-20.55%
PKA2	Hormone signaling	1.34:1	1.11:1	-15.88%
NYP1	NAD diphosphatase	16.82:1	28.99:1	73.02%
CCR4	Glucose-repressible alcohol dehydrogenase	10.08:1	24.78:1	115.13%
AKG2*	Inositol phosphate metabolism	0.94:1	1.08:1	15.34%
DAK101	Carbon metabolism	1.12:1	0.95:1	-23.98%
GUT1	Glycerolipid metabolism	1.02:1	0.72:1	-30.08%
IGI2	Pyrimidine metabolism	1.18:1	0.71:1	-38.59%

Scanning Electron Microscopy Analysis of *C. neoformans* Following MPO Treatment

- SEM was used to evaluate surface morphology following MPO treatment.
- Untreated: Cells displayed normal spherical morphology with intact surfaces.
- MPO-treated: Cells exhibited pronounced structural abnormalities including surface collapse, deformation and aggregation.
- MPO + sorbitol: Cells retained a more intact and rounded morphology.
- These results suggest that osmotic stabilization protects cells from MPO-induced structural damage.

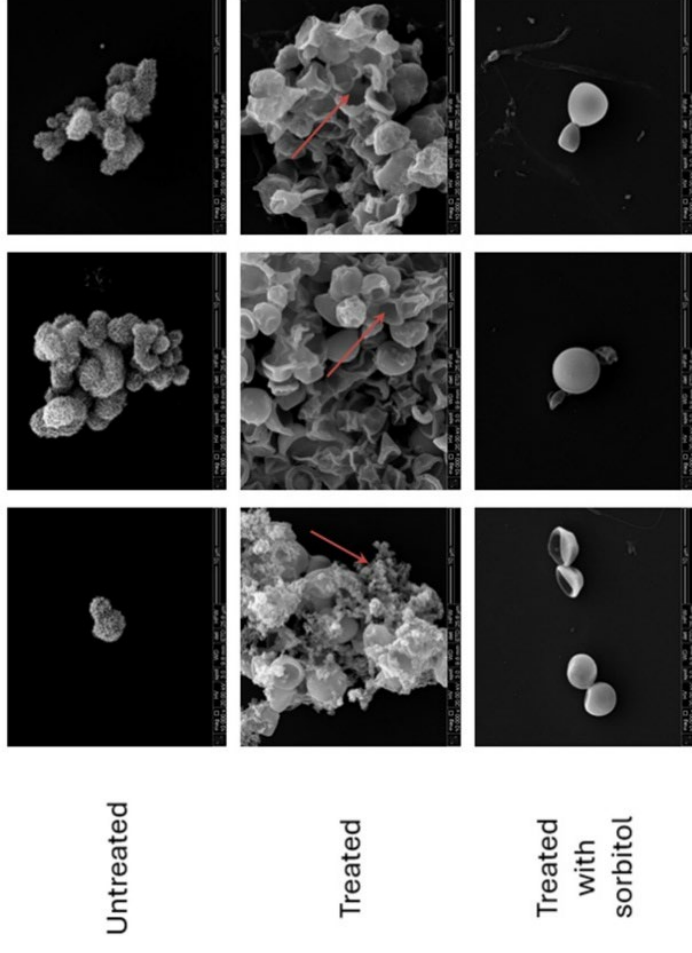
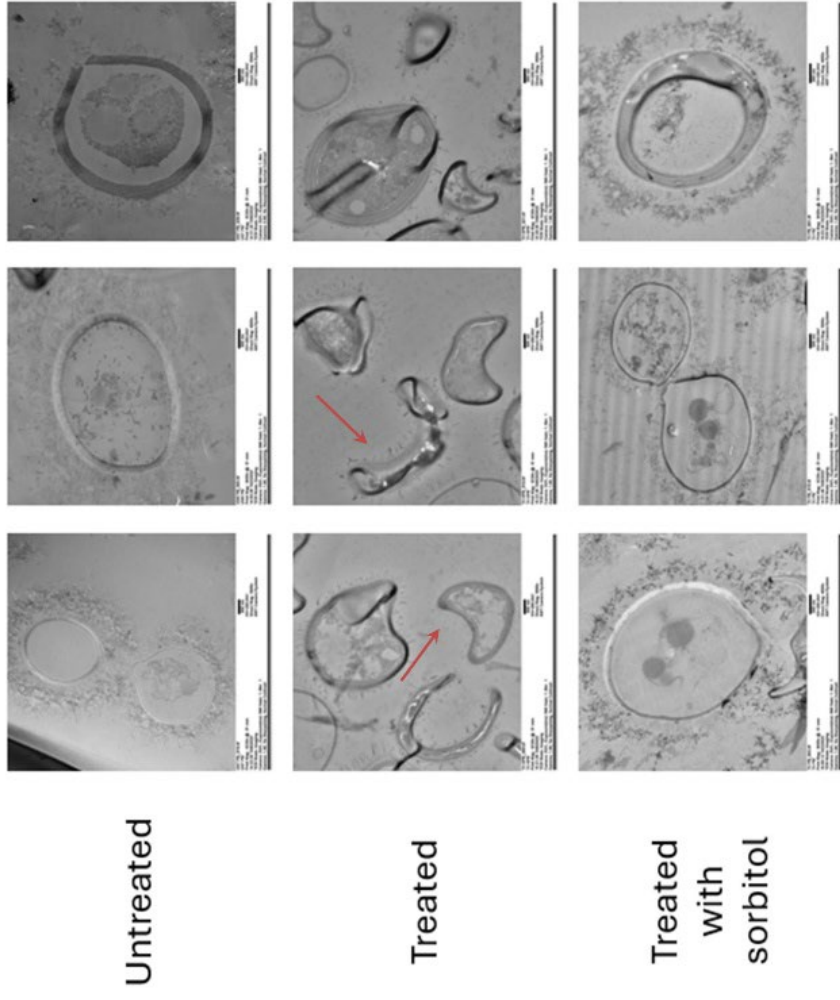


Figure 2: Scanning electron microscopy (SEM) analysis of *C. neoformans* morphology following MPO treatment in phosphate buffer. SEM was used to evaluate structural changes in *C. neoformans* cells following treatment with myeloperoxidase (MPO) in phosphate buffer. Untreated cells displayed normal spherical morphology with smooth and intact cell surfaces. MPO-treated cells exhibited pronounced structural abnormalities (red arrows). MPO treatment in phosphate buffer supplemented with sorbitol, an osmotic stabilizer, shows a more intact and rounded morphology.

Transmission Electron Microscopy Analysis of *C. neoformans* Following MPO Treatment

- TEM was used to examine internal structure changes following MPO treatment.
- Untreated: Cells exhibited intact cellular architecture with well-defined cell walls and membranes.
- MPO-treated: Cells showed disrupted membranes, loss of cellular integrity and cellular collapse.
- MPO + sorbitol: Cells retained more intact structural features with reduced membrane and cell wall damage.
- These findings further support MPO-mediated disruption of fungal cell wall/membrane integrity.



Sorbitol Rescued MPO Killing of *C. neoformans*

- Sorbitol was used as an osmotic stabilizer to determine whether osmotic instability contributes to MPO-mediated killing.
- MPO treatment in phosphate buffer resulted in a significant reduction in CFUs.
- Addition of sorbitol restored fungal survival to levels comparable to untreated controls.
- Therefore, osmotic stabilization protects *C. neoformans* from MPO-mediated killing.
- These results support a mechanism involving cell wall/membrane disruption and osmotic stress

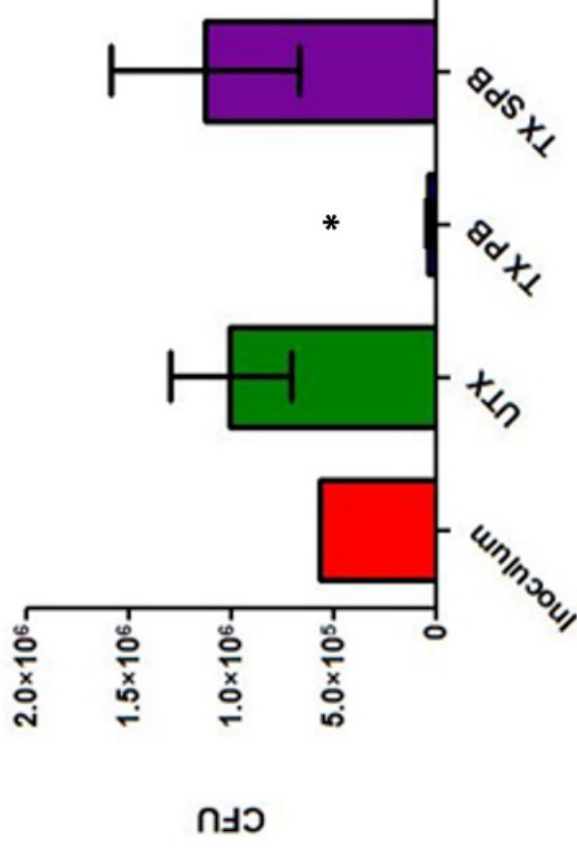


Figure 4: Effect of buffer conditions on *C. neoformans* survival following treatment. Colony-forming units (CFUs) of *C. neoformans* were quantified in the untreated inoculum control, treatment in phosphate buffer (TX PB), and treatment in sorbitol-containing phosphate buffer (TX SPB), with untreated cells in phosphate buffer (UTX) included as a comparison control.

Summary

- MPO inhibited *C. neoformans* growth with an MIC of 6.25 µg/mL.
- Mutant library screening identified pathways associated with:
 - Inositol phosphate metabolism, NAD metabolism, glycerolipid metabolism, GPI-anchor biosynthesis
- SEM and TEM revealed substantial structural damage following MPO treatment.
- Sorbitol reduced structural damage and restored fungal survival.
- Together, these findings indicate that MPO-mediated killing involves disruption of fungal cell wall/membrane integrity and osmotic instability.

Conclusion

- **MPO exhibits antifungal activity against *C. neoformans* by disrupting fungal cell wall and membrane integrity, resulting in osmotic instability and cellular damage.**
- The combination of mutant library analysis, electron microscopy and osmotic rescue experiments provides evidence that cell envelope integrity is an important component of MPO- mediated antifungal activity.
- Understanding this mechanism may help identify novel therapeutic strategies targeting fungal cell envelope integrity in cryptococcosis.

Future directions

- Further characterize MPO-resistant cryptococcal mutants.
- Determine the precise cellular targets of MPO.
- Investigate whether MPO directly oxidizes:
 - Membrane lipids
 - Cell wall polysaccharides
 - Cell wall/membrane-associated proteins
- Examine interactions between MPO and other antifungal lysosomal proteins.
- Further investigate pathways involving in fungal stress response and survival during MPO exposure.

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 - Fungal Genetics Stock Center- Cryptococcal mutant libraries
 - Microscopy Facility Staff- SEM and TEM assistance

Diversity and Evolutionary Significance of Anaerobic Gut Fungi in Testudines

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Microbiology and Molecular Genetics

Faculty Sponsor: Mostafa Elshahed

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ABSTRACT

Anaerobic gut fungi (AGF; phylum Neocallimastigomycota) are strict anaerobes that aid in plant biomass degradation in herbivorous animals. Their diversity and evolutionary history in non-mammalian hosts remain poorly understood. My Niblack project focused on two connected goals: 1) isolating and characterizing novel tortoise-affiliated AGF from sulcata tortoise fecal samples and 2) helping evaluate the broader fungal community structure of Testudines, the order including turtles and tortoises. Targeted anaerobic isolation yielded strains SR0.6 and TM0.3 from sulcata tortoises. These isolates, together with the Texas tortoise isolate GXA2, represent three novel genera and species: *Gopheromyces tardescens*, *Gigasporangiomyces pilosus*, and *Kelyphomyces adhaerens*, respectively. Morphological, physiological, and phylogenetic analyses supported the placement of the isolates into distant deep-branching tortoise-affiliated lineages and led to the proposal of a new family, Testudinimycetaceae. In parallel, analysis of 71 fecal/cloacal samples from 27 Testudines species revealed a highly diverse gut mycobiome containing 17 fungal phyla and 157 orders. Neocallimastigomycota was detected in nearly all samples but was most abundant in tortoises, suggesting that host family, feeding preference, and gut conditions influence fungal community assembly. Together, these findings expand the known diversity of AGF, support the importance of reptiles in AGF evolution, and provide a broader understanding of fungal communities in underexplored animal hosts.

1. Introduction

Anaerobic gut fungi (AGF) are members of the phylum Neocallimastigomycota and are the only fungi currently known to be strict anaerobes. They live in the gastrointestinal tracts of herbivorous animals, where they contribute to the physical and enzymatic breakdown of plant biomass. Although AGF are ecologically important, much of what is known about them has come from mammalian hosts, particularly ruminants and hindgut fermenters. This leaves a major gap in understanding how AGF diversity evolved and how the fungi interact with non-mammalian hosts.

