ORAL PRESENTATION ABSTRACTS
QGCS 18TH ANNUAL SYMPOSIUM

Temperature-Dependent Optical and Electronic Study of Single-Crystal Cs_2TeX_6 (X = Cl, Br)

Michael P. Lewis[1], Trinanjan Dey[2], Arthur Mar[2], Damien Wlodarczyk[3], Andrzej Suchocki*[3], Serge Nagorny[4,5], Stefano Nisi[4], and Peng Li Wang[1*]*

[1]Queen's University, 90 Bader Lane, Kingston (Canada)
[2]University of Alberta, 4-181 Centre for Interdisciplinary Science, Edmonton (Canada)
[3]Institute of Physics, Polish Academy of Sciences, al. Lotnikow 32/46, 02-668 Warsaw, Poland
[4]INFN - Laboratori Nazionali del Gran Sasso, Assergi, 1-67100 L'Aquila, Italy
[5]Gran Sasso Science Institute, 1-67100 L'Aquila, Italy

The anti-fluorite type A_2BX_6 materials, where A is a monovalent cation, B is a smaller tetravalent cation, and X is a halide, have garnered significant interest in the past decade due to their highly interchangeable structure influencing their optoelectronic properties. Anti-fluorite A2BX6 materials have a wide range of applications, including solar cells, light-emitting diodes, impedimetric sensors, and radiation detectors[1]. The vibrational and optical centers of these materials originate from the Jahn-Teller distorted $[BX_6]^{2-}$ octahedra, and changing the atoms in the octahedra affects the materials' properties. For example, Cs_2ZrCl_6 and Cs_2ZrBr_6 microcrystals have been synthesized[2], and the emission peak, caused by self-trapped excitons, shifts from 454 to 528 nm for the chloride and bromide analogues, respectively. Thus, since the $[BX_6]^{2-}$ dominates the electronic and phonon dispersion in these materials, there must be a deep fundamental understanding of how the local environment of the octahedra influences the optoelectronic properties of the studied materials. In the present study, Cs_2TeX_6 (X = Cl, Br) was synthesized using low-purity reagents (TeO_2 (>99%) and Cs_2CO_3 (99%)) by a cheap, facile solution synthesis method and grown by the Bridgman-Stockbarger method with a temperature gradient of approximately 15℃ cm^{-1}. A □10□40 mm Cs_2TeCl_6 crystal and an □8□45 mm Cs_2TeBr_6 crystal were grown. The concentration of impurities in the components involved in the production of the crystals was analyzed by ICP-MS. The synthesized powders and crystals show excellent phase purity, verified through powder X-ray Diffraction (p-XRD) patterns. The Cs_2TeCl_6 crystal was grown with no bubbling or inclusions in the crystal bulk and a final yield of 90.7%. The Cs_2TeBr_6 crystal had slight cracking and plane misalignment throughout the crystal, and a final yield of 85.2%. The optical-electronic properties of the Cs_2TeX_6 (X = Cl, Br) crystals were evaluated at different temperatures using single-crystal XRD, temperature-dependent photoluminescence, and temperature-dependent Raman Spectroscopy.

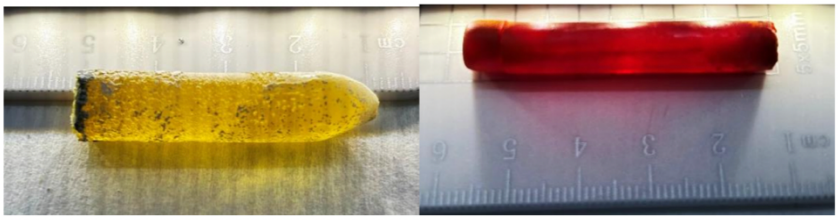

Fig. 1. Grown single-crystal ingots of Cs_2TeCl_6 (left) and Cs_2TeBr_6 (right)

References:

(1) Faizan, M.; Wang, X.; Abdelmohsen, S. A. M.; Bhamu, K. C.; Sappati, S.; Laref, A.; Muhammad, N.; Mushtaq, M.; Abdelbacki, A. M. M.; Khenata, R. Understanding the Electronic Structure and Optical Properties of Vacancy-Ordered Double Perovskite A2BX6 for Optoelectronic Applications. *Energy Fuels* **2022**, *36* (13), 7065–7074.

