Tenth Annual

BEE Research Symposium

Friday, March 9, 2018

Abstract Book

Poster Session	**Riley Robb 400**	**12:30 – 2:30PM**

Lunch Available

Group A presenters will be at their posters from 1:00-1:30; Group B 1:30-2:00

Closing Reception	**Riley Robb 400**	**2:15PM**

Poster Awards

[1A] **It's All Uphill From Here: Shewanella's Reverse Electron Transfer Chain**

Bram Baxter*, Buz Barstow; Cornell University
Department of Biological and Environmental Engineering
*: Presenter, bab362@cornell.edu

Electroactive microbes contain specialized extracellular electron transfer (EET) machinery that allows them to transfer electrons between external substrates like metals and even our electrodes. A growing body of evidence suggests that microbes using this process to oxidize iron laid down some of the earliest features in the geological record, and powered the Earth's carbon cycle prior to the advent of oxygenic photosynthesis some 2.7 billion years ago. Our theoretical calculations indicate that solar renewable electricity can drive this process with a maximum efficiency far exceeding the theoretical peak efficiency of C3 and C4 photosynthesis, allowing the combination of biology's highly flexible metabolism with the efficiency of renewables. However, the exact molecular mechanisms used to orchestrate this process, and hence its real world performance, remains a mystery. Using the *Shewanella* whole genome knockout collection developed by our team, we have begun to identify the components of this pathway that channels electrons into intracellular reductants needed for carbon fixation, growth and biofuel synthesis.

[2A] **Rapid Creation of Whole Genome Knockout Collections with Knockout Sudoku**

Bram Baxter*, Buz Barstow; Cornell University
Department of Biological and Environmental Engineering
*: Presenter, bab362@cornell.edu

Whole-genome knockout collections are invaluable for connecting gene sequence to function, yet traditionally their construction has required an extraordinary technical endeavor. Despite enormous advances in gene sequencing over the last decade enabled by next-generation sequencing the percentage of genes of unknown function in a genome sequenced today remains about the same, 30 to 40%, as it did a decade ago. This situation is most acute for the most esoteric microbes that offer the most unique resources to genetic engineering and synthetic biology. Knockout Sudoku enables the creation of a whole-genome knockout collection for a single microorganism for as little as three weeks of dedicated labor and a cost of only approximately $10,000. Knockout Sudoku uses manual 4D combinatorial pooling, next-generation sequencing, and a Bayesian inference algorithm to rapidly process and then accurately annotate the extremely large progenitor transposon insertion mutant collections needed to achieve saturating coverage of complex microbial genomes. Knockout Sudoku is approximately 100 times faster and around 30 times lower in cost than the next comparable method (IN-SEQ) for annotating transposon mutant collections by combinatorial pooling and next-generation sequencing. The method facilitates the rapid, algorithmically guided condensation and curation of the progenitor collection into a high-quality, non-redundant collection that is suitable for rapid genetic screening and gene discovery.

[3A] **Applying Biology to the Challenge of Sustainable Energy**

Bram Baxter*, Buz Barstow; Cornell University
Department of Biological and Environmental Engineering
*: Presenter, bab362@cornell.edu

Biology has the potential to make contributions to sustainable energy from the synthesis of advanced materials for construction and transportation to the ultra-low-cost, high-efficiency solar power. Advances in sustainable energy enabled by applied biology could have a transformative effect on human health by mitigating the effects of climate change, revolutionizing access to energy, and improving air and water quality. If fully realized, the impact of these advances could be as large those made by antibiotics, vaccines, and recombinant protein drugs. Over the next 5 years we will use applied biology to find solutions to pressing problems in sustainable

energy including facilitating the widespread adoption of battery energy storage; enabling the environmentally friendly extraction of rare earth elements for energy efficient electronics; producing better biofuels; and increasing the efficiency of photosynthesis. And, we aim to lay a new foundation for further advances in applying biology to sustainable energy by developing new genomic tools including the democratization of the creation of double-gene knockout collections to enable genome-wide genetic interaction studies in organisms useful for sustainable energy.

[4A] **Porous Media Characterization of Foods: Estimation of Hydraulic Permeability**

Younas Dadmohammadi*[1], Ashim K. Datta[1]; Cornell University
[1]*Department of Biological and Environmental Engineering*
*: Presenter, e-mail yd345@cornell.edu

The biological structure of food comprised of cells, cell walls, and extracellular spaces. The integrity and compaction of these compartments generate micro size channels and pores. Therefore, the food can be treated as porous media characterized with descriptors which dynamically changing during the food storage and processes. One of the food vital descriptor, which is highly influencing water and vapor flow transport through the porous media, is hydraulic permeability. The pressure driven flow, which can be experienced, in most of food processes such as cooking and microwave heating, is the essential driving force for hydraulic permeability. This descriptor has been ignored in the food transport modeling widely or lumped into diffusivity coefficient descriptor.

In this study, the simple but realistic Carman-Kozeny correlation, which has been successfully applied in different fields, is accurately tuned up to estimate the food hydraulic permeability descriptor at various food states and during the food processing considering the food cellular structure evolution. The permeability descriptor is critically controlled by other porous media descriptors such as porosity, accessible internal surface area, and tortuosity; these descriptors are well defined and estimated considering the cellular structure of food. Lack of accuracy in aforementioned descriptors is considerably shifting the hydraulic permeability prediction.