Two recently described AGF genera (*Testudinimyces* and *Astrotestudinimyces*) obtained from tortoise fecal samples represent the first AGF isolates from non-mammalian hosts (4,5). The novel tortoise-affiliated AGF (T-AGF) display an ecological preference to tortoises, representing the predominant AGF taxa in the tortoise fecal samples examined, while being absent in mammalian feces. This suggests that tortoises may harbor lineages that are evolutionarily distinct from mammal-associated AGF (M-AGF). Molecular dating from previous work further suggested that T-AGF may represent some of the oldest cultured AGF, raising the possibility that reptiles hosted AGF before the rise of mammalian herbivores. Because only a small number of tortoise fecal samples (n=11) had previously been examined, we hypothesized that additional T-AGF lineages would be discovered through targeted isolation from new tortoise samples.

This project also examined the broader fungal community of Testudines. Previous Testudines gut microbiome studies have primarily focused on bacteria, despite the functional importance of fungi in animal-associated microbiomes (7,8). By combining culture-dependent isolation with culture-independent sequencing, the study aimed to describe novel tortoise-affiliated AGF and place those findings into the wider context of fungal diversity across turtles and tortoises.

My work specifically contributed to the isolation and characterization of novel AGF strains from sulcata tortoises. These isolates were evaluated alongside a Texas tortoise isolated recently isolated by another undergraduate student to determine whether they represented new genera and whether their placement required changes to the current taxonomy of Neocallimastigomycota. Because this project was part of a larger collaborative effort in the Elshahed and Youssef laboratory, my report focuses primarily on the portions of the study I directly contributed to, including targeted anaerobic isolation, culture maintenance, substrate utilization testing, and temperature preference experiments. Additionally, I was heavily involved in the laboratory work of microscopy, sequencing, and phylogenetic and community-level analyses, but their interpretation and significance were concluded higher-level individuals, and they are included only as needed to explain the broader significance of my work.

2. Experimental Details

Targeted isolation efforts were guided by prior sequencing data that identified tortoise fecal samples with high relative abundance of potentially novel AGF (5,6). Samples used for my isolation work were obtained from a sulcata tortoise (*Centrochelys sulcata*) at the Tanganyika Wildlife Park in August 2024 and from another sulcata tortoise at the Amarillo Zoo in February 2025. Fecal samples were collected in sterile 50 mL Falcon tubes, frozen on site, and stored at -20°C until used for isolation.

Isolation enrichments were prepared in an anaerobic chamber using rumen-fluid cellobiose medium (RFC) with antibiotics (50 µg/mL chloramphenicol, 50 µg/mL penicillin

G sodium salt, 20 µg/mL streptomycin sulfate). Enrichments were incubated at 30°C and monitored for signs of fungal growth, including biomass formation, sticking to glass, and gas production. Once growth was observed, roll tubes containing RFC medium, antibiotics, and 2% agar were prepared from the enrichment tubes and incubated again until colony growth was present. Single colonies were picked in an anaerobic chamber and transferred to fresh RFC medium with antibiotics. Roll tubing and colony-picking were repeated twice to ensure culture purity.

Morphological characterization was conducted because the isolates appeared distinct from previously described AGF. Cultures were evaluated using microscopy to observe thallus structure, rhizomycelia, sporangia, zoospores, and zoospore-release mechanisms. For selected samples, DAPI staining, confocal microscopy, and scanning electron microscopy were used to examine nuclear localization and fine structural details.

To examine substrate utilization, cultures were grown in Balch tubes containing rumen fluid (RF) media with individual carbon sources. Each tube had a total volume of 10 mL, consisting of 7 mL medium and 3 mL fungal culture. Three replicates were tested per substrate and strain, along with uninoculated controls. Cultures were followed for four consecutive subcultures. Growth was evaluated by visual inspection and gas pressure measurements (MediaGauge). Gas pressure readings were conducted at 35 °C to eliminate any that influence temperature changes might have on the pressure reading. Gas pressure measurements and visual growth evaluation were condensed into a qualitative growth scale of – (no growth), + (very slight growth), ++ (slight growth), +++ (good growth), and ++++ (very good growth), given that visually well-growing cultures did not produce noticeable gas pressure on some substrates (Table 1).

To determine the optimal growth temperature, isolates were grown in RFC medium at six different temperatures (22, 28, 32, 35, 37, and 39°C), and growth was evaluated using the scale described above (Table 1). For each isolate, three replicates and one uninoculated control were investigated at each temperature for six consecutive subcultures.

DNA was extracted from cultures grown in RFC medium and used for phylogenetic analyses. The D1-D2 region of the LSU rRNA gene was amplified for initial identification. For more detailed analysis, the region including ITS-1, 5.8S rRNA, ITS-2, and the D1/D2 domains of the 28S (LSU) rRNA gene was amplified (approximately 1.3 Kb). Sequencing results were aligned to reference sequences, and phylogenetic trees were generated to determine whether the isolates clustered with known AGF genera or represented novel lineages.

For the broader Testudines mycobiome analysis, DNA was extracted from 71 additional fecal/cloacal samples from 27 Testudines species. Primers targeting the D2 region of the LSU rRNA gene were used to amplify fungal sequences, which helps recover early diverging fungal lineages that can be underrepresented by some commonly used fungal markers (9). The samples were sent for Sanger sequencing at the Oklahoma State University

DNA Protein Core Facility. Sequence data were processed using the Mothur MiSeq standard operating procedure (10). Sequences were quality filtered, clustered at 94% similarity as a genus-equivalent threshold and classified using fungal LSU reference databases supplemented with anaerobic fungal sequences. Unclassified sequences were compared to additional databases and inserted into reference fungal trees to improve taxonomic placement. Sequences clustering with Chytridiomycota outside described orders were assigned putative new order designations, while Rozellomycota-affiliated sequences were assigned at higher taxonomic ranks when necessary.

Statistical analyses were used to identify host factors associated with fungal community differences. The relative abundance of major fungal phyla was compared across host family, feeding preference, food retention time, carapace length, and domestication status. Community assembly patterns were evaluated to distinguish deterministic processes, such as host selection, from more stochastic patterns that may reflect transient environmental fungi.

Table 1: Growth of strains GXA2, SR0.6, and TM0.3 on different substrates and temperatures. ‘-’ refers to no growth, ‘++++’ refers to very good growth. (6)

Substrates		GXA2	SR0.6	TM0.3
Monosaccharides	Arabinose	-	+	-
	Fructose	+++	+++	++
	Galactose	-	+++	++
	Glucose	+++	+++	+++
	Xylose	-	-	-
Disaccharides	Cellobiose	+++	+++	+++
	Lactose	+++	+++	-
	Trehalose	-	-	+
Polysaccharides	Cellulose	+++	+++	+++
	Chitin	++	++	-
	Inulin	++	++	-
	Pectin	+	+	-
	Polygalacturonic Acid	+	+	-
	Xylan	++	++	++
Temperature (°C)		GXA2	SR0.6	TM0.3
	22	-	-	+
	28	+	++	++
	32	+++	++++	+++
	35	++++	++++	++++
	37	++	+++	++
	39	-	++	+

3. Results

Targeted anaerobic isolation yielded three viable AGF isolates for further characterization (6): GXA2 from an OKC Zoo Texas tortoise (isolated by another undergrad in the lab, Alex Morris), SR0.6 from a Tanganyika Wildlife Park sulcata tortoise, and TM0.3 from an Amarillo Zoo sulcata tortoise. All three strains have remained viable on RFC media with weekly transfers at 30°C.

The isolates displayed distinct morphological characteristics. GXA2 produced filamentous, polycentric thalli with nucleated rhizomycelia and elongated, unbranched nucleated structures (Figure 1). SR0.6 produced filamentous, monocentric thalli with mostly anucleated rhizomycelia and globose sporangia with hair-like projections. Sporangia increased in size with age, with some reaching diameters of more than 100 µm. SEM microscopy revealed pores in the sporangial wall as a potential zoospore release mechanism (Figure 2). TM0.3 produced filamentous, polycentric thalli with less branched but nucleated filaments. Sporangia were irregularly shaped. Zoospore release was observed through a rupture of the sporangial wall (Figure 3). These features helped distinguish the isolates from previously described AGF genera.

Figure 1. GXA2 microscopy. Further figure description in reference 6.

Figure 2. SR0.6 microscopy. Further figure description in reference 6.

Figure 3. TM0.3 microscopy. Further figure description in reference 6.

Substrate utilization experiments showed the substrates glucose, fructose, cellobiose, cellulose, and xylan supported growth of all three isolates through repeated subculturing. Lactose supported the growth of GXA2 and SR0.6 but not TM0.3. Chitin supported growth of GXA2, while galactose supported the growth of SR0.6 and TM0.3. Temperature trials showed that all three isolates grew best at 35°C, with SR0.6 also showing very good growth at 32°C. GXA2 grew between 28 and 37 °C (Table 1). Strain SR0.6 grew between 28 and 39°C. TM0.3 grew across the widest tested range, from 22 to 39°C.

Phylogenetic analyses placed the GX, SR, and TM isolates into three deeply branching clades related to previously described tortoise-affiliated genera but distinct from both each other and M-AGF. The three novel genera and species are proposed as *Gopheromyces tardescens* (GXA2), *Gigasporangiomyces pilosus* (SR0.6), and *Kelyphomyces adhaerens* (TM0.3) (6). Based on phylogenetic, physiological, and ecological evidence, four T-AGF genera (*Testudinimycetes*, *Gopheromyces*, *Gigasporangiomyces*, and *Kelyphomyces*) were placed into the proposed family Testudinimycetaceae. Previous molecular dating analysis with the two tortoise AGF genera pushed the Neocallimastigomycota divergence to the early Cretaceous. The addition of the three novel taxa to the AGF phylogenomic tree further suggested an earlier divergence time of AGF, to the early Cretaceous/late Jurassic (Figure 4).

Figure 4. Updated phylogenetic tree of AGF. Further figure description in reference 6.

The broader Testudines gut mycobiome analysis revealed a highly diverse fungal community. Across 71 additional fecal/cloacal samples, representatives of 17 phyla and 157 orders were identified (11). Four major phyla (Neocallimastigomycota, Chytridiomycota, Ascomycota, and Basidiomycota) made up most of the community. Neocallimastigomycota represented 19.56% of the total dataset and were identified in nearly all (68/71) samples examined. Its relative abundance was higher in family Testudinidae (tortoises) ($42.36 \pm 37.99\%$), compared to all other host families ($3.31 \pm 4.28\%$) combined, and it was significantly associated with feeding preference, gut retention time, host family, carapace length, and domestication status. Comparisons of different feeding preferences showed species with a primarily herbivorous diet harbored significantly more Neocallimastigomycota than those that are primarily carnivorous or omnivorous with a carnivore diet preference (Wilcoxon test p -value <0.05).

The sequencing survey also identified representatives of several recently described yet-uncultured fungal phyla (11,12) (Figure 5). The analysis also identified putative novel orders in Chytridiomycota and higher-rank lineages in Rozellomycota. Because many of these lineages occurred at low relative abundances, they may represent rare community members, transient fungi passing through the GI tract, or environmental fungi associated with sample collection.

Community assembly analyses suggested that tortoise fungal communities are more strongly shaped by deterministic processes than those of several other turtle families. This pattern indicated that host-associated selection may be especially important in Testudinidae. In contrast, more stochastic patterns in other turtle families may reflect a larger contribution of transient environmental fungi. Overall, differences in Testudines fungal community structure were largely driven by the abundance and identity of Neocallimastigomycota.

Figure 5. Phylogenetic fungal tree of life based on D1-D2 regions of LSU rRNA. Further figure description in reference 11.

4. Discussion and Conclusions

This project expands the known diversity of anaerobic gut fungi by describing tortoise-affiliated AGF and the characterization of the Testudines fungal gut microbiome. The isolation of SR0.6 and TM0.3 from sulcata tortoises, together with the Texas tortoise isolate GXA2, increased the number of known T-AGF genera from two to five. Their distinct morphology, substrate utilization patterns, temperature preferences, and phylogenetic positions support their recognition as three novel genera. The discovery of *Gopheromyces*, *Gigasporangiomyces*, and *Kelyphomyces* also has taxonomic significance (6). The T-AGF form deep-branching lineages that differ from M-AGF in host preference, phylogenetic placement, and physiology. These differences support the proposal of Testudinimycetaceae to accommodate several of the T-AGF genera. This taxonomic revision reflects the growing evidence that reptile-associated AGF are not simply extensions of M-AGF diversity but instead represent distinct evolutionary lineages.

The physiological results further suggest that T-AGF may be adapted to their host environment. Compared with many M-AGF, these isolates grew across lower and broader

temperature ranges, which help them persist in the poikilothermic hosts. Differences in substrate use including growth on galactose by SR0.6 and TM0.3 and growth on chitin by GXA2 suggest that these fungi may have metabolic capabilities worth further investigation. Future genomic work could clarify the genetic basis of these traits.