(2) Abfalterer, A.; Shamsi, J.; Kubicki, D. J.; Savory, C. N.; Xiao, J.; Divitini, G.; Li, W.; Macpherson, S.; Gałkowski, K.; MacManus-Driscoll, J. L.; Scanlon, D. O.; Stranks, S. D. Colloidal Synthesis and Optical Properties of Perovskite-Inspired Cesium Zirconium Halide Nanocrystals. *ACS Mater. Lett.* **2020**, *2* (12), 1644–1652.

Exploring the Nucleocidin Biosynthetic Pathway: A Dive into Sulfamation

Alessia Rizzello[1], and Dr. David L Zechel[1]
[1]Department of Chemistry, Queen's University, Kingston, Ontario, K7L
Tel.: +1 4163015467, E-mail: 17ar59@queensu.ca

Natural products often possess potent bioactivities that benefit producing organism and humanity as source molecules with useful applications. The pharmaceutical industry is one sector where these bioactivates are utilized in a wide variety of therapeutic treatments. Currently, the majority of modern drugs are generated from natural products or direct derivatives. One natural product at the beginning stages of the drug discovery pipeline is nucleocidin. Originally isolated from *Streptomyces calvus*, nucleocidin is an adenosine analogue with antimicrobial and antitrypanosomal activity. This natural product is especially valued by due to the 4'-fluoro and 5'-O-sulfamoyl substituents. Deciphering the nucleocidin biosynthetic pathway may lead to biocatalytic applications in the synthesis of drug-like molecules. Recent studies have identified the biosynthetic gene cluster for nucleocidin. Additionally, mutagenesis experiments identified three genes essential for fluorination and at least nine genes essential for sulfamation.[1,2] Beyond this, however, little is known about the biosynthetic pathway for nucleocidin. The following research covers recent efforts to characterize one of the sulfamate enzymes, NucN. Through computational programs (i.e. AlphaFold, and Dali), NucN presented high similarity to an arginine deiminase (PBD:1S9R) originally isolated from *Mycoplasmopsis arginini.* The known arginine deiminase is responsible for converting arginine into citrulline, releasing ammonia.[3] The conditions of the known enzyme will be replicated for NucN and samples will be dansylated to better visualize arginine on UPLC-MS with a C18 column. Additionally, ammonia release will be monitored by coupling the NucN enzyme reaction with a GDH enzyme, which uses ammonia to aminate α-ketoglutarate resulting in the reduction of NADH to NAD^+ which is can be detected at an absorbance of 32m.[4]

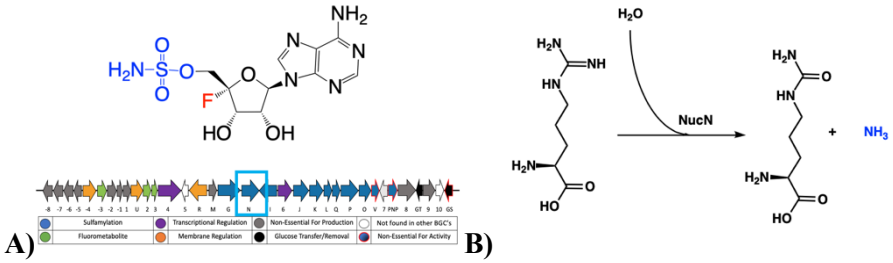

Fig. 1. A) Nucleocidin structure with the biosynthetic gene cluster and **B)** general scheme showing the predicted function of NucN

References:

1. Pasternak, A. R.; Bechthold, A.; Zechel, D. L. *ChemBioChem* **2022**, *23*.
2. Wojnowska, M.; Feng, X.; Chen, Y.; Deng, H.; O'Hagan, D. *ChemBioChem* **2023.**
3. Das, K.; Butler, G. H.; et al. *Structure* **2004**, *12*, 657–667.
4. Oteng-Pabi, S. K.; Keillor, J. W. *Analyt BioChem* **2013,** 169-173.