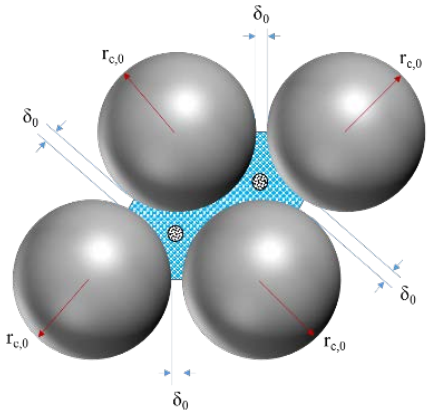


Illustration of Cellular Structure. *Microstructure of food in which the polylateral cell geometry is approximated by sphere. The extracellular space filled with water and gas.*

[5A] **Mechanisms of bacteria infiltration into leafy greens during water film evaporation**

Mohsen Ranjbaran*, Ashim K. Datta
Biological and Environmental Engineering Department, Cornell University
*: Presenter, e-mail: mr963@cornell.edu

Evaporation of a water film at a leaf surface is a process that frequently happens as leafy greens move from field to fork. As surface water evaporates, capillary forces generated by the surface tension of water can push microorganisms present in water toward the leaf surface, facilitating their adhesion to the substrate surface and infiltration into the leaf openings and crevices. Therefore, this pathway can play a strong role in the infiltration of leafy greens by pathogenic bacteria before and during minimal processing chain. We model transport of bacteria within an evaporating sessile droplet at a leaf surface. The model includes fluid flow within the droplet and gas phase, gas-water interface tracking during evaporation, transport of vapor in the gas, transport of bacteria within water, and heat transfer. The model results for bacteria infiltration are to

be validated by conducting drop evaporation experiments on patterned micro-fabrications and real leaf surfaces. Our results indicate that evaporation can cause internal flows within the droplet that can move and accumulate bacteria close to the leaf surface and infiltrate the leaf tissue.

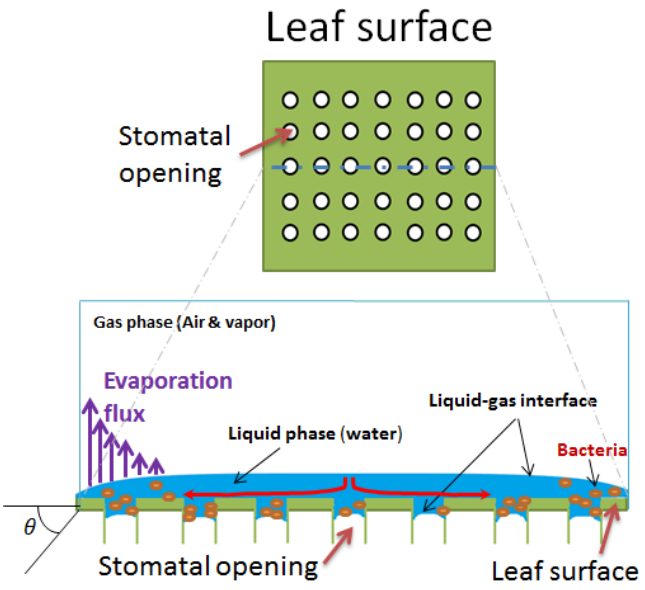
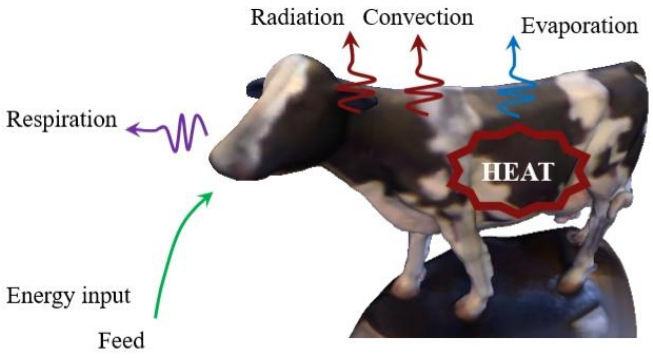


Fig 1. Transport of bacteria into stomatal openings due to evaporation-driven flows within water film at the leaf surface

[6B] **Bio-energetic Model of a Dairy Cow Using Transmission-Line Modeling (TLM) Method**

Hugo FM Milan*, Kifle G Gebremedhin; Cornell University
Department of Biological and Environmental Engineering
*Presenter, e-mail hm496@cornell.edu, scholarship from Brazilian National Counsel of Technological and Scientific Development (CNPq; Proc. 203312/2014-7)

Bio-energetic models are used to predict heat exchange between animals and the environment. The prediction from these models can be used to evaluate animal comfort through partitioning the energy of metabolism into energy for maintenance, production and reproduction potential. Bio-energetic models are based on fundamental principles of conservation of heat, mass, and energy, animal geometry, and animal physiological responses. The limitations of existing models include: (a) simplification of the animal geometry by either a cylinder, ellipse or sphere, (b) simplification or incomplete formulation of the transport mechanisms between the animal and the environment, and (c) not validating the model predictions against physiological data. These simplifications introduce errors in the predictions. The model developed in this study will address the simplification of the animal geometry by using a true representation of a cow by using time-of-flight 3D scanners to obtain cow geometries. The 3D scans were post-processed in MeshLab and Autodesk ReCap Photo. Gmsh was used to convert the 3D scans into meshes. For the internal transport, the Pennes bio-heat energy conservation equation with boundary conditions at the skin surface and hair coat-air interface will be used. For the external transport, all relevant modes of heat and mass transfer (convection, radiation, latent heat loss by cutaneous evaporation and respiration; see Figure) will be considered. The partial differential equations resulting from these formulations will be solved using transmission-line modeling (TLM) method. We have developed a general TLM method that solves 3D heat transfer problems. The TLM method is fast and accurate compared to a numerical solution.