The Testudines mycobiome survey placed the culture-based finding in a broader ecological context (11). Neocallimastigomycota was not limited to strict herbivorous tortoises but was detected across nearly all Testudines samples examined. This challenges the previous assumption, based largely on mammalian hosts, that AGF colonization requires strict herbivory and a dedicated fermentation chamber. Although turtles are not strict herbivores and do not possess the same gut structures as ruminants, they often have long food retention times, anaerobic conditions in the large intestine, and at least some plant material in the diet. These features may allow Neocallimastigomycota to colonize plant material within the turtle GI tract.

At the same time, the much higher abundance of Neocallimastigomycota in Testudinidae suggests that tortoises provide especially favorable conditions for resident AGF communities. In contrast, many other fungal lineages detected in the broader survey were rare or variably distributed, suggesting that some may be transient members introduced through diet or the environment. This distinction between resident and transient fungi is important for interpreting animal mycobiomes, especially in hosts that interact closely with soil, sediment, aquatic environments, and diverse diets.

Together, these results show that Testudines are valuable hosts for understanding fungal evolution and animal-associated mycobiomes. The project broadens the fungal tree of life by identifying novel cultured AGF lineages as well as additional representatives of uncultured fungal groups. Future work should continue sampling a wider range of tortoise and turtle species, improve isolation strategies for difficult to culture lineages, and use comparative genomics to understand how host ecology shapes AGF evolution and function.

5. Summary

My Niblack project contributed to the discovery and characterization of novel tortoise-affiliated anaerobic gut fungi and to the broader description of fungal diversity in Testudines. Targeted isolation from sulcata tortoise fecal samples yielded novel AGF strains that, together with a Texas tortoise isolate, support the description of three new genera and species: *Gopheromyces tardescens*, *Gigasporangiomyces pilosus*, and *Kelyphomyces adhaerens*. These isolates expand the known diversity of tortoise-associated AGF and support the proposal of the new family Testudinimycetaceae. Sequencing of 71 Testudines fecal/cloacal samples revealed a diverse gut mycobiome and showed that Neocallimastigomycota occurs across nearly all sampled host families but is most abundant in tortoises. Overall, this work supports the importance of reptile hosts in AGF evolution and highlights Testudines as an underexplored source of fungal diversity.

6. Appendices

6a. Acknowledgements

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6b. Papers Published

1. Morris, A., Mills, T., Miller, S. L., Pratt, C. J., Wang, Y., Elshahed, M. S., Youssef, N. H., and Vinzelj, J. Description of *Gopheromyces tardescens* gen. nov., sp. nov., *Gigasporangiomyces pilosus* gen. nov., sp. nov., *Kelyphomyces adhaerens* gen. nov., sp. nov., proposal of *Testudinimycetales* ord. nov. and *Testudinimycetaceae* fam. nov., and emended description of the order *Neocallimastigales*. Manuscript in publication process.
2. Mills, T., Vinzelj, J. M., Cook, E. R., Mills, E., Rurik, A. J., Dallas, J. W., Walker, D. M., Stone, P. A., Siler, C. D., Eichelberger, H., Elshahed, M. S., and Youssef, N. H. Exploring the diversity and community structure of the Testudines fecal mycobiome. Manuscript in preparation.
3. Poster presentation of “[Description of three novel anaerobic gut fungal genera from tortoise feces](#)” at The Oklahoma Academy of Science at ORU (Fall 2025) and the OSU Undergraduate Research Symposium (Spring 2026).

6c. Literature Cited

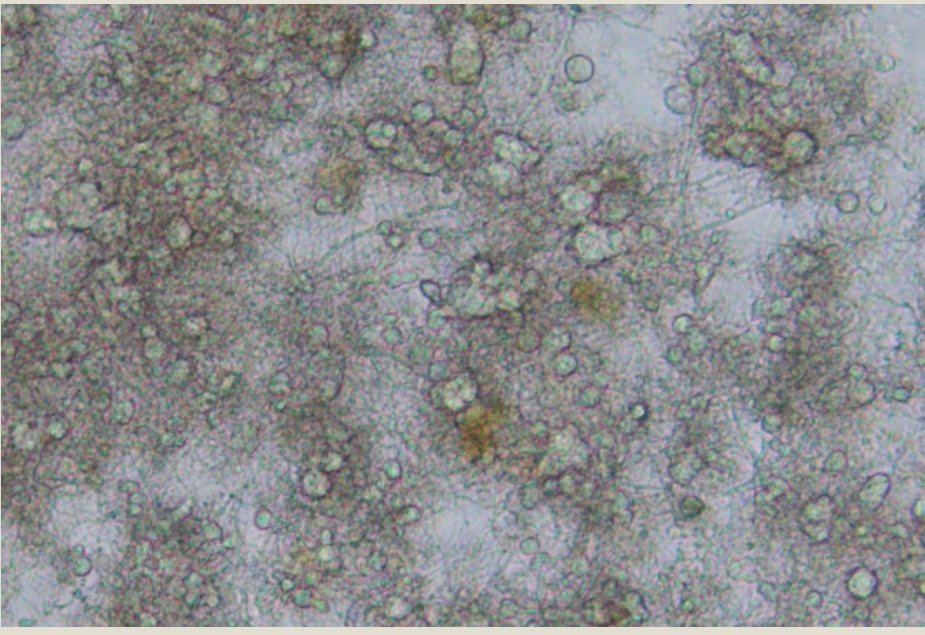
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Diversity and Evolutionary Significance of Anaerobic Gut Fungi in Testudines

Taylor Mills
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2025 - 2026 Niblack Research Scholar



BACKGROUND

Anaerobic gut fungi (AGF):

- Phylum Neocallimastigomycota
- Strict anaerobes
- Aid in plant biomass degradation in the gastrointestinal tract of herbivorous animals
- Much of what is known about them has come from mammalian hosts

This leaves a major gap in understanding how AGF diversity evolved and how the fungi interact with non-mammalian hosts.

Two recently described AGF genera (*Testudinimycetes* and *Astrotestudinimycetes*) obtained from tortoise fecal samples represent the first AGF isolates from non-mammalian hosts.

Family Bovidae

- Cattle
- Goat
- Sheep
- Buffalo
- Bison
- Chamois
- Markhor
- Miniature Zebu
- Mountain Goat

Family Cervidae

- White-tail Deer
- Elk
- Pere David's Deer
- Red deer

Family Camelidae

- Alpaca
- Llama
- Camel

Family Caviidae

- Mara
- Capybara

Family Giraffidae

- Okapi
- Giraffe

Family Rhinocerotidae

- Rhinoceros

Family Elephantidae

- Elephant

Family Trichechidae

- Manatee

Family Equidae

- Horse
- Mule
- Donkey
- Przewalski's Horse
- Zebra

NIBLACK PROJECT FOCUS

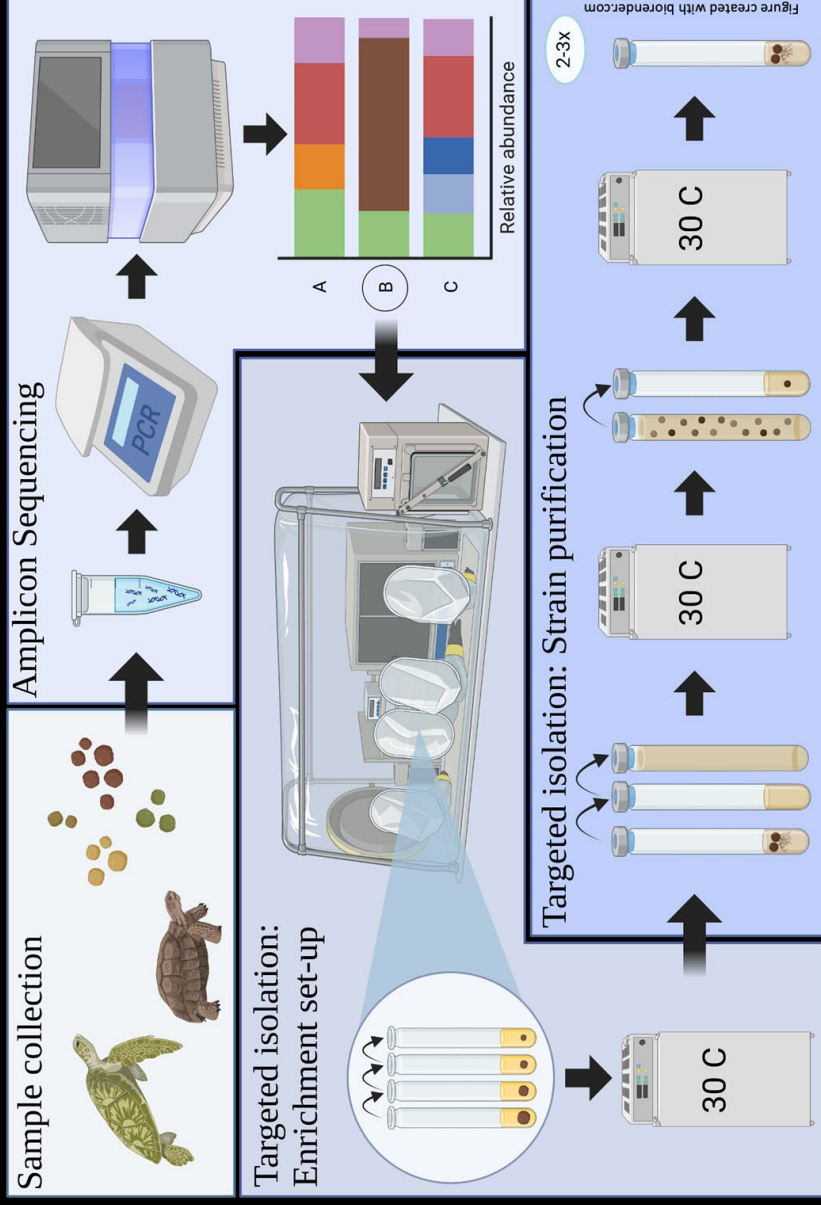
Novel tortoise -affiliated AGF (T-AGF):

- ecological preference for tortoises
- suggests that tortoises may harbor lineages that are evolutionarily distinct from mammal-associated AGF (M-AGF).
- molecular dating from previous work further suggested that T-AGF may represent some of the oldest cultured AGF

We hypothesized that additional T-AGF lineages would be discovered through targeted isolation from new tortoise samples.

My Niblack project focused on isolating and characterizing novel tortoise-affiliated AGF from sulcata tortoise fecal samples.

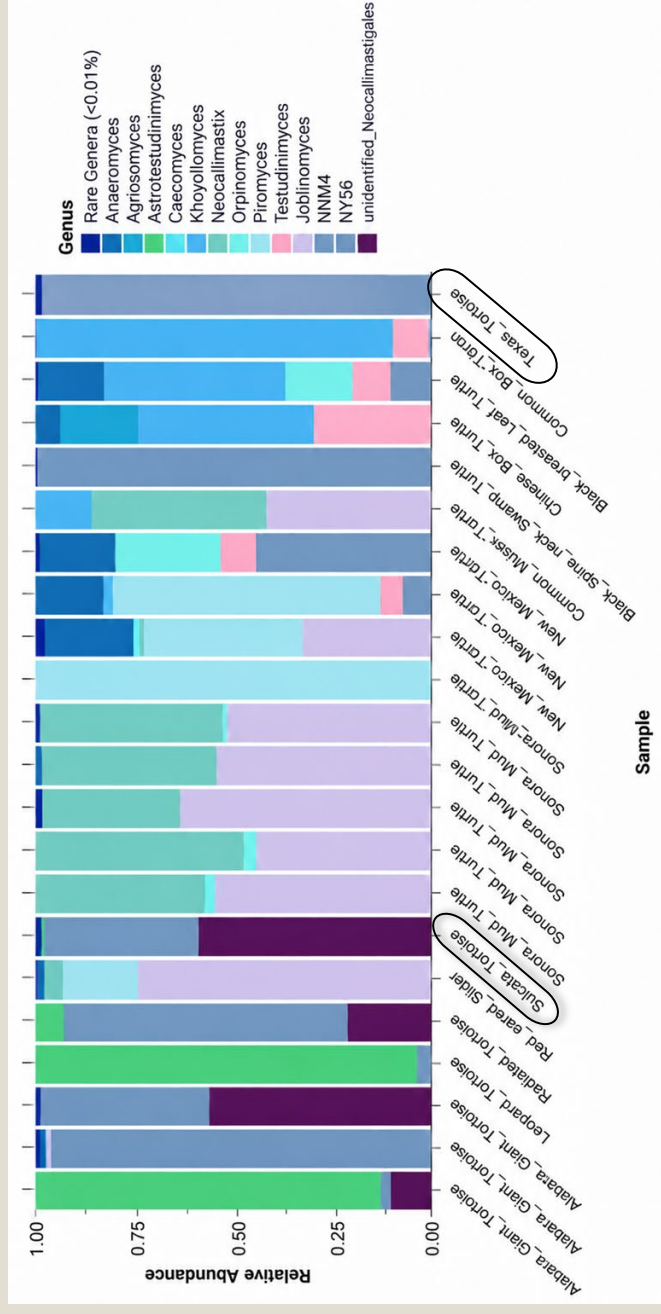
ISOLATION



- Fecal samples were collected, stored, and used for isolation.
- Focus on samples with higher abundance of novel genera.
- Isolation enrichments were prepared in an anaerobic chamber using rumen-fluid cellobiose medium (RFC) with antibiotics.
- Enrichments were monitored for signs of fungal growth: biomass formation, sticking to glass, and gas production.
- Roll tubes were used for colony picking and culture purification.