Modifying and Breaking Down Polystyrene Using Peroxidase–Mediator Systems

Liwah Keller[1] and David Zechel[1]

[1]Department of Chemistry, Queen's University, Kingston, Ontario, K7L 2S8

E-mail: liwah.keller@queensu.ca

Current methods of recycling plastics are inefficient, and only 10-20% of plastic waste is recycled.[1] More effective pathways are needed, as hundreds of millions of tonnes of plastic are accumulating and harming both the environment and our health. A potential solution to this problem is using enzymatic catalysis, since enzymatic reactions require more moderate conditions than the industrial reactions currently used to break down plastics. Non-hydrolyzable plastics, such as polyethylene, polypropylene, and polystyrene, which have carbon-carbon polymer backbones, are highly resistant to degradation. However, there have been reports that various enzymes act on these plastics or similar substrates. Among these enzymes are peroxidases, a diverse group of enzymes which use peroxides to oxidize their substrates.[2,3]

In this presentation, we will detail our progress exploring the activity of peroxidase systems on polystyrene, specifically horseradish peroxidase (HRP). Using small molecular mediators, HRP can perform reactions such as adding oxygen to benzylic positions.[4] Specifically, HRP oxidizes mediator compounds to generate a radical that reacts with the substrate, which then reacts with molecular oxygen.[4] Further radical-initiated breakdown of polystyrene can produce useful small molecules, like benzoic acid, acetophenone and benzaldehyde, which are naturally-occurring and are used to produce flavourings, fragrances and pharmaceuticals.[5,6] Here, we will showcase our research on the reactions of HRP-mediator systems with polystyrene dimers and polymers, where we have characterized the products by various analytical methods including GC-MS, FT-IR and NMR.

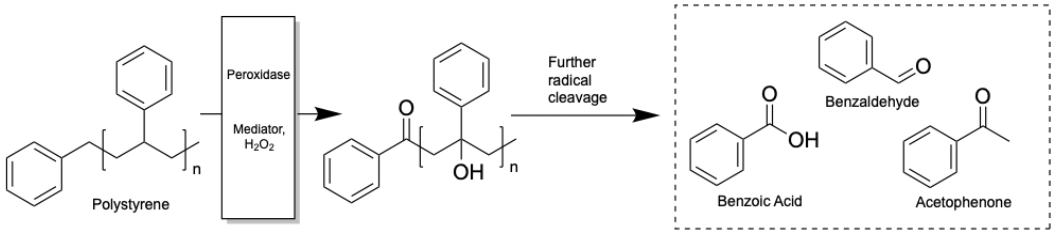

Fig. 1. Peroxidase-catalyzed oxygen addition to PS and breakdown to small molecule products.

References:

(1) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3* (7), e1700782.

(2) Du, Y.; Yao, C.; Dou, M.; Wu, J.; Su, L.; Xia, W. Oxidative Degradation of Pre-Oxidated Polystyrene Plastics by Dye Decolorizing Peroxidases from Thermomonospora Curvata and Nostocaceae. *J. Hazard. Mater.* **2022**, *436*, 129265.

(3) Nakamiya, K.; Sakasita, G.; Ooi, T.; Kinoshita, S. Enzymatic Degradation of Polystyrene by Hydroquinone Peroxidase of Azotobacter Beijerinckii HM121. *J. Ferment. Bioeng.* **1997**, *84* (5), 480–482.

(4) Cribari, M. A.; Unger, M. J.; Martell, J. D. A Horseradish Peroxidase–Mediator System for Benzylic C–H Activation. *ACS Catal.* **2022**, *12* (19), 12246–12252.