Heat exchange between a cow and the environment.

[7B] **Monitoring environmental stress in New York State dairy barns**

Kristen Perano*[1,2], Hugo Milan[1], Jennifer Bockhahn[2], Kifle Gebremedhin[1], Curt Gooch[2]; Cornell University
[1]*Department of Biological and Environmental Engineering*
[2]*Pro-Dairy Program*
*: Presenter, e-mail kmp263@cornell.edu

Environmental conditions and cow responses were measured at two commercial dairy barns in New York State. One barn was naturally ventilated and the other was tunnel ventilated. Both barns used sprinkler systems to help cool cows during warm weather. Temperature/humidity recording sensors were set up in four locations inside both of the barns as well as in one location outside each barn to quantify the temperature-humidity index (THI) the cows experienced and to compare indoor to outdoor environmental conditions. Cows were also monitored for respiration rate by randomly choosing 30 cows at each farm on four different afternoons and taking respiration rates (RR). During hotter weather (88^{o}F) cows were moderately heat stressed in both barns, with herd average RR of 73 ± 17 breaths per minute (bpm) in the naturally-ventilated barn and 79 ± 15 bpm in the tunnel-ventilated barn. For more moderate weather (daily high of 77^{o}F), the herd average RR was 50 ± 15 bpm in the naturally-ventilated barn and 52 ± 13 bpm in the tunnel-ventilated barn, but many individual cows were over the heat-stress threshold of 60 bpm. Increasing environmental temperature led to higher mean and higher variation in cows' RR response.

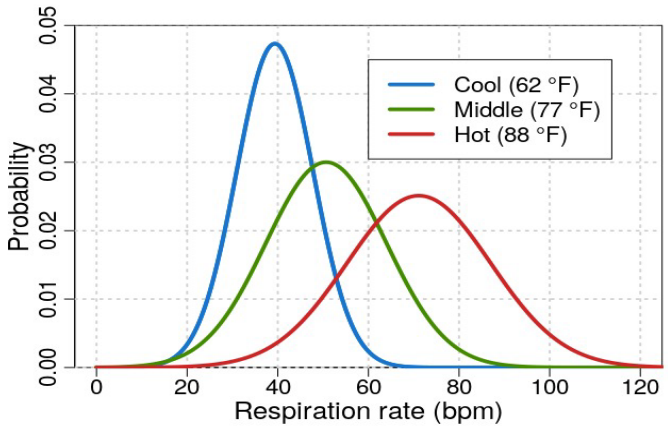


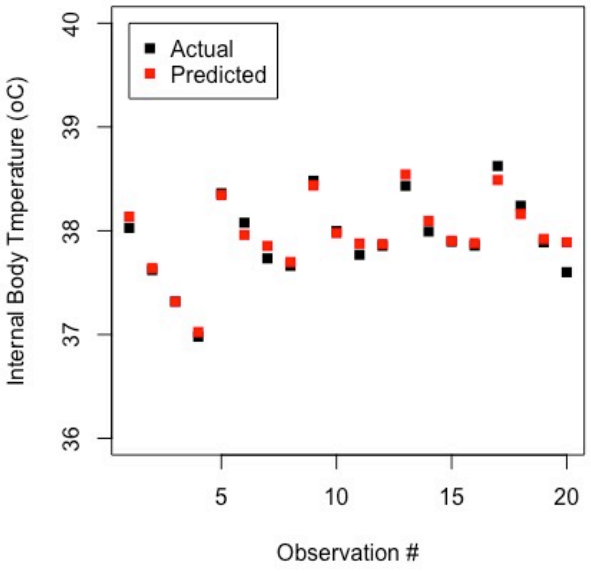
Figure 1: Increase in mean and variability of RR in breaths per minute (bpm). *As cows were exposed to higher heat stress, the mean and variance of RR increased.*

[8B] **Predicting Body Temperature of a Pig Using Machine Learning**

Michael T Gorczyca[1]*, Hugo FM Milan[2], Alex SC Maia[3], Kifle G Gebremedhin[2]; Cornell University
[1] *Department of Information Science, Cornell University, Ithaca, NY 14853*
[2]*Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853*
[3]*Laboratory of Animal Biometeorology, Department of Animal Science, Faculty of Agricultural and Veterinary Sciences, State University of São Paolo, Jaboticabal, SP, Brazil*
*Presenter, e-mail: mtg62@cornell.edu. Funding: CNPq (Proc. 203312/2014-7) and FAPESP (Proc. 17.519/14).

Heat stress costs the livestock industry billions of dollars each year. This problem is especially critical for the swine industry because pigs lack the ability to sweat to alleviate heat stress by cutaneous evaporation, and have lungs that are too small to make panting an effective mode of cooling. As a consequence, the pork industry loses an estimated $300 million each year due to heat stress. Several researchers have developed prediction models of bioenergetics for pigs. These models are typically linear regression models or mechanistic models. The limitation of linear regression models is that the relationship between the input variables are assumed to be independent, and the relationship between the input and output variables is also assumed linear.