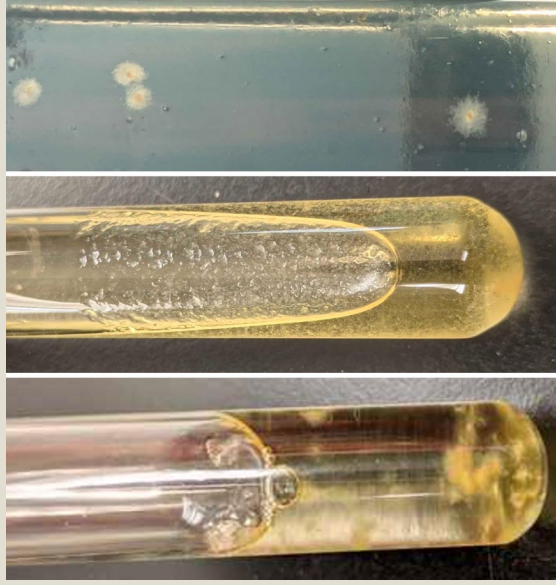
EXPERIMENTAL DETAILS

- A few tortoise samples harbored novel AGF diversity in high abundance
- Samples with the greatest relative abundance of novel fungi were selected to undergo isolation efforts.

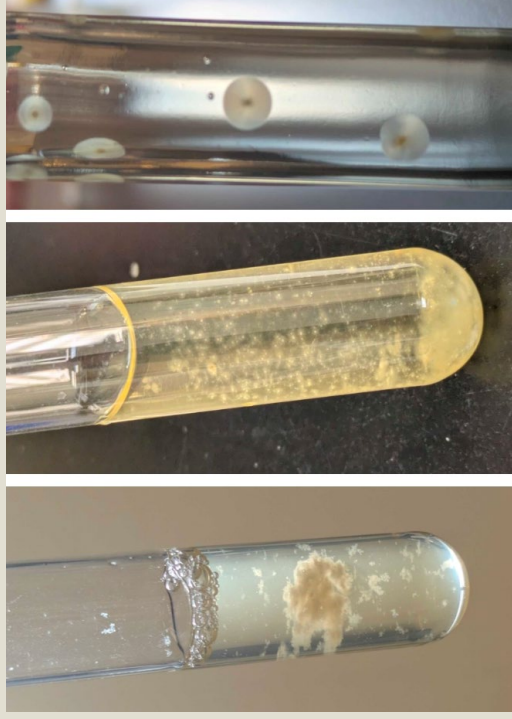


SUCCESSFUL ISOLATION

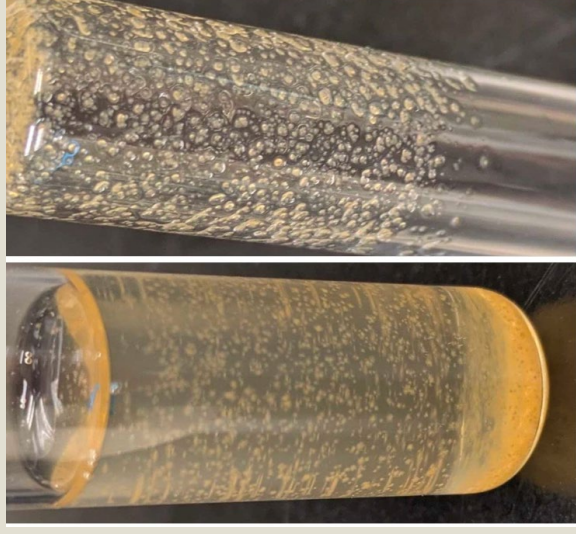
*Gopheromyces
tardescens*
(GXA2)



*Gigasporangiomyces
pilosus*
(SR0.6)



*Kelyphomyces
adhaerens*
(TM0.3)



MORPHOLOGICAL CHARACTERIZATION OF THE ISOLATES

- Microscopy was used to observe physical characteristics.
- For selected samples, DAPI staining, confocal microscopy, and scanning electron microscopy were used to examine nuclear localization and fine structural details.
- The isolates displayed distinct morphological characteristics.

GXA2:

- Filamentous, polycentric thalli
- Nucleated rhizomycelia
- Elongated, unbranched nucleated structures

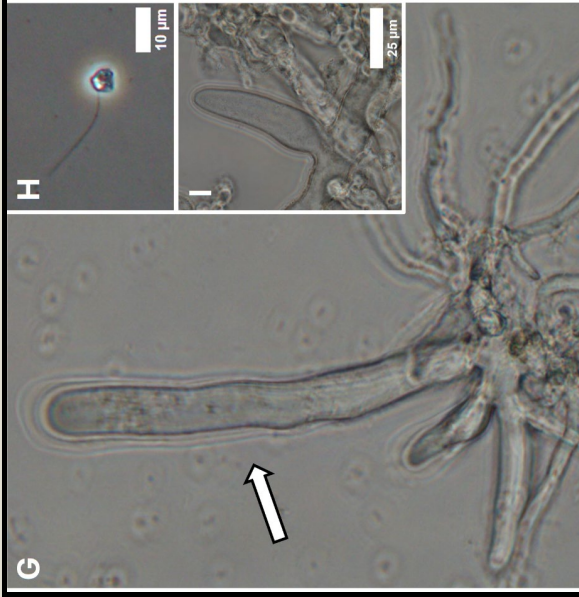
SR0.6:

- Filamentous, monocentric thalli
- Mostly anucleated rhizomycelia
- Sporangia with hair-like projections
- Some sporangia reached diameters of more than 100 μm
- Pores in the sporangial wall release zoospores

TM0.3:

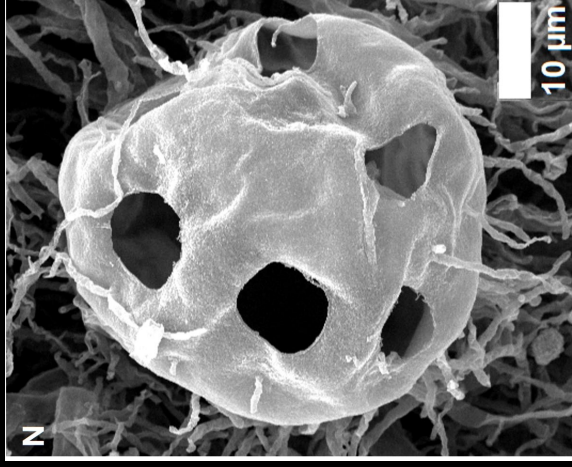
- Filamentous, polycentric thalli
- Less branched but nucleated filaments
- Sporangia are irregularly shaped
- Zoospore release through a rupture of the sporangial wall

THREE NOVEL ISOLATES



Gopheromyces
tardescens
(GXA2)

OKC Zoo Texas
tortoise



Gigasporangiomyces
pilosus
(SR0.6)

Tanganyika Wildlife Park
sulcata tortoise



Kelyphomyces
adhaerens
(TM0.3)

Amarillo Zoo sulcata
tortoise

SUBSTRATE UTILIZATION

- All isolates: glucose, fructose, cellobiose, cellulose, and xylan
- GXA2: lactose and chitin
- SR0.6: lactose and galactose
- TM0.3: galactose

Substrates		GXA2	SR0.6	TM0.3
Monosaccharides	Arabinose	-	+	-
	Fructose	+++	+++	++
	Galactose	-	+++	++
	Glucose	+++	+++	+++
	Xylose	-	-	-
Disaccharides	Cellobiose	+++	+++	+++
	Lactose	+++	+++	-
	Trehalose	-	-	+
Polysaccharides	Cellulose	+++	+++	+++
	Chitin	++	++	-
	Inulin	++	++	-
	Pectin	+	+	-
	Polygalacturonic Acid	+	+	-
	Xylan	++	++	++

Table 1: Growth of strains GXA2, SR0.6, and TM0.3 on different substrates. ‘-’ refers to no growth, ‘++++’ refers to very good growth.

TEMPERATURE OPTIMIZATION

- All three isolates grew best at 35°C.
- GXA2: grew between 28 and 37 °C
- SR0.6: grew between 28 and 39°C, optimal growth also at 32°C.
- TM0.3: grew across the widest tested range, from 22 to 39°C.

Temperature (°C)		GXA2	SR0.6	TM0.3
	22	-	-	+
	28	+	++	++
	32	+++	++++	+++
	35	++++	++++	++++
	37	++	+++	++
	39	-	++	+

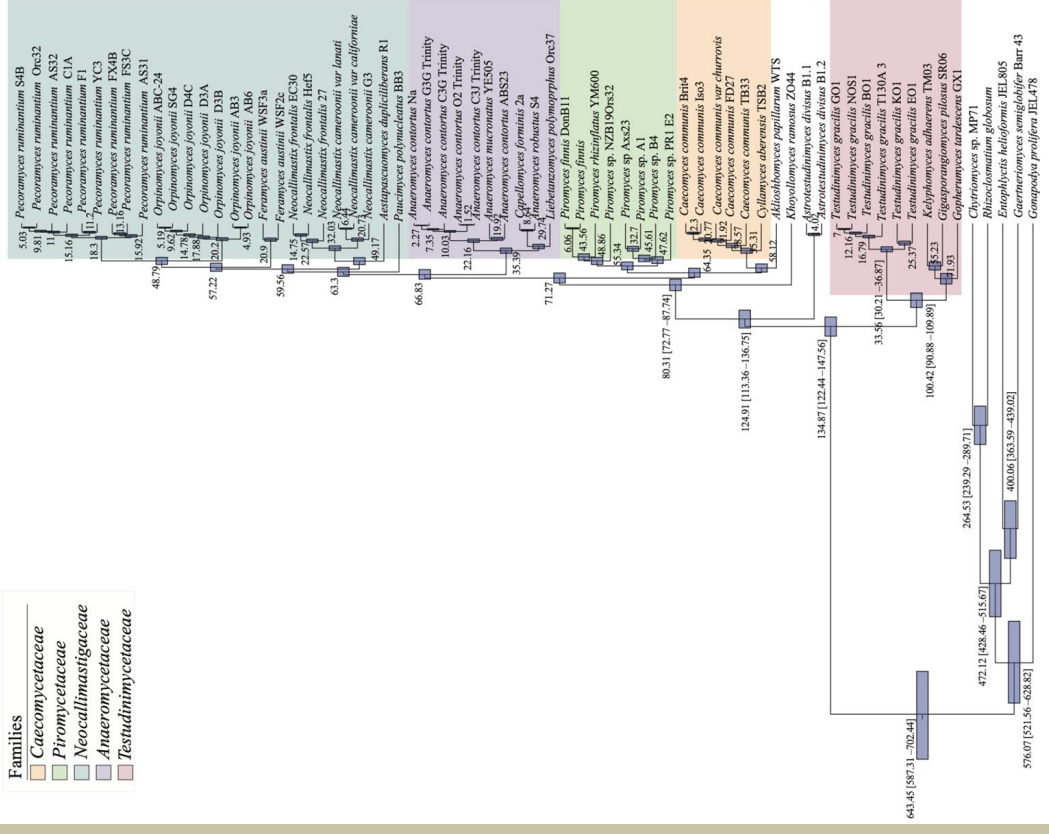
Table 2: Growth of strains GXA2, SR0.6, and TM0.3 at different temperatures. ‘-’ refers to no growth, ‘++++’ refers to very good growth.



- Initial identification: D1/D2 region of the LSU rRNA
- Detailed analysis: 1.3 Kb region including ITS-1, ITS-2, and D1/D2 domains

- GX, SR, and TM:
- related to previously described tortoise-affiliated genera
 - distinct from each other and M-AGF

- Based on phylogenetic, physiological, and ecological evidence, four T-AGF genera (*Testudinimycetes* , *Gopheromyces* , *Gigasporangiomycetes* , and *Kelyphomyces*) were placed into the proposed family Testudinimycetaceae.