(5) Nikitas, N. F.; Skolia, E.; Gkizis, P. L.; Triandafillidi, I.; Kokotos, C. G. Photochemical Aerobic Upcycling of Polystyrene Plastics to Commodity Chemicals Using Anthraquinone as the Photocatalyst. *Green Chem.* **2023**, *25* (12), 4750–4759.

(6) Yan, B.; Shi, C.; Beckham, G. T.; Chen, E. Y. -X.; Román-Leshkov, Y. Electrochemical Activation of C−C Bonds through Mediated Hydrogen Atom Transfer Reactions. *ChemSusChem* **2022**, *15* (6), e202102317.

Designing CO_2-Responsive and Degradable Polymers for Coating Applications

Shannon Whitty[1] and Philip Jessop[1]
Department of Chemistry, Queen's University, Kingston, Ontario, K7L 2S8
E-mail: liwah.keller@queensu.ca

Billions of liters of volatile organic solvents are consumed annually for paints and coatings. The organic solvents used release volatile organic compounds (VOC's) into the atmosphere, harming the environment by contribution of smog and ozone formation, and to humans via organ damage with long-term exposure. Ho et al. previously designed water-based paints that had the mechanism of solvent-based paints using carbonated water as the bulk solvent carrier. The proposed paint uses CO_2-responsive polymers which can reversibly change properties, becoming protonated in the presence of carbonated water and neutral in the absence of CO_2. The paint proves to be a promising alternative to offset the environmental harm from organic solvent-based paints.

The second largest source of microplastics is paints and coatings, with ~1300 kilotonnes entering the marine environment annually. The particles can be from dispersed polymer particles present in water-based paints or the breakdown of dried or cracked paint at the end of the product lifetime. The particle size (>5 mm) means a high surface-to-volume ratio, making it difficult for seawater to penetrate the particle centre once in the marine environment. Penetration by seawater helps accelerate particle degradation, as it can assist with swelling and allow breakdown into smaller pieces. The lack of seawater penetration for microplastics results in higher resistance to degradation, contributing to persistence of the particles.

This research aims to design a polymer that is degradable and CO_2-responsive. A polymer with both capabilities would allow break down into smaller fragments at the end of its lifetime while the CO_2-responsiveness may further assist with degradation by increasing polymer swelling in response to pH changes from external conditions. Bulky secondary amines are used to install CO_2-responsive functionality, becoming protonated in the presence of CO_2. Functional groups such as esters undergo hydrolysis under varying conditions, and incorporating esters in the polymer backbone will allow breakdown of the polymer into smaller fragments. A polymer with both functionalities may be used for coating applications, building on the research from Ho et al. by introducing degradable functionality in addition to the use of carbonated water as a solvent. Such a polymer would be able to address the issues of microplastic and VOC contribution from paints and coatings, offering an alternative to currently used products.

Exploring students' use of chemical diagrams via exam analysis

***Steven Ganescu,*[1] *Caleb Fung,*[1] *and Amanda Bongers*[1]**
[1]**Department of Chemistry, Queen's University, Kingston, Ontario, K7L 3N6**
E-mail: steven.ganescu@queensu.ca