For this reason, linear regression models do not account for non-linearity that may exist in the data. Mechanistic models, however, account for non-linearity but require input variables that are difficult to obtain in practice, such as, physical properties of tissue and internal-body temperature of the biological object. This study explores how well non-linear machine learning algorithms (random forests, gradient boosted machines, and deep learning) predict internal-body temperature, skin-surface temperature, and hair coat-surface temperature of a pig. The preliminary results show that machine learning models predict the considered temperatures reasonably well (see Figure). The results obtained from the machine learning algorithms (such as internal-body temperature) could be used in conjunction with mechanistic models to determine the thermal comfort of an animal through partitioning of the energy of metabolism.



Predicted (a deep learning model) vs. measured internal-body temperature (mean absolute error 0.072 °C).

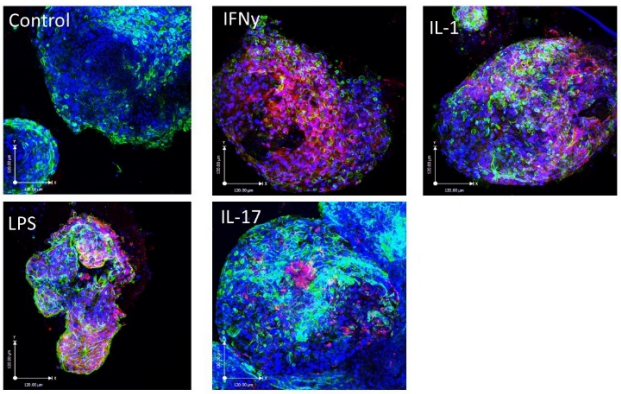
[9B] **Investigating Sal-4 igA as a Potential Treatment for Salmonella Typhimurium Infection**

Kjersti Anderson[*1], Cait Costello[1], John March[1]; Cornell University[1]Department of Biological and Environmental Engineering
[*]Presenter, email kma57@cornell.edu

Our lab has come up with a novel three-pronged approach to study and develop treatment against infection by *Salmonella typhimurium* (**ST**), an intestinal pathogen. The current treatments against ST include inducing the production of *Salmonella* lipopolysaccharide (LPS)-specific (Sal-4) Secretory IgA (SIgA)[2] *in vivo*, or direct treatment with the specific antibody by oral gavage. It is believed that the antibody IgA acts as a competitive inhibitor to pathogen binding on epithelial cells and thus can combat ST infection.

First, we grew differentiated pluripotent stem cells with well-defined media to create human intestinal organoids (HIOs) that allow us to study the effects of IgA on ST. The HIOs are able to grow undifferentiated in a matrigel and WERNAS media until seeded into monolayers. Second, our cell culture techniques have allowed us a better understanding and study of ST infection in the intestine. The experiments and data collected would not be able to be conducted in *in vivo* animal models without genetic modification. We induced polymeric immunoglobulin receptor (pIgR), using cytokine stimulation, which facilitates the transportation of IgA across the intestinal epithelium. The cytokine IFNy had a higher increase than our chosen IL-1 cytokine, but caused tight junction atrophy. Further experiments include using CRISPR gene editing in the organoids to create pIgR knockout (KO) culture to verify pIgR-mediated transport in our cell culture models. Lastly, with our collaborator, we will create a 3-D printed small intestine bioreactor, which will allow

us to study the effects of IgA protection under flow conditions. The geometry of the bioreactor allows the seeding of epithelial cell and bacteria cultures.



Effect of cytokines on pIgR induction in organoids .
Organoids were stimulated with cytokines for 24 hr to induce pIgR
Red = pIgR
Blue = nuclei
Green = actin

[10B] **Roles of Ribose Glycation in 3D Tumor Cell Motility and Collective Dynamics**

Young Joon Suh[1]*, Matthew S. Hall[1], Farid Alisafaei[2], So Youn Moon[1], Min Seo Kang[4], Chung-Yuen Hui[3], Vivek B. Shenoy[2], and Mingming Wu[1]
[1]Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, 14853
[2]Department of Materials Science and Engineering, University of Pennsylvania, Philadelphia, PA, 19104
[3]Field of Theoretical and Applied Mechanics, Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, 14853
[4]Division of Nutritional Sciences, Cornell University, Ithaca, NY, 14853
*: Presenter, e-mail ys668@cornell.edu

Cancer metastasis is a process in which cancer cells break away from the primary tumor, enter the blood or lymph vessels, and establish a secondary tumor in a non-adjacent organ. Current studies report higher risk of cancer metastasis in diabetic than nondiabetic patients. However, due to myriad of overlapping risk factors of diabetes and cancer, the mechanism or the reason behind the correlation is not known. In this study, we explore the role of ribose glycation of collagen matrices in cancer cell invasion. We fabricated six different types of gels with variation of collagen concentration and presence of glycation treatment. A malignant breast cancer cell line, MDA-MB-231, was embedded in each gel and observed. Cancer cells exhibited higher motility rate in gels with glycation treatment and gels with lower concentration. Additionally, cell-cell communication and collective movements were observed much more frequently in glycated collagen gels compared to non-glycated gels. These results reveal the possible biomechanical connections between high concentration of sugar in diabetics and cancer metastasis.