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Description of *Gopheromyces tardescens*, gen. nov., sp. nov., *Gigasporangiomyces pilosus*, gen. nov., sp. nov., *Kelyphomyces adhaerens*, gen. nov., sp. nov., and proposal of *Testudinimycetaceae*, fam. nov.

Alexandria Morris¹, Taylor Mills¹, Samuel L. Miller¹, Carrie J. Pratt^{1,2}, Stephen Marek³, Yan Wang², Mostafa S. Elshahed¹,

Noha H. Youssef¹ and Julia Vinzejj^{1,*} 

 View Affiliations

<https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.007196>

Gopheromyces tardescens :
● *Gopherus* - the gopher tortoise (*Gopherus berlandieri*)
● *myces* - “fungus”
● *tardescens* - “to grow slowly”

Gigasporangiomyces pilosus :
● *gigas*- Greek, “giant”
● *sporangion* - “spore vessel”
● *myces* - “fungus”
● *pilosus* - Latin, “hairy”

Kelyphomyces adhaerens :
● *kelyphos* - Greek, “shell, covering”, host
Centrochelys sulcata
● *myces* - “fungus”
● *adhaerens* - Latin, “sticking, clinging”

New Results

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Exploring the diversity and community structure of the Testudines fecal mycobiome

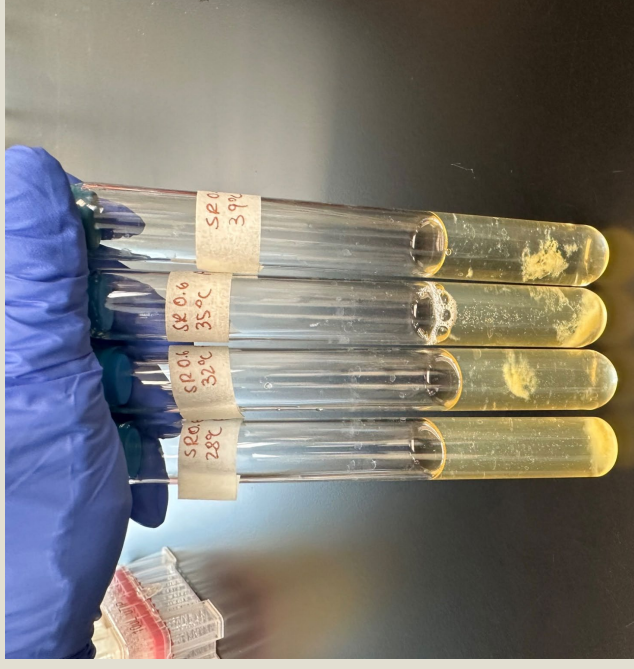
Taylor Mills, Julia M. Vinzeli, Emma R. Cook, Emma Mills, Alexander J. Rurik, Jason W. Dallas, Donald M. Walker, Paul A. Stone, Cameron D. Siler, Mostafa S. Elshahed,  Noha H. Youssef

doi: <https://doi.org/10.64898/2026.04.22.720109>

<https://doi.org/10.64898/2026.04.22.720109>

CONCLUSION/SIGNIFICANCE

- Three additional tortoise genera were isolated from Texas tortoise and Sulcata tortoise.
- A new family is proposed to accommodate these three novel genera and one of the previously isolated tortoise genera.
- All T-AGF genera are evolutionarily older than the M-AGF genera (Testudines are older than Mammalia).
- T-AGF are distinct from M-AGF.



Overall, this work supports the importance of reptile hosts in AGF evolution and highlights Testudines as an underexplored source of fungal diversity.

THANK YOU!!

I gratefully acknowledge Dr. and Mrs. Niblack and the Niblack Research Scholars Program for supporting and funding this research.

I also thank Dr. Mostafa Elshahed, Dr. Noha Youssef, Sakalya De Silva, Julia Vinzeli, Alexandria Morris, and additional members of the laboratory for their mentorship, training, and assistance throughout this project.



Comparative Metagenomic Analysis of the Gut Community of Ratites

Ty Young

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Faculty Sponsor: Mostafa Elshahed

Graduate Student Mentor: Hasan Mehedi

ABSTRACT

Ratites are a commonly overlooked group of large flightless birds including the emu, ostrich, and rhea that each possess a unique gastrointestinal makeup. Here we present a comprehensive, comparative metagenomic analysis of the gut microbiome of these three Ratites. We recovered 494 high-quality genomes from 3.2 Gbp of sequencing data. These genomes represented 17 phyla, 21 classes, 55 orders, and 103 families. Each Ratite displayed a significantly distinct microbial community with host phylogeny, gastrointestinal architecture, and diet being identified as the main drivers of unique community composition. The phyla, *Bacillota* and *Bacilloita* dominated the ostrich and rhea samples while *Bacillota* and *Actinomycetota* dominated the emu community. Despite the phyla overlap, each Ratite saw vast divergence at the family level. Carbohydrate-active enzyme (CAZyme) profiling revealed a shared composition of GH13, GH43, and GH5 across the Ratites, though the distribution of these CAZyme classes varied between each animal. These findings contribute to the classification of the uncharacterized Ratite gut microbiome and the CAZyme profile within them.

Introduction

Animals harbor a wide array of microorganisms, including bacteria, fungi, protozoa viruses and archaea within their gastrointestinal tract (Barko et al., 2018). Collectively, these microorganisms make up an animal's gut microbiome. The gut microbiome plays a multitude of roles in the health of an animal such as nutrient absorption (Negash, 2022), immune system regulation (Choden and Cohen, 2022), disease progression (Yoshida et al., 2018), and brain health (Mohajeri et al., 2018). The study of the gut microbiome makes vast contributions to expanding the known diversity of microorganisms, understanding the intricacies of co-evolutionary relationships between a host and its microbiome, identifying the impact specific taxa have on their host, and displaying the relation between the health of other animals to humans. Over the past two decades, progress in studying the animal gut microbiome has been remarkable (Peixoto et al., 2021). This progress has been marked with

an emphasis on human health (Liang et al., 2018), animals of economic importance (e.g. cattle) (Zhou et al., 2018), and studying common animal models such as mice (Park and Im, 2020) and zebra fish (Zhong et al., 2022). Beyond this, research into more unusual host, such as pets and zoo animals, to address antimicrobial resistance and expand the known diversity of microbes, has received relatively less attention (Youngblut et al., 2020) (Schmartz et al., 2024). However, more is needed as a plethora of animals remains unstudied.

Birds (Aves) are an extremely diverse class of animals that potentially contain up to 18,000 species (Barrowclough et al., 2016). Within Aves, the order Struthioniformes (commonly referred to as Ratites) are large, flightless birds that lack a kneel and are comprised of the ostrich, emu, rhea, cassowary, and kiwi (Kummrow, 2015). Within the Ratites, the ostrich, emu, and rhea possess differing gastrointestinal makeup. The ostrich possesses a unique gastrointestinal makeup that relies on hindgut-fermentation in their enlarged cecum and colon (Matsui et al., 2010). The rhea has a paired cecum but lacks a distinct colon (Frei et al., 2015). The emu, more in-line with a typical Ave digestive system, lack both a paired cecum and distinct colon (Frei et al., 2015). These differences in gastrointestinal architecture point to potentially well adapted microbial communities fitted to these birds' diets and gastrointestinal makeup.

To date, examination of the microbial gut communities of these ratites has been sparse and focused on identification of the cause of mortality in a farm setting, mostly using 16s rRNA amplicon-based surveys (Videvall et al., 2020) (Curry et al., 2022) (Kim et al., 2023). Nevertheless, a comprehensive detailed metagenomic analysis of the gastrointestinal tract microbiome of these Ratites remains currently lacking.

To this end, we conducted a metagenomic based survey on three Ratite species, the ostrich, emu, and rhea to address the microbial communities' differences between and in species, compare this to Aves as a whole, identify key organisms within their respective communities, and highlight specific and prevalent metabolic functions.

Experimental Details

Ostrich fecal samples (n=7), emu fecal samples (n=7), and rhea fecal samples (n=7) were passively collected in 2025 from various private farms located in Vermont, Arkansas, Arizona, and Illinois within the USA. The fecal samples were aseptically collected in 50-mL falcon tubes and put on ice for transport. Once in the laboratory, the fecal samples were placed at -80°C for storage until being processed for DNA extraction.

DNA was extracted from each of the fecal samples using the Quick DNA fecal/soil microbe miniprep kit according to the manufacturer's instructions (Zymo, Irvine, California, USA) Once extracted, the DNA was subjected to quantity and quality analysis using Qubit

TM dsDNA HS Assay Kit (Thermo Fisher, Waltham, Massachusetts, USA) and the 4200 TapeStation System (Agilent, Santa Clara, California, USA) to determine the concentration and fragment length of the extracted DNA.

Extracted DNA was subjected to shotgun metagenome DNA sequencing using the Illumina NextSeq 2000 platform (San Diego, California, USA). The metagenomic reads were trimmed and quality filtered using Trimmomatic v.0.39 (Bolger et al., 2014). The high-quality trimmed metagenome reads were then assembled into metagenomic contigs using Megahit v.1.2.9, using base parameters, with all samples being combined on a per animal basis (Li et al., 2016). The metagenomic contigs were assessed for quality using QUAST v.4.4, confirming large and complete contigs (Gurevich et al., 2013).

The high-quality contigs were binned into draft genomes using CONCOCT v1.1 (Alneberg et al. 2013), MetaBAT2 v1.7 (Kang et al., 2019), and MaxBin2 v2.2.4 (Wu et al., 2016). DAS Tool v1.1.2 was used to combine and optimize the bins from each of the previously listed programs (Sieber et al., 2018). CheckM v1.0.18 was used to estimate the genome completeness and contamination of the draft genomes (Parks et al., 2015). Low-quality bins (>10% contamination and/or <50% completeness) were not evaluated further.

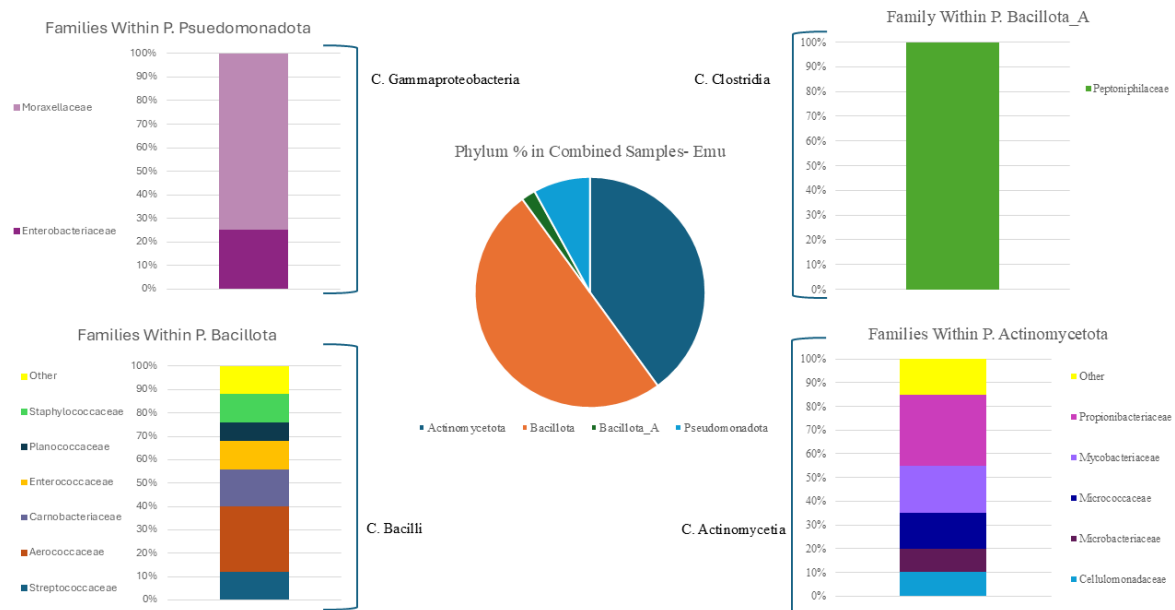
Taxonomic classification and novelty assessment followed the Genome Taxonomy Database (GTDB) release 226 (Parks et al., 2022) and were carried out using GTDB-Tk v2.3.2 on default parameters (Chaumeil et al., 2022). The phylogenetic analysis evaluated the alignment and identification of 120 bacterial and 122 archaeal single-copy marker genes generated within GTDB-Tk (Chaumeil et al., 2022), using this data, a phylogenetic tree was assembled using FastTree 2 (Price et al., 2010).

Common genomic features including genome size, coding density, GC content, number of genes, and average gene length were gathered using a custom-made Pearl Script. Other common features such as the number of ribosomal RNA and transfer RNA genes were gathered using Barrnap v.1.10.5 (<https://github.com/tseemann/barrnap>) and tRNAscan-SE (Chan and Lowe, 2019), respectively.