Students struggle to learn chemistry, particularly when it comes to using visual representations like diagrams of molecules.[1] These diagrams comprise the *visual language* of chemistry and are used routinely by chemists to reason about chemical reactivity.[1] With our research, we wish to understand how chemistry instructors might best support their students' development of *fluency* in using visual diagrams of molecules. Prior research has demonstrated that students who use the problem-solving strategies of chemistry experts perform better on predict-the-product and mechanism problems.[2,3] Because many of these strategies include annotating and drawing diagrams of molecules, analysis of the *off-hand* writing of students during problem-solving can provide insights into their fluency in using diagrams of molecules.[4] This study explores how undergraduate students learning organic chemistry use diagrams of molecules to problem-solve. Specifically, we aim to explain how different types of drawings and annotations are used together by students and in what contexts. We analyzed the off-hand drawings and annotations on exams (N = 427) taken by undergraduate students in a second-year organic chemistry course. We identified which multiple-choice and predict-the-product questions each student had annotated provided diagrams, drawn new diagrams, and included various features in their diagrams, such as explicitly drawn lone pairs and hydrogen atoms. A total of 23 types of annotation/drawing features (examples in **Fig. 1**) were examined for their use on each question across all the exams. To understand the relationships among the uses of each feature, we performed a multiple correspondence analysis, an exploratory multivariate statistical technique for categorical data akin to principal component analysis.[5] Through our analysis, we reveal common patterns in students' use of chemical diagrams in their drawings and annotations, how these patterns vary with context (e.g., question format, reaction type), and the relationship between the observed patterns and question accuracy. Notably, our preliminary analyses have revealed that there is no single superior method for using these drawings and annotations to successfully problem-solve, suggesting that tailored instructional approaches may accommodate multiple student approaches and preferences.

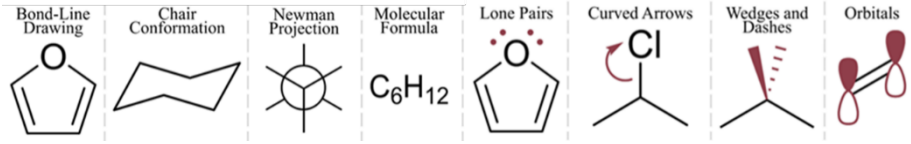

Fig. 1. Examples of features examined in students' annotations and drawings.

References:

1. Talanquer, V. The Complexity of Reasoning about and with Chemical Representations. *JACS Au* **2022**, *2* (12), 2658–2669.
2. Bodé, N. E.; Flynn, A. B. Strategies of Successful Synthesis Solutions: Mapping, Mechanisms, and More. *J. Chem. Educ.* **2016**, *93* (4), 593–604.
3. Webber, D. M.; Flynn, A. B. How Are Students Solving Familiar and Unfamiliar Organic Chemistry Mechanism Questions in a New Curriculum? *J. Chem. Educ.* **2018**, *95* (9), 1451–1467.
4. Flynn, A. B.; Featherstone, R. B. Language of Mechanisms: Exam Analysis Reveals Students' Strengths, Strategies, and Errors When Using the Electron-Pushing Formalism (Curved Arrows) in New Reactions. *Chem. Educ. Res. Pract.* **2017**, *18* (1), 64–77.
5. Husson, F.; Le, S.; Pagès, J. *Exploratory Multivariate Analysis by Example Using R*; Chapman and Hall/CRC: Boca Raton London New York, 2017.

Atomically Precise NHC-Protected Au_{13} Nanoclusters: Synthesis and Properties

Kayla Boire[1], Kumar Siddhant[1], and Cathleen Crudden[1]
[1]Department of Chemistry, Queen's University, Chernoff Hall, Kingston, Ontario, K7L 3N6
Tel.:(613) 533-2616, E-mail: kayla.boire@gmail.com

Gold (Au_{13}) NHC-stabilized nanoclusters have been recently notable for their significant properties, including but not limited to fluorescence, stability, and biomedical applications.[1] The investigation of atomically precise NHC-decorated nanoclusters is a growing field, due to the tunabilities of NHCs, affecting the arrangement of the gold core, as well as the photophysical properties observed. The synthesis of the highly fluorescent, chiral Au_{13} cluster have introduced further investigations to delve into the properties of Au NCs.[2]

While phosphines and thiols both present a high stability and notable properties, the preparation of NHCs enables precise control of the gold core arrangement and photophysical properties through rational ligand design.[3] Previously reported research has determined the variation of functionality for NHCs; (1) the wingtips, which can be further functionalized for applications or used to increase fluorescence; (2) the linkers, thereby the amount of NHCs connected to increase stabilization, as well as the fluorescence; (3) the backbone, which is able to modify solubility, increase fluorescence, and increase stability; and (4) the NHC core, which can vary electronic properties and fluorescence.[3]

The modifications in the NHC-decorated wingtip has been the focus of recent research to determine causes of an increased PLQY; via electronic properties, steric properties, and interactions via intramolecular forces.[4] The primary interactions, mainly through electron-donating or withdrawing nature, have been found to suggest a significant correlation between properties and structure. A significant increase in QY from 30% to 49% was observed, underscoring the significance of ligand design for the development of highly luminescent nanomaterials for photonics and biological application.