[11A] **Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes**

Duo An*[1], Alan Chiu[1], James A. Flanders[2], Wei Song[1], Dahua Shou[3], Yen-Chun Lu[1], Lars G. Grunnet[4], Louise Winkel[4], Camilla Ingvorsen[4], Nicolaj Strøyer Christophersen[4], Johannes Josef Fels[5], Fredrik Wolfhagen Sand[4], Yewei Ji[6], Ling Qi[6], Yehudah Pardo[7], Dan Luo[1, 8, 9], Meredith Silberstein[10], Jintu Fan[3], Minglin Ma[1]*

1. Department of Biological and Environmental Engineering, Cornell University
2. Department of Clinical Sciences, Cornell University
3. Department of Fiber Science and Apparel Design, Cornell University
4. Diabetes & Cardiovascular Disease, Novo Nordisk A/S, Denmark.
5. Research Bioanalysis, Novo Nordisk A/S, Denmark.
6. Department of Molecular and Integrative Physiology, University of Michigan Medical School
7. Meinig School of Biomedical Engineering, Cornell University
8. Kavli Institute at Cornell for Nanoscale Science
9. Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, P. R. China.
10. Sibley School of Mechanical and Aerospace Engineering, Cornell University

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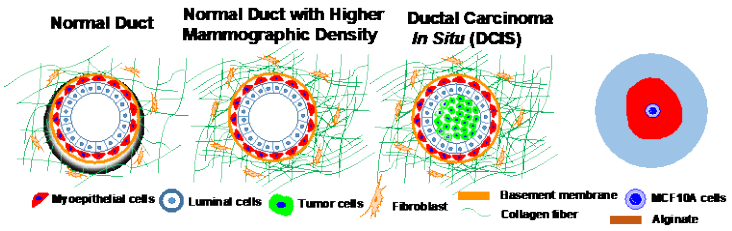
Cell encapsulation has been shown to hold promise for effective, long-term treatment of type 1

diabetes (T1D). However, challenges remain for its clinical applications. For example, there is an unmet need for an encapsulation system that is capable of delivering sufficient cell mass while still allowing convenient retrieval or replacement. Here, we report a new and simple cell encapsulation design that is readily scalable and conveniently retrievable. The key to this design was to engineer a highly wettable, Ca^{2+}-releasing nanoporous polymer thread that promoted uniform *in situ* crosslinking and strong adhesion of a thin layer of alginate hydrogel around the thread. The device provided immunoprotection of rat islets in immunocompetent C57BL/6 mice in a short-term (1-month) study, similar to neat alginate fibers. However, the mechanical property of the device, critical for handling and retrieval, was much more robust than the neat alginate fibers due to the reinforcement of the central thread. It also had facile mass transfer due to the short diffusion distance. We demonstrated the therapeutic potential of the device through the correction of chemically-induced diabetes in C57BL/6 mice using rat islets for 3 months as well as in immunodeficient SCID-Beige mice using human islets for 4 months. We further showed, as a proof of concept, the scalability and retrievability in dogs. After 1 month of implantation in dogs, the device could be rapidly retrieved through a minimally invasive laparoscopic procedure. This encapsulation device may contribute to a cellular therapy for T1D because of its retrievability and scale-up potential.

[12A] **Malignant transformation of mammary epithelium induced by physical confinement**

Yen-Chun Lu*[1], Minglin Ma[1]; Cornell University
[1]*Department of Biological and Environmental Engineering*
*: Presenter, e-mail yl2347@cornell.edu

Many tumorigenesis factors such as mechanical stiffness of extracellular matrices and paracrine signaling in microenvironment have been studied in tumor formation. Moreover, cancer cells could interact with immune cells, stromal cells to build their own microenvironment. Taking breast cancer as example, altered microenvironment induces the malignancy of epithelial cells, such as uncontrollable cell growth and disruption of apical-basal polarized structure which are preliminary malignant features. For example, the extracellular matrix fibrils surrounding tumor exhibits higher stiffness than normal tissues because breast tumor can be detected by palpation. The standard in-vitro model for studying the relationship between altered microenvironment and tumor development is based on human benign mammary epithelial cells (MCF-10A) within basement membrane matrix. The growth-arrested acinus is formed by a single layer of mammary epithelial cell within the reconstituted basement membrane (Matrigel™). However, stiffer extracellular matrix could disrupt the acini hollow structure and induce malignant behavior. In current culture models, changes in stiffness are always coupled with altering composition of the matrix; it has been challenging to independently control and study the mechanical stiffness and matrix composition. Given this great challenge, our newly developed Matrigel/alginate core-shell decoupled hydrogel microparticles to develop a scalable 3D in vitro tumor model where the effect of mechanical confinement imposed by the alginate shell can be studied within unaltered Matrigel core. We have shown that even within chemically unaltered Matrigel matrix, the confinement from hydrogel layer could induce malignant transformation of MCF-10A cells. Under reducing confinement, MCF10A cells still could form nice hollow acini structure but larger than normal condition.