Due to the primarily herbivorous nature of the evaluated Ratites, a portion of their carbohydrate metabolism was examined through looking at the community of carbohydrate-active enzymes (CAZymes) produced by members of their gut microbiome. CAZymes are a class of enzymes that are involved in the breakdown of complex polysaccharides, such as cellulose, hemicellulose, and starch (Gavande et al., 2023). To see which CAZyme families were present within the gut microbiome of each animal, the previously assembled metagenomic contigs were annotated, using Prokka v1.14.5 (Seemann, 2014), and combined for each Ratite then input into the meta server, dbCAN2 v10 (Zhang et al., 2018), whose database includes ~500,000 individual CAZyme sequences (Zheng et al., 2023).

Results

Metagenomic sequencing of the different Ratite fecal samples yielded a total of 3.2 Gbp of reads, with an average of 94.96% of the reads being assembled into contigs for each animal. A total of 651 draft genomes were binned, of which, 494 genomes remained after quality control. These high-quality metagenome-assembled genomes (MAGs) belonged to 17 phyla, 21 classes, 55 orders, and 103 families across the three Ratites. Each Ratite displayed a unique distribution and composition of taxa within their respective gut communities. At the phylum level, the emu samples were dominated by the phyla, *Bacillota* and *Actinomycetota*. While the ostrich was comprised of, *Bacillota_A*, *Bacillota*, and *Bacteroidota*. Lastly, the rhea samples displayed a large distribution of, *Bacillota_A* and to a lesser extent, *Bacillota*. At the family level, the three Ratites display a wider divergence when compared to each other. Within the emu microbiome, the prevalent families are *Aerococcaceae* and *Propionibacteriaceae*. While the ostrich samples are comprised of *Lachnospiraceae* and the uncultured family, UBA660. Similarly, the rhea samples are vastly dominated by the family, *Lachnospiraceae*. The communities between these three Ratites, as displayed on an exploratory analysis, shows a clear distinction in community composition between them, with the emu and ostrich communities sharing the most similarity. Along with taxonomic evaluation, assessment of the novelty of the MAGs was executed, displaying 1 novel family, 23 novel genera, and 262 novel species based on relative evolutionary divergence (RED) value.



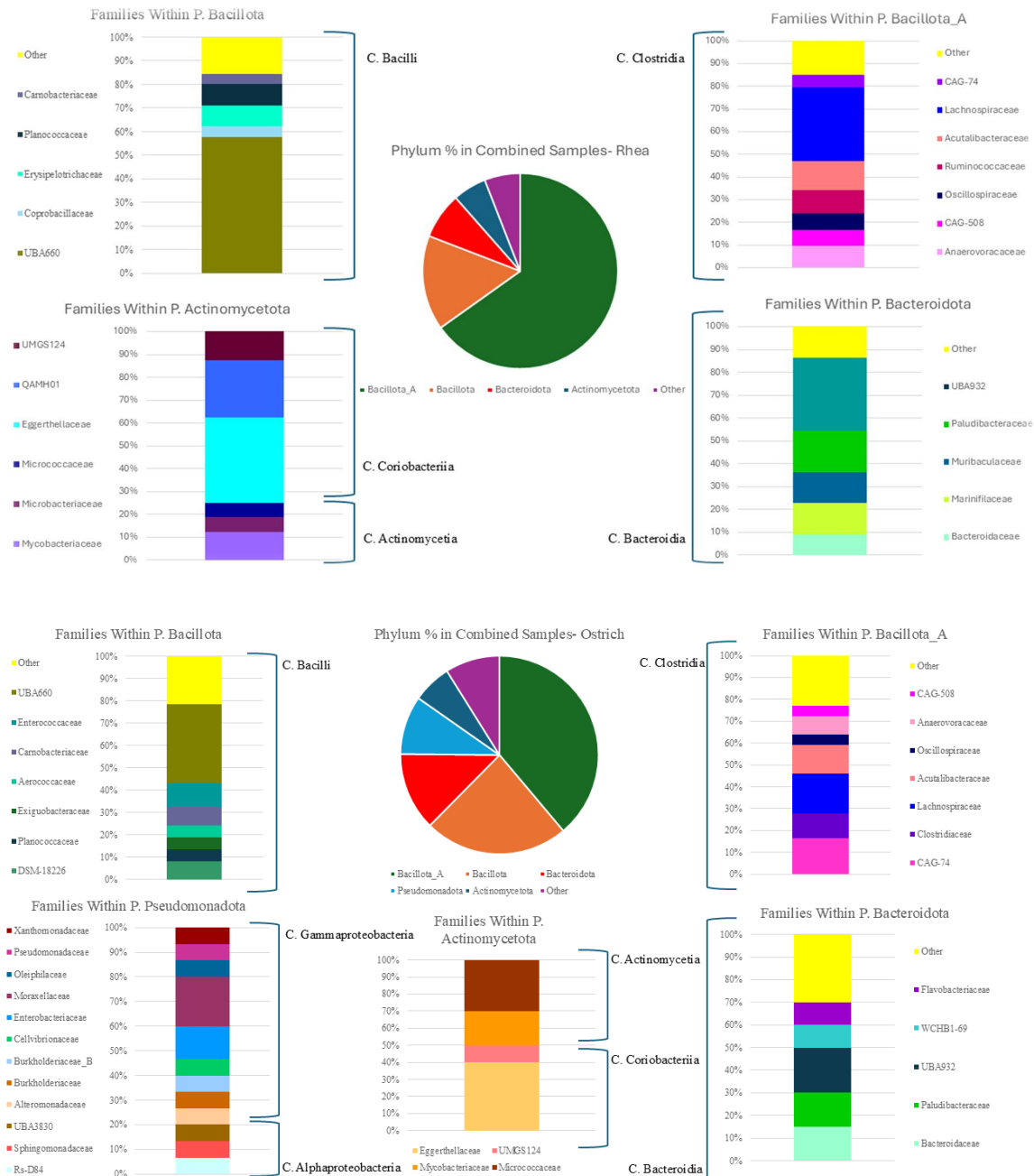


Figure 1- Microbial community composition within each Ratite (Top- Emu, Middle- Rhea, Bottom- Ostrich) fecal sample at the phylum (pie chart, colors indicate different phyla), class (brackets), and family (stacked bar plots, colors indicate different families) level.

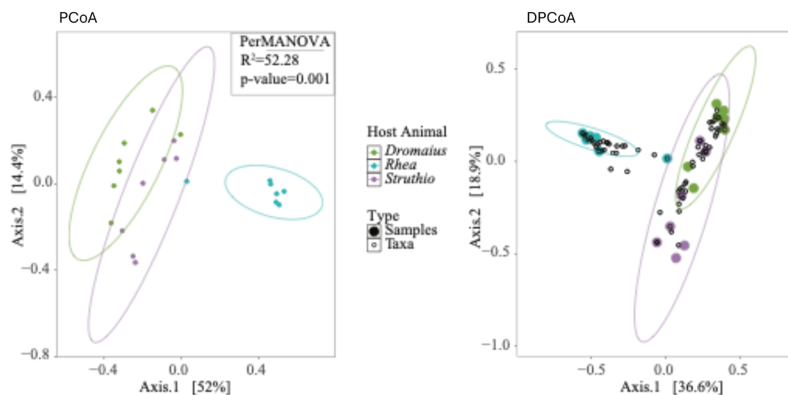


Figure 2- Beta diversity of gut microbial communities across Ratites. Green dots are emu, blue dots are rhea, and purple dots are ostrich. Full circles indicate host, open circles indicate either sample (black) or taxa (white). Left plot- Axis 1 shows 52% variation, Axis 2 shows 14.4% variation. Right plot- Axis 1 shows 36.6% variation, Axis 2 shows 18.9% variation. Communities are significantly different between ratites.

Analysis of the composition of CAZymes within each Ratite sample was conducted, displaying that the samples shared a similar community of CAZyme families but varied in the distribution of said families. Glycoside hydrolases (GHs) are a family of degradative CAZymes that break down a variety of polysaccharides through hydrolyzing glycosidic bonds (Gavande et al., 2023). This CAZyme family was the most dominate among the samples, specifically, GH13, GH43, and GH5. GH13 is a group that is mostly comprised of α -amylases which break the internal α 1-4 link in starch and glycogen (Gavande et al., 2023). GH43 is a group of enzymes that break down hemicellulose, an abundant polysaccharide in plant cell walls, through the activity of β -xylosidases and α -arabinofuranosidases (Morais et al., 2021). GH5 are a group that participates in the breakdown of cellulose, the main component of plant cells walls, through the activity of endo- β -1-4-glucanase (endoglucanase) (Gavande et al., 2023). The emu samples displayed a large domination of GH13 and GH43. Similarly, the ostrich samples also contained large amounts of GH13 and GH43 but presented a more even distribution of these two families when compared to the emu and contained a higher amount of GH5. More in line with the ostrich, the rhea samples were dominated by GH13 and GH43 but had a higher distribution of GH13 and less GH43, as compared to the ostrich. The amount of GH5 was similar between the ostrich and emu.

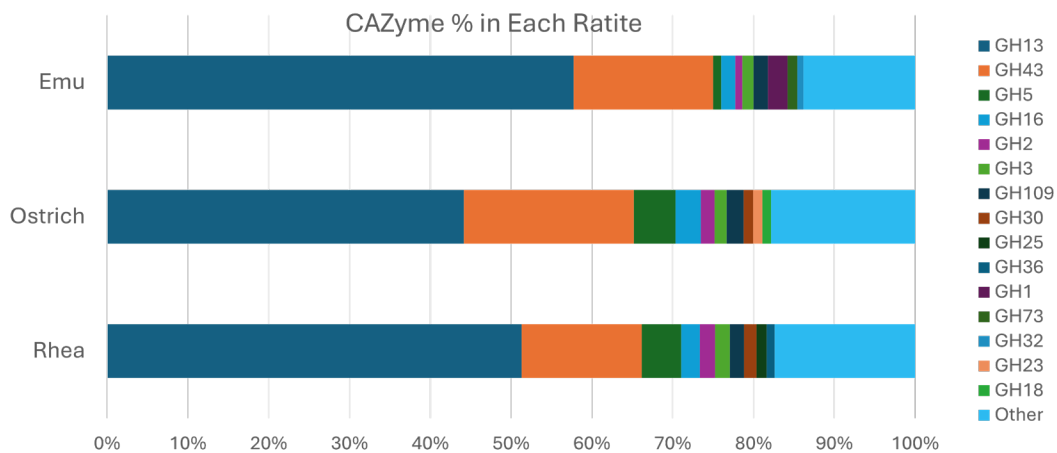


Figure 3- GH class CAZyme distribution across each Ratite (emu, ostrich, rhea). Each bar correlates to a different Ratite, colors indicate different GH classes.

Discussion and Conclusions

As displayed above, across the three Ratites, there lies a distinct microbiome community and distribution within their respective gastrointestinal tracts. The taxonomic composition of these Ratites displays the contributions made by microorganisms within their digestive systems. At the phylum level, each of the hosts are dominated by *Bacillota*, which aligns with the established role of these phyla in the greater avian gut microbiome previously described in wild birds by Grond et al. (2018). In domestic chickens, *Bacillota* abundance has been associated with mass gain, potentially due to the production of short-chain fatty acids as byproducts of fermentation, supplying the animal with an additional energy source (Grond et al., 2018). Notably, this pattern has not been displayed in non-poultry birds. In the context of these ratite diets, this fermentation capacity is especially important as these Ratites are primarily herbivores, having diets of high-fibrous plant biomass and seeds, reflecting need for the *Bacillota* phylum within their gut microbiome. Strikingly, the rhea and ostrich microbiomes show a high representation of *Bacillota_A* (which includes lineages previously classified within *Clostridia*). Commensal *Clostridia* constitutes a large portion of the gut microbiome of vertebrates, where they play a crucial role in gut homeostasis through the release of short-chain fatty acids as an end production of fermentation, providing a primary energy source from consumed plant material (Lopetuso et al., 2013). On the other hand, the emu has a more even split between the phyla *Bacillota* and *Actinomyces* within their gut community. Since emu's consume a larger portion of small invertebrates along with plant biomass, when compared to rheas and ostriches (Sales, 2006), the presence of *Actinomyces* could potentially be attributed to the omnivorous feeding pattern of emus. Overall, the taxonomic differences between these ratites can be drawn back to their differences in feed patterns, gastrointestinal makeup, and evolutionary history.