References:

1. Jin, R.; Zeng, C.; Zhou, M.; Chen, Y. Atomically Precise Colloidal Metal Nanoclusters and Nanoparticles: Fundamentals and Opportunities. *Chemical Reviews* 2016, *116* (18), 10346–10413. https://doi.org/10.1021/acs.chemrev.5b00703.
2. Albright, E. L.; Malola, S.; Jacob, S. I.; Yi, H.; Takano, S.; Mimura, K.; Tsukuda, T.; Häkkinen, H.; Nambo, M.; Crudden, C. M. Enantiopure Chiral Au13 Nanoclusters Stabilized by Ditopic *N*-Heterocyclic Carbenes: Synthesis, Characterization, and Electrocatalytic Reduction of CO_2. *Chemistry of Materials* 2024, *36* (3), 1279–1289. https://doi.org/10.1021/acs.chemmater.3c02288.
3. Sullivan, A. I.; Steele, E. A.; Takano, S.; Elham Zeinizade; Chen, J.; Sami Malola; Kumar Siddhant; Hannu Häkkinen; Stamplecoskie, K. G.; Tatsuya Tsukuda; Zheng, G.; Crudden, C. M. Diving into Unknown Waters: Water-Soluble Clickable Au13 Nanoclusters Protected with N-Heterocyclic Carbenes for Bio-Medical Applications. *Journal of the American Chemical Society* 2025. https://doi.org/10.1021/jacs.4c14240.
4. Yi, H.; Osten, K. M.; Levchenko, T. I.; Veinot, A. J.; Aramaki, Y.; Ooi, T.; Nambo, M.; Crudden, C. M. Synthesis and Enantioseparation of Chiral Au13 Nanoclusters Protected by Bis-*N*-Heterocyclic Carbene Ligands. *Chemical Science* 2021, *12* (31), 10436–10440. https://doi.org/10.1039/d1sc03076k.

Forward osmosis followed by reverse osmosis for the removal of contaminants of emerging concern using a CO_2-responsive draw agent

Rachel S. Korchinsky[a], José Giovanni L. Brito[a], Louis Fieldhouse[c], Pascale Champagne[a], Michael F. Cunningham[b], and Philip G. Jessop[a]*

[a]Department of Chemistry, Queen's University, Kingston, ON, K7L 3N6, Canada.
[b]Department of Chemical Engineering, Queen's University, Kingston, ON, K7L 3N6, Canada.
[c]Department of Chemistry, University of Sheffield, Sheffield, S10 2TN, UK.

Contaminants of emerging concern (CECs) are an unmonitored and unregulated class of chemicals found in the environment that negatively impact the health of living organisms. CECs are pervasive in private and public domains as consumer products and enter the environment primarily through wastewater effluent streams from wastewater treatment plants, which remove harmful organisms but cannot remove most CECs. This study's chosen method for removing CECs from an aqueous solution was forward osmosis followed by reverse osmosis (FORO) using a CO_2-responsive draw agent, poly(N,N-dimethylallylamine (PDMAAm). A solution of this CO_2-responsive material has a high osmotic pressure (π) in the presence of CO_2 for ease of filtration by FO and a low π in the absence of CO_2 for ease of recovery by RO. The CECs investigated were ciprofloxacin (CIP), sulfamethoxazole (SMX), atenolol (ATN), trimethoprim (TMP), acetaminophen (ACM), and carbamazepine (CBZ). Using a CO_2-responsive draw agent resulted in high CEC removal efficiencies over multiple filtration cycles.