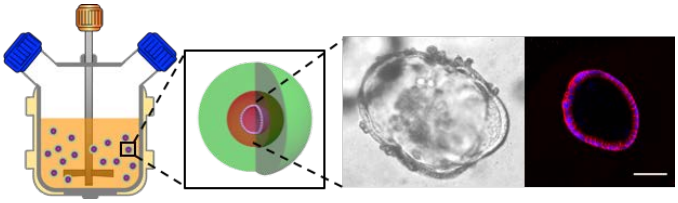


Mammary epithelium transformed progress mimicked by hydrogel microcapsules

[13A] **Scalable Production and Cryo-Storage of Organoids Using Core-Shell Decoupled Hydrogel Capsules**

Yen-Chun Lu*[1], Minglin Ma[1]; Cornell University
[1]*Department of Biological and Environmental Engineering*
*: Presenter, e-mail yl2347@cornell.edu

Organoids, organ-mimicking multicellular structures derived from pluripotent stem cells or organ progenitors, have recently emerged as an important system for both studies of stem cell biology and development of potential therapeutics; however, a large-scale culture of organoids and cryopreservation for whole organoids, a prerequisite for their industrial and clinical applications (such as, regenerative medicine), has remained a challenge. Current organoid culture systems relying on embedding the stem or progenitor cells in bulk extracellular matrix (ECM) hydrogels (e.g., Matrigel™) have limited surface area for mass transfer and are not suitable for large-scale productions. Here, we demonstrate a hydrogel capsule-based, scalable organoid production, and cryopreservation platform. The capsules have a core-shell structure where the core consists of Matrigel™ that supports the growth of organoids, and the alginate shell forms robust spherical capsules, enabling suspension culture in stirred bioreactors. Compared with conventional, bulk ECM hydrogels, the capsules, which could be produced continuously by a two-fluidic electrostatic co-spraying method, provide better mass transfer through both diffusion and convection. The core-shell structure of the capsules also leads to better cell recovery after cryopreservation of organoids probably through prevention of intracellular ice formation.



Scalable organoids biomanufacturing in stirred bioreactor

[14A] **Developing High-Performance, Low-Cost and Rechargeable Antimicrobial Surface Coatings for Food Safety Applications**

Mingyu Qiao*[1], Qingsheng Liu[1], You Yong[1,3], Randy Worobo[2], Minglin Ma[1]
[1]*Department of Biological and Environmental Engineering, Cornell University*
[2]*Department of Food Science, Cornell University*
[3]*Department of Chemical Engineering, Tsinghua University, Beijing, China*
* Presenter, e-mail mq76@cornell.edu

Each year in the US, more than 90% of foodborne illnesses were due to microbiological contamination. Environmental surfaces (including equipment and facilities) are important avenues for microbial inhabitation and transmission in the food processing establishments. Making environmental surfaces with antimicrobial function is of great importance for food safety preventive control across the food supply chain. We recently developed an innovative polymer-based coating material which could be easily applied on various surfaces found in the food-associated environments. In this study, a copolymer which contains both *N*-halamine antimicrobial and dopamine adhesive functional groups was successfully synthesized and fabricated on the surface of stainless steel 316L through a dip-coating method. The *N*-halamine functional group on the coated surface can be easily activated through treating with a diluted chlorine bleach solution and

this “charging” procedure can be repeated after surface chlorines were consumed (“rechargeable” antimicrobial function). This *N*-halamine modified stainless steel surface was able to completely inactivate both gram-positive (*Staphylococcus argues*) and gram-negative (*Escherichia coli* O157:H7) pathogenic bacteria within 10 min of contact. The antimicrobial function could be maintained for at least 100 “recharge-discharge” cycles. More importantly, this reported coating method can be easily scaled up for real application. The halamine-dopamine copolymer was also successfully coated on various real equipment components (metal pipes and plastic conveyor belts) using a spray-coating method. In summary, this reported coating material and method is high-efficacy, low-cost and easy-to-use; therefore, it has great potential as protective coating for environmental surfaces of the food processing plants.

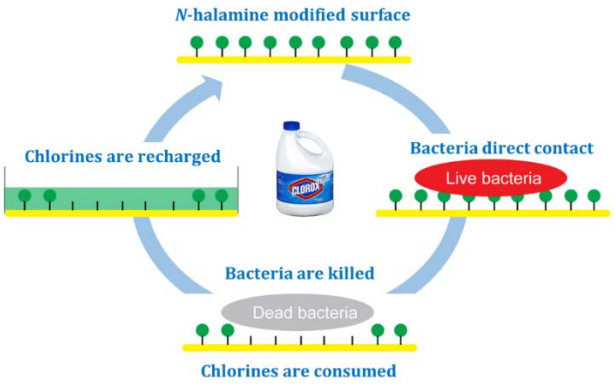


Illustration of N-halamine modified surface with rechargeable antimicrobial function.

[15A] **Statistical Bus Ranking for Robust Unit Commitment**

Amandeep Gupta*[1], C. Lindsay Anderson; Cornell University
[1]*Department of Biological and Environmental Engineering*
*: Presenter, e-mail ag729@cornell.edu

As the level of uncertain renewable capacity increases on power systems worldwide, industrial and academic researchers are seeking efficient and flexible stochastic methods for unit commitment under uncertainty. This work describes a formulation that ranks the buses of an electrical network to compute a robust unit commitment solution. Specifically, the methodology utilizes statistical analysis to identify the most critical buses based on criteria such as economic dispatch cost, or ramping needs thus providing much needed flexibility to the robust formulation.

Compared to traditional robust unit commitment models, the proposed model combines machine learning tools with analytical framework of power system networks. The resulting formulation is easily implementable and customizable to the needs of the system operator. The method and its applications are illustrated via a case study on the IEEE 30-bus system and compared to other established approaches to demonstrate the efficacy of obtained solutions. Results show that the bus ranking method performs as well as the best of these methods, with the provision of additional flexibility and potential for parallelization.