The CAZyme profiles recovered from each of the ratite gut microbiomes display a functional dimension to the observed differences in community composition, with all the ratites sharing a broadly similar community of enzymes while displaying a differing in the distributions of the families. The abundance of the GH family of CAZymes, namely, GH13, GH43, and GH5 are consistent with the primarily herbivorous diets of these animals. The dominance of GH13 within every sample, a class comprised mostly of α -amylases, reflects the high starch content of the plant biomass and seeds that form the basis of these animals' diets. Similarly, the high representation of GH43 displays the importance of hemicellulose degradation in the breakdown of the plant cell wall, through the activity of β -xylosidases and α -arabinofuranosidases. The elevated GH5 composition in the ostrich and rhea compared to the emu, is potentially due to their fermentative architecture, namely the cecum and colon, in the gastrointestinal tract of these ratites, with the ostrich and to a lesser extent the rhea favoring plant biomass retention within their gut. The alignment between the differing distribution of CAZymes and the differing taxonomic composition of the Ratites potentially points to varying contribution from the well-adapted microorganisms within their respective gut microbiome, with the functional differences between hosts mirroring the compositional differences.

This study provides one of the first comprehensive, comparative metagenomic analysis of the Ratite gut microbiome across three different ratite species that differ in their gastrointestinal makeup. We recovered 494 high-quality MAGs from these Ratite samples, displaying a unique taxonomic and enzymatic composition and distribution within each Ratite. The alignment between the differing taxonomic composition and CAZyme distribution suggests that the Ratites microbes are well adapted to their respective metabolic functions. The taxonomic novelty observed, including 1 novel family, 23 novel genera, and 262 novel species displays the uncharacterized nature of these Ratites gut microbiome, warranting further investigation into the community composition and metabolic capabilities of the animals. Future work evaluating a broader view of carbohydrate metabolism within these Ratites can provide a necessary insight into potential unique enzyme production, creating a more mechanistic understanding of the Ratite gut microbiome.

Summary

This study conducted a comparative metagenomic analysis of the gut microbiome of three Ratites, the emu, ostrich, and rhea. These animals differ in their gastrointestinal makeup and feeding patterns, leading them to possess unique taxonomic composition and a unique CAZyme arsenal adapted to their gut anatomy and microbiome. We classified the specific composition of these Ratites gut microbiome, a lacking area of microbiome research. While displaying a portion of carbohydrate metabolism through an enzymatic analysis of the CAZyme community and how taxonomic composition influences CAZyme composition.

Appendices

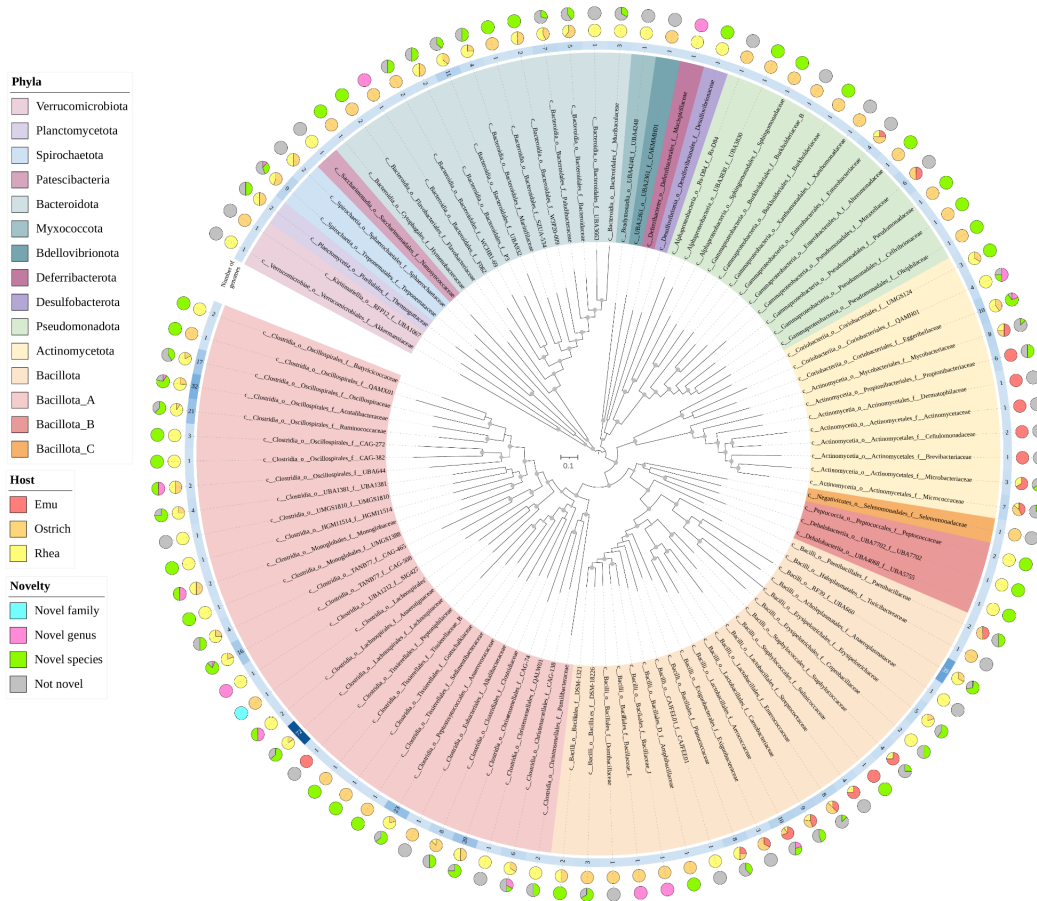


Figure 4- Entire microbial community composition ordered by animal (middle circle, colors indicate ratite), arranged by phylogenetic similarity (inner most circle) and assayed for novelty (outer circle, color indicates novelty)

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Papers Published

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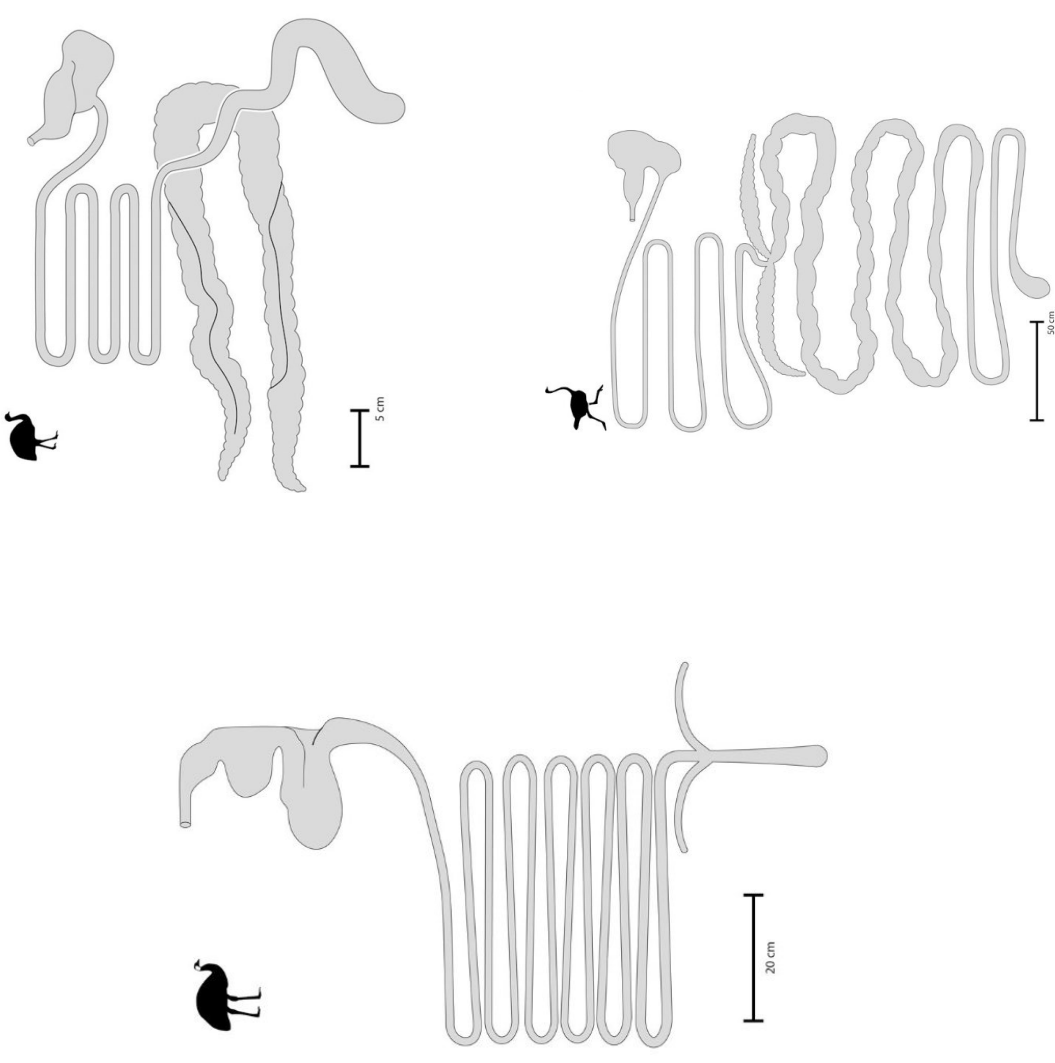


Metagenomic Analysis of the Gut Community of Ratites

Ty Young, Sam Miller, Noha Youssef, and Mostafa Elshahed

Background

- The gut microbiome plays a significant role in the health of animals
- Gut microbiome of ratites (large flightless birds) remains vastly understudied
- The emu, ostrich, and rhea contain unique gastrointestinal composition, pointing to a specialized microbial community within their gut



Objectives

- Determine microbial community composition within the selected ratites
 - Compare their respective communities between each other and identify significant changes
- Describe capabilities of carbohydrate metabolism
 - Specifically, through evaluation of carbohydrate-active enzymes (CAZymes) given their role in the breakdown of complex polysaccharides
- Examine the antimicrobial resistance (AMR) gene content
 - Identify AMR gene classes present in each ratite

Methodology- DNA Extraction and Sequencing

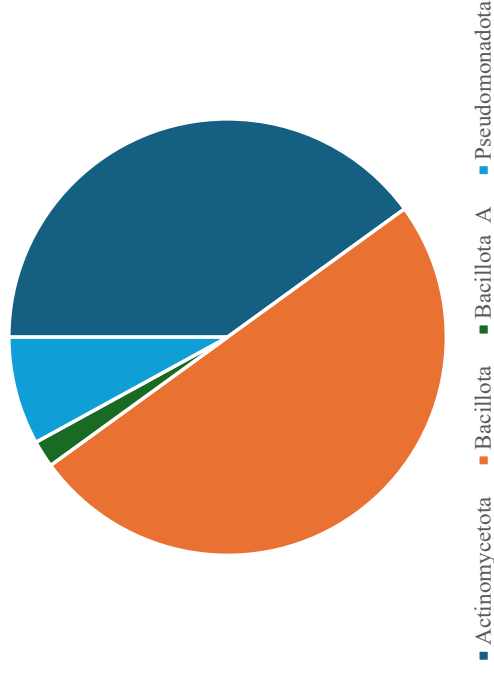
- Fecal matter was aseptically collected from various farms
 - 7 samples per animal
- Each fecal sample was subjected to DNA extraction
 - Quality and quantity of DNA was measured using a TapeStation and Qubit
- Shotgun metagenomic DNA sequencing using the Illumina platform was executed on extracted DNA

Methodology- MAG formation and Analysis

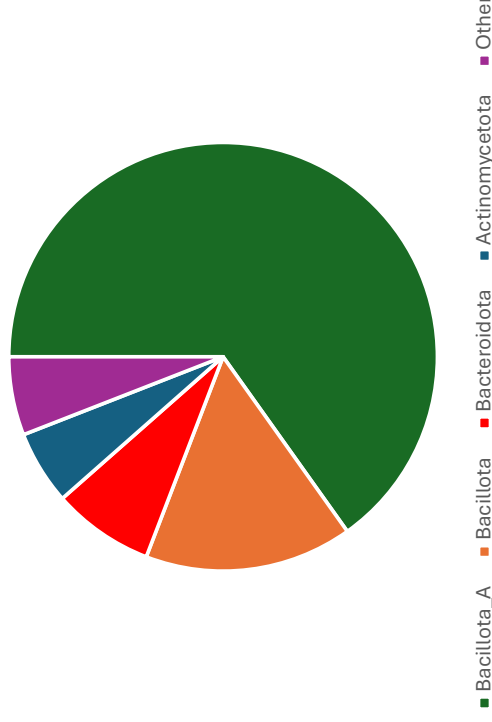
- Metagenomic reads from DNA sequencing were trimmed and assembled into Contigs
 - Contigs were grouped on a per animal basis and used to determine CAZyme arsenal and AMR genes
- Metagenomic contigs were binned into draft genomes, then further combined into metagenome assembled genomes (MAGs)
 - These MAGs were taxonomically classified to identify the specific members of the microbial gut community

Results- Community Distribution (Phylum)