Improving the performance of anaerobic co-digestion of food waste and sludge via biochar addition

Fatemeh Sadat Arghavan[1], Louise Meunier[1], and Ehssan Koupaie[2]
[1]**Chemical Engineering Department, Queen's University, Kingston, Ontario, K7L 3N6**
[2]**Chemical Engineering Department, Queen's University, Kingston, K7L 3N6**
Tel.:+13439892627, E-mail: ehssan.koupaie@queensu.ca

The generation of organic wastes, such as sludge from wastewater treatment plants and food waste, is an inevitable byproduct of human activity. In line with the Federal Pollution Prevention Act of 1990 and the principles of Green Chemistry, waste that cannot be prevented or recycled must be managed in an environmentally sustainable manner. Conventional waste management methods, including incineration and landfilling, pose significant environmental risks, including greenhouse gas emissions and soil and groundwater contamination.

Anaerobic digestion (AD) is a sustainable method for treating organic waste while producing valuable products such as biogas and fertilizer. The AD process consists of three main stages: hydrolysis, acidogenesis, and methanogenesis. Pretreatment techniques and co-digestion are commonly applied to AD feedstocks to enhance the soluble organic fraction, improving the hydrolysis and acidogenesis. However, increasing the organic loading rate can lead to the volatile fatty acids (VFA) accumulation, reducing the AD performance. For instance, when the feed-to-microorganisms ratio (F:M) increased from 1 to 5 gVS/gVS, the soluble chemical oxygen demand (SCOD) increased from 3.3 to 14.2 g/L which led to an increase in VFA concentration from 3 to 5 g/L. This accumulation occurs due to the slower growth rate of methanogenic archaea (0.02–0.06 1/d) compared to acidogenic bacteria (0.6–1.0 1/d). This imbalance can delay biogas production or even result in system failure.

A promising strategy to address this issue is to add materials with porous structure and electron transfer capabilities. In this study, wood-derived biochar (BC), a green and non-toxic alternative to conventional nanoparticles (such as iron, manganese and cobalt), was selected for this purpose.

Batch anaerobic digesters were set up with food waste and thermally pretreated sludge, and supplemented with 15 g/L of biochar. These digesters were operated under three different food-to-microorganism ratios (F/M) of 1, 3, and 5 (g/g volatile solids) representing low (L), medium (M), and high (H) organic loading conditions. Control digesters without biochar were also operated under the same conditions. Soluble organics and VFA concentrations were monitored over the first 16 days. The two-phase Gompertz model was used to calculate the kinetics of biogas production.

The performance of control digesters declined as F:M increased. The high F:M, no biochar (H-CO) reactor produced 460 mL biogas/g volatile solids (VS) at a rate of 6.9 mL/gVS·d, which is lower than the 570 mL/gVS with a rate of 9.8 mL/gVS·d values observed in the low F/M, no biochar (L-CO) reactor. The lag phase was also 70% longer in H-CO (17 d) compared to L-CO (10 d), indicating delayed microbial activity under high organic loading. When comparing digesters with and without BC, the greatest improvement was observed under the highest F:M condition. The high F:M with biochar (H-BC) reactor achieved a significantly higher biogas yield of 470 mL/gVS compared to 590 mL/gVS in the H-CO reactor ($p < 0.001$). Furthermore, the lag phase in H-BC reactor was four days shorter than that in H-CO reactor, and the biogas production rate was 1.3 times higher than the control. This improved performance in H-BC is likely attributed to lower VFA concentrations, which decreased by half from 5.6 g/L on day 3 to 2.3 g/L on day 7. The used BC has a porous structure (pore volume of 0.23 cm^3/g) that can adsorb VFA and release them gradually, allowing methanogenic archaea sufficient time to utilize VFA effectively.

In conclusion, the addition of 15 g/L of biochar improved AD performance by 28% in terms of biogas yield under high organic loading conditions. For the next step in the present research, the focus will be on estimating the effects of BC on biogas composition, to maximize the methane content and reduce impurities. This innovative approach will enhance the sustainability of the AD process by lowering the costs associated with biogas upgrading and purification.