[16B] **The role of nutrients on growth, foreign protein yield, and resource allocation in chloroplast-engineered tobacco for large-scale recombinant protein production**

Jen A. Schmidt[1], Maureen R. Hanson[2] and Beth A. Ahner[1];
[1]*Department of Biological & Environmental Engineering, Cornell University*
[2] *Department of Molecular Biology & Genetics, Cornell University*

The demand for large quantities of high value proteins has seen a sharp increase in recent years in both industrial and pharmaceutical spheres. Enzymes play critical roles in industrial manufacturing of detergents, textiles, and biofuels as well as food, beverage, and animal feed processing. Additionally, large quantities of medical proteins are synthesized

for ailments from the common flu to rare threats like anthrax, botulism, and Ebola. Chloroplast-engineered plants, like tobacco, can offer a cheaper, safer, and more flexible method of producing these valuable proteins than the current cell culture methods. However, chloroplast-engineering research has only recently turned from novel, proof-of-concept experiments to targeted research into developing economically-practical large-scale application techniques. Here, we report a series of growth studies of chloroplast-transformed tobacco plants, TetC-*cel6*A. The goal of this research is to better understand the resource allocation strategies these plants adopt to accommodate foreign protein synthesis when challenged with varying nutrient inputs. From these experiments, we find that TetC-Cel6A production and total plant growth are enhanced by increasing ammonium nitrate input as well as elevating atmospheric carbon dioxide. This increase in Cel6A production at elevated CO_2 is concurrent with a decrease in Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco). Furthermore, we identify the best harvest time to maximize Cel6A yield while minimizing purification and production costs. Altogether, these experiments will provide insight into the metabolic constraints and resource demands of chloroplast-engineered plants that will enable us to develop better cultivation strategies tailored for high production of the valuable foreign protein.

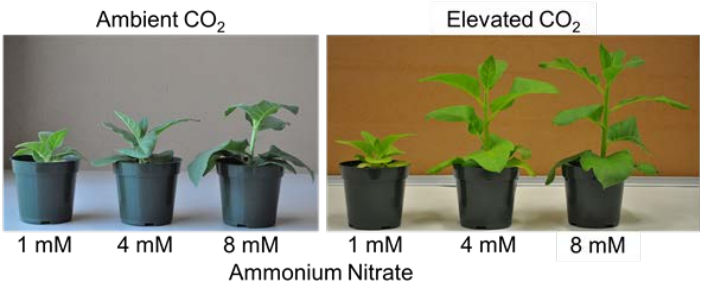


Figure 1. Harvest photos of 2-month-old TetC-*cel6*A tobacco grown with 1, 4, and 8 mM ammonium nitrate and at ambient (400ppm) or elevated (1600 ppm) atmospheric carbon dioxide.

[17B] Constitutive expression of OR protein in algae in order to improve nutritional quality

Mohammad Yazdani*, Beth A. Ahner
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Carotenoids are natural pigments distributed widely in plants, algae, fungi, and bacteria. They confer orange, yellow, and red colors to flowers, fruits, and roots. Carotenoids play essential roles in the assembly and stabilization of photosynthetic complexes in plants and algae. They are precursors for the biosynthesis of phytohormones and also act as signaling molecules to response to environmental cues. Beside their pivotal functions in plants, carotenoids play important roles in human nutrition and health. They provide dietary sources of provitamin A and serve as antioxidants to reduce the onset of some chronic diseases, such as cardiovascular diseases, cancers, and age-related eye diseases. The finding of ORANGE (OR) protein as a regulator of carotenoid biosynthesis provided an opportunity to understand the regulatory mechanisms of carotenoid biosynthesis and accumulation in order to develop crop plants with enhanced nutritional value. In addition, recent studies define a 'golden SNP' in OR that alters an highly-conserved amino acid to promote carotenoid biosynthesis. It has been shown that overexpression of the *OR* gene promotes carotenoid accumulation in a number of crops including potato tuber and tomato. Although the role of OR protein in accumulation of β-carotene in higher plants has been shown in several studies, the function of this regulatory protein in algae is not clear. The goal of our study is to elucidate the function of mutated OR protein in β-carotene over-production in algae. To achieve this goal, we took advantage of *Chlamydomonas reinhardtii* as a model organism to transform with wild-type and mutated *OR* genes. Next, the positive transformants will be used to analyze the *OR* gene and protein expression levels as well as the analysis of carotenoid levels.

[18B] **Nanoliter habitats for studying harmful algal blooms**

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Harmful Algal Blooms (HABs) impact humans by degrading drinking water resources and endangerment of aquatic life forms. HABs are the sudden explosive growth of cyanobacteria often occurring in surface waters. The frequency and duration of HABs are influenced by eutrophication and increased temperatures. Given that cyanoHAB formation is sudden and explosive, we suspect cell-cell communication may be a factor in bloom formation. Using a nanoliter fluidic habitat device, we exposed a common cyanoHAB forming, *Microcystis aeruginosa*, to a concentration gradient of a quorum sensing (QS) molecule OOHL. We predicted that OOHL would cause cell aggregation that leads to colony formation. Our results indicate *M. aeruginosa* increases the speed of motility and aggregates in the presence of OOHL. Our ultimate goal is to develop a model to more carefully examine and quantitatively analyze the role of QS in the growth and aggregation of cyanobacteria to discover an effective control solution for HABs.