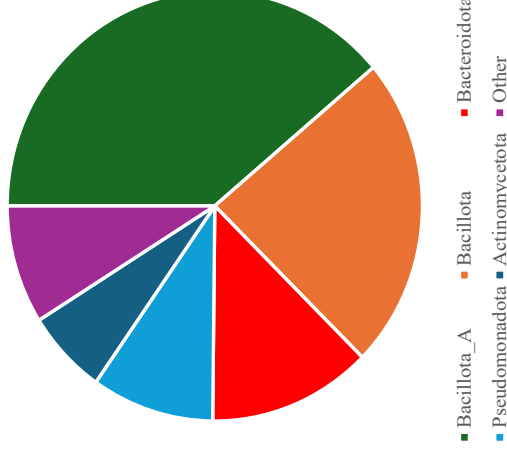
Phylum % in Combined Samples- Emu



Phylum % in Combined Samples- Rhea



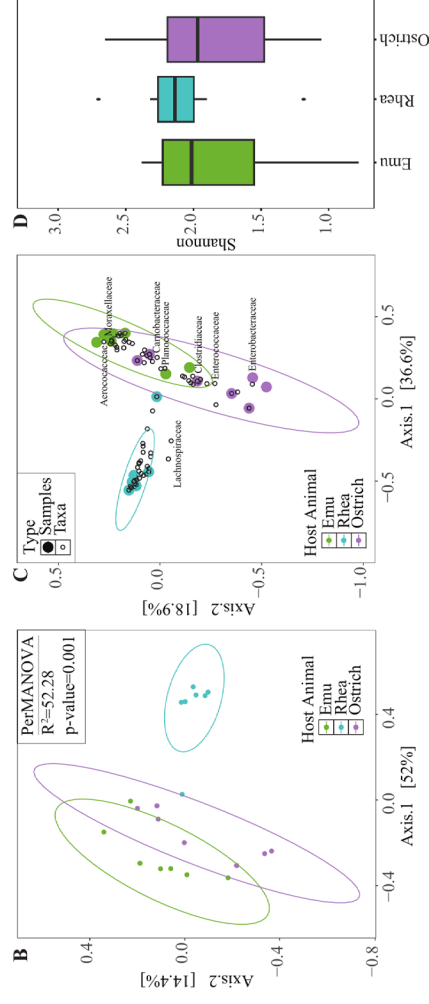
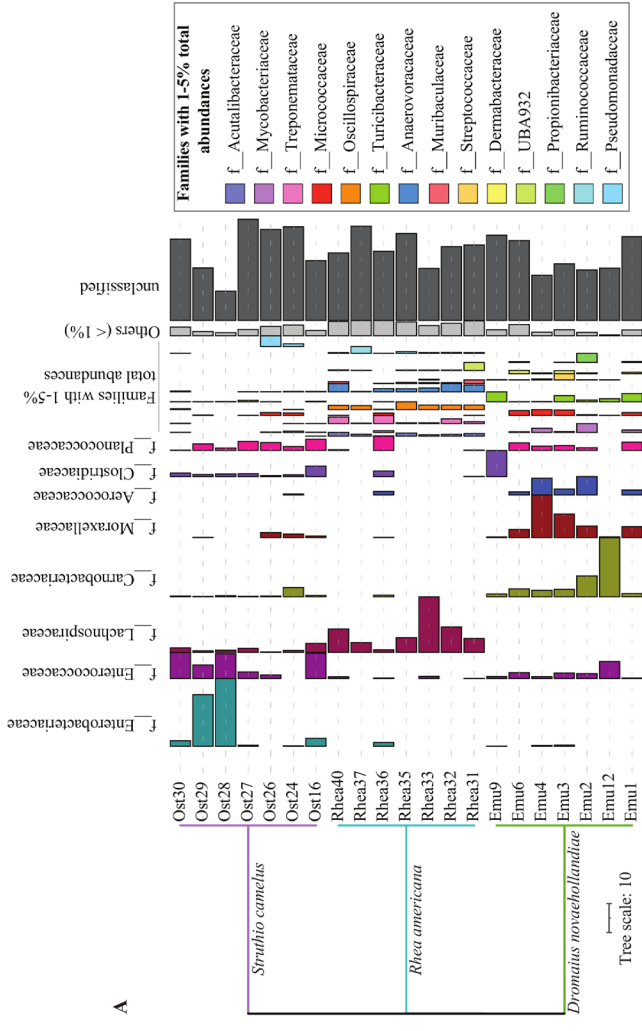
Phylum % in Combined Samples- Ostrich



- Every ratite displays a unique distribution and composition of phyla within their respective microbial gut communities
 - Emu samples are dominated by *Bacillota* and *Actinomycetota*
 - Rhea samples are mainly composed of *Bacillota_A*
 - Ostrich samples encompass *Bacillota_A* and *Bacillota*

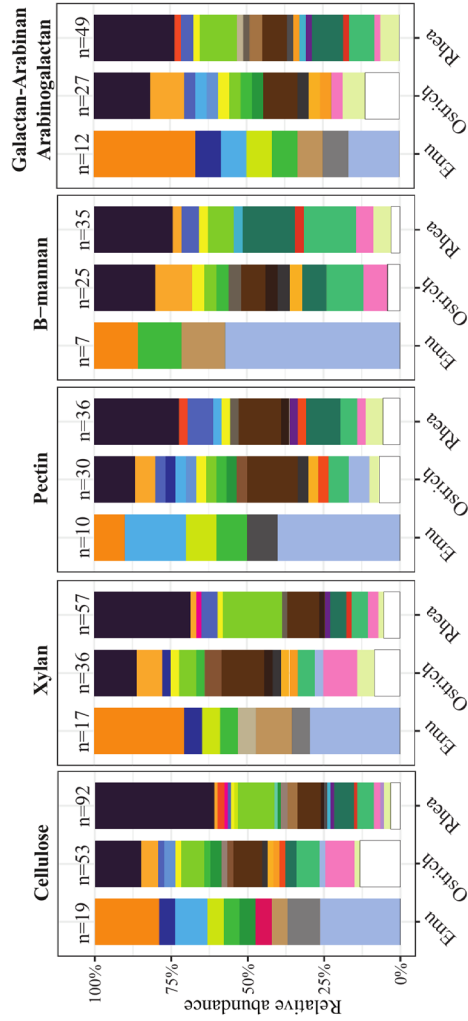
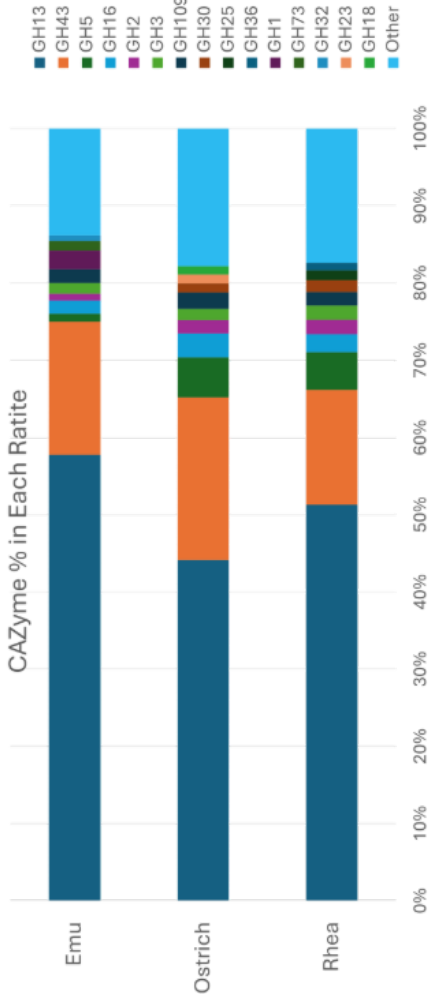
Results- Community Distribution (Family)

- Each ratite encompasses differing taxa at the family level
 - Ostriches are dominated by *Enterococcaceae* and *Enterobacteriaceae*
 - Rheas are comprised mostly of *Lachnospiraceae*
 - Emus contain more *Carnobacteriaceae* and *Moraxellaceae*
- The gut community within each ratite differs significantly from each other
 - While having low divergence within the respective ratite samples



Results- CAZyme Analysis

- GH13 and GH43 are the most prevalent CAZyme classes
- Emus are lacking in GH5, compared to the ostrich and rhea
- GH13, GH43, and GH5 classes of CAZymes correspond to breakdown of starch, hemicellulose, and cellulose within plant cell walls

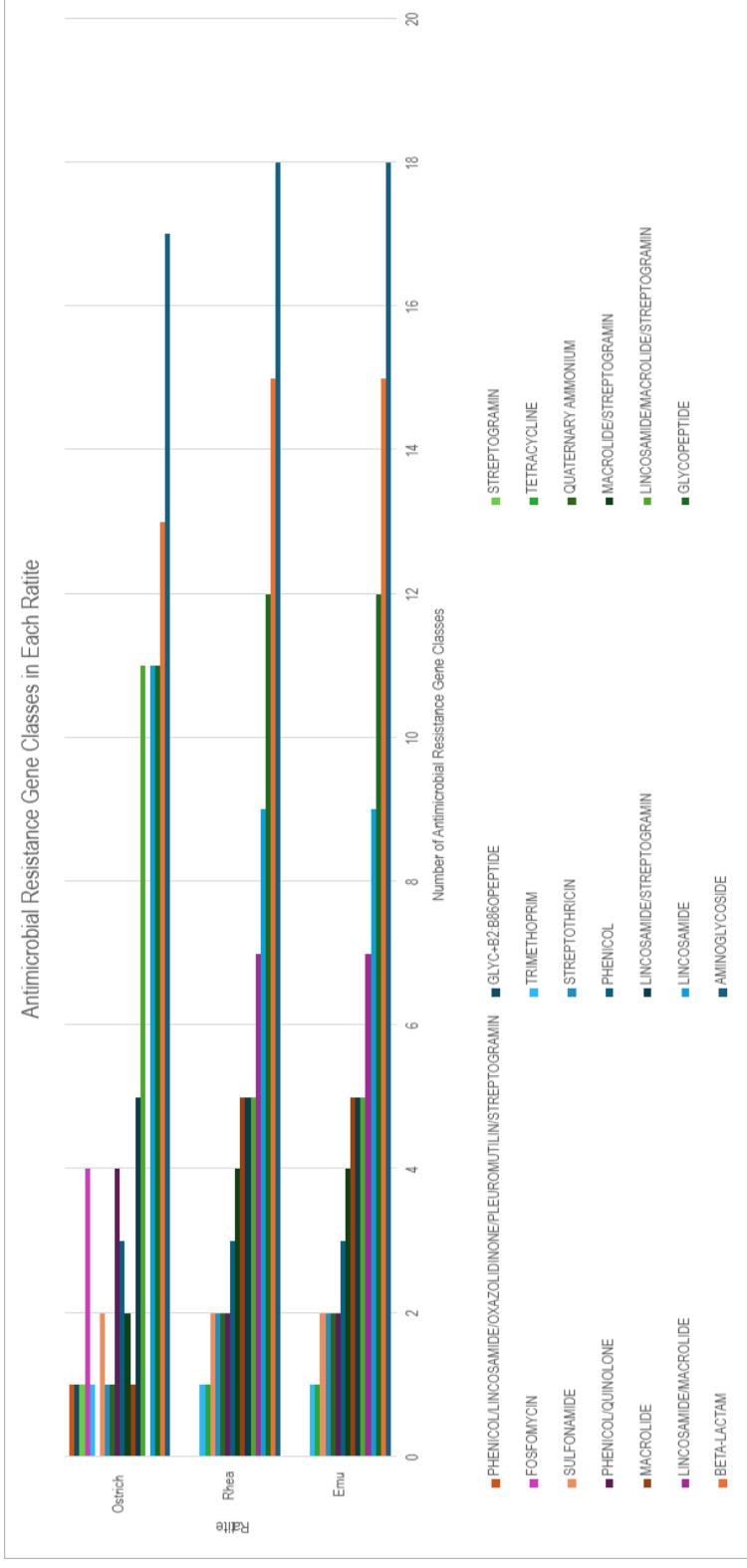


- Despite similar makeup, distribution of these CAZyme classes differs between each ratite
- Displaying separate contribution of these CAZymes from their respective microbial communities



Results- Antimicrobial Resistance Genes

- Individual ratites have a similar load of AMR genes
 - Ostrich- 90
 - Emu- 93
 - Rhea- 93
- Resistance to aminoglycoside and β -lactam classes is most dominant
 - Emu and rhea share identical composition and distribution
- The ostrich has a distinct distribution
 - Higher amounts of lincosamide/macrolide/streptogramin, phenicol/quinolone, and fosfomycin classes of resistance genes



Discussion

- Each ratite displays a unique composition and distribution of taxa within their respective microbial gut community
- Differences in CAZyme distribution can be attributed to differing contribution from microbes within the gut of each ratite
 - Community composition could be driven by the gastrointestinal makeup of each ratite
- AMR gene profiles in the emu and rhea are identical but distinct from the ostrich
 - Similarities between emu and rhea AMR genes could be attributed to similar environmental pressures and horizontal gene transfer

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Questions?