Cas12a-mediated (CAPTURE) cloning and heterologous expression of biosynthetic gene clusters from *Pseudoalteromonas luteoviolacea* 2ta16 in *E. coli*

Julia E. Tropak[1], and Avena C. Ross[1]

[1]Department of Chemistry, Queen's University, Kingston, ON, K7L 2S8

E-mail: 17jet11@queensu.ca

Species from the marine proteobacterial genus *Pseudoalteromonas* have proven their ability to produce bioactive molecules, but their secondary metabolite profiles have not been well-characterized, leaving many strains with uninvestigated potential. One strain, *P. luteoviolacea* 2ta16, shows *in vivo* antibiotic activity, and genome analysis using antiSMASH[1] reveals the presence of 18 biosynthetic gene clusters (BGCs), 15 of which are not associated with known compounds (i.e. cryptic BGCs). Despite its potential, the sheer number of compounds that this strain produces makes it very difficult to isolate individual molecules from the native producer. We used an augmented version of Cas12a-assisted precise targeted cloning using *in vivo* Cre-lox recombination (CAPTURE)[2] to heterologously express select BGCs from *P. luteoviolacea* 2ta16 in *E. coli* BL21(DE3) under control of the T7 promoter without further modifications to the resulting plasmid (Figure 1). We began with proof-of-concept testing using the BGC for violacein, a highly pigmented purple compound. We were not only successful in cloning this BGC, but we also discovered that, contrary to other publications, it is possible to express the violacein BGC from *P. luteoviolacea* 2ta16 in *E. coli* under its native promoter, although the production is temperature dependent. We see negligible production at 37ºC, visible production at 30ºC and the most at 18ºC. It is unclear whether it is the promoter that is temperature-inducible or if the biosynthesis itself is temperature sensitive. Regardless, given the strength we have seen from this promoter in both *E. coli* DH10β and *P. luteoviolacea* 2ta16, it represents one of the first promoters from *Pseudoalteromonas* that is also active in *E. coli*. Given our success with the violacein BGC, we have now proceeded to our first target cryptic BGCs, ranging from 10kb to 85kb in size and predicted to produce a variety of classes of natural products, from hybrid non-ribosomal peptide-polyketide molecules to β-lactones, all of which have the potential to be antibiotics.

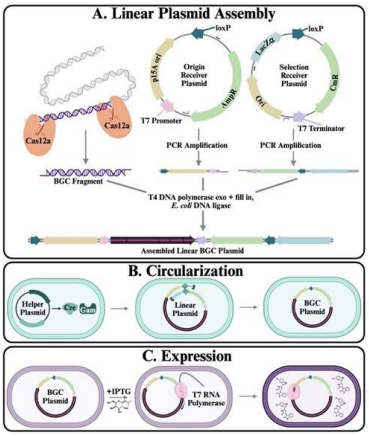

Figure 1. Augmented CAPTURE strategy. **1.** Linear plasmid assembly, adapted from Enghiad et al. (2022). **2.** Transformation into helper competent cells and circularization by Cre recombinase. **3.** Transformation into *E. coli* BL21(DE3) and IPTG-induced expression of the BGC by T7 RNA polymerase, resulting in production of the NP.

References:

1. Blin, K.; Shaw, S.; Augustijn, H. E.; Reitz, Z. L.; Biermann, F.; Alanjary, M.; Fetter, A.; Terlouw, B. R.; Metcalf, W. W.; Helfrich, E. J. N.; et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Research* **2023**, *51* (W1), W46-W50.
2. Enghiad, B.; Huang, C.; Guo, F.; Jiang, G.; Wang, B.; Tabatabaei, S. K.; Martin, T. A.; Zhao, H. Cas12a-assisted precise targeted cloning using in vivo Cre-lox recombination. *Nature Communications* **2021**, *12* (1).