[19B] **Precision fertilizer and buffer injection with In-line Real-time Injection Strategy (IRIS)**

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Research and Commercial plant production facilities relay on nutrient (E.C.) and buffer (pH) monitoring and injection technologies to provide regular and repeatable fertigation based on the specificities of the particular crop being cultured.

Currently, fertilizer and buffering agent injection methodology is formed on three primary strategies: 1) inline manual injection, 2) monitor, dose and wait, and 3) batch-mix and pump; all of these approaches have particular application restrictions and achievable degrees of accuracy.

- Inline Manual Injection(IMI) is prone to variability based on source water flow rates, concentration of stock, and sliding-calibration over time; and is only intended for open (drain to waste) systems.
- Monitor Dose and Wait (MDS) systems are primarily used in closed (recirculating) systems and rely on user set points to determine injection and wait times, and the consistency of stock solutions making them prone to variability and slow to react to changing system conditions.
- Mix and Pump (MP) systems are a hybrid of the previous two iterations; MP uses MDS logic to maintain a holding tank of irrigation ready solution and then delivers solution to the crop based on time or medium moisture content; MP requires large holding tanks and is prone to stagnation and compounding reactions between constituents.

We are developing In-line Real-time Injection Strategy (IRIS) to address the shortcomings of preexisting systems and to deliver accurate and repeatable irrigation methodology, without the potential for user error, across a myriad of crops, with a single device. This work ensures constituent nutrients are applied efficiently and accurately, regardless of implementation in open or closed systems. This system will be paired with other monitoring technologies to more accurately respond to a plant's nutritional needs over the entire course of its culture.

[20B] **Vorticles: a new class of particles through vortex ring freezing**

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A vortex ring is a torus-shaped fluidic vortex. During its formation, the fluid experiences a rich variety of intriguing geometrical intermediates from spherical to toroidal. Here we discovered that these constantly changing intermediates could be "frozen" at controlled time points into particles with various unusual and unprecedented shapes. These novel vortex ring-derived particles, termed collectively as Vorticles, were mass-produced by employing a simple and inexpensive electrospraying technique, with their sizes well controlled from hundreds of microns to millimeters. Guided further by theoretical analyses and a laminar multiphase fluid flow simulation, we showed that this freezing approach was applicable to a broad range of materials from organic polysaccharides to inorganic nanoparticles. We demonstrated the unique advantages of Vorticles in several applications including cell encapsulation, 3-D cell culture, and cell-free protein production. Moreover, compartmentalized Vorticles and ordered-structures composed of directionally assembled Vorticles were all achieved, creating tremendous opportunities to engineer Vorticle-based materials.

[21A] **A water balance based, spatiotemporal evaluation of terrestrial evapotranspiration products across the contiguous United States**

Elizabeth Carter[1]*, Christopher Hain[2], Martha Anderson[3], Scott Steinschneider[4]

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2. Research Scientist, NASA Short-term Prediction Research and Transition Center
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Accurate gridded estimates of evapotranspiration (ET) are essential to the analysis of terrestrial water budgets. In this study, ET estimates from three gridded energy-balance based products (ET_{EB}) with independent model formations and data forcings are evaluated for their ability to capture long term climatology and inter-annual variability in ET derived from a terrestrial water budget (ET_{WB}) for 671 gaged basins across the CONUS. All three ET_{EB} products have low spatial bias and accurately capture inter-annual variability of ET_{WB} in the central US, where ET_{EB} and ancillary estimates of change in total surface water storage (ΔTWS) from the GRACE satellite project appear to close terrestrial water budgets. In humid regions, ET_{EB} products exhibit higher long-term bias, and the covariability of ET_{EB} and ET_{WB} decreases significantly. Several factors related to either failure of ET_{WB}, such as errors in ΔTWS and precipitation, or failure of ET_{EB}, such as treatment of snowfall and horizontal heat advection, explain some of these discrepancies. These results mirror and build on conclusions from other studies: on inter-annual timescales, ΔTWS and error in precipitation estimates are non-negligible uncertainties in ET estimates based on a terrestrial water budget, and this confounds their comparison to energy balance ET models. However, there is also evidence that in at least some regions, climate

and landscape features may also influence the accuracy and long-term bias of ET estimates from energy balance models, and these potential errors should be considered when using these gridded products in hydrologic applications.

[22A] **Summer co-variability of surface climate for renewable energy across the contiguous United States: role of the North Atlantic subtropical high**

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This study examines the joint spatiotemporal variability of summertime climate linked to renewable energy sources (precipitation and streamflow, wind speeds, insolation) and energy demand drivers (temperature, relative humidity, and heat index) across the contiguous United States (CONUS) between 1948 and 2015. Canonical correlation analysis is used to identify the primary modes of joint variability between wind speeds and precipitation and related patterns of the other hydrometeorological variables. The first two modes exhibit a pan-US dipole with lobes in the eastern and central CONUS. Composite analysis shows that these modes are directly related to the displacement of the western ridge of the North Atlantic subtropical high, suggesting that a single, large-scale feature of atmospheric circulation drives much of the large-scale climate co-variability related to summertime renewable energy supply and demand across the CONUS. The implications for the impacts of climate variability and change on integrated renewable energy systems are discussed.

[23A] **Teleconnection patterns associated with winter precipitation in Great Lakes**

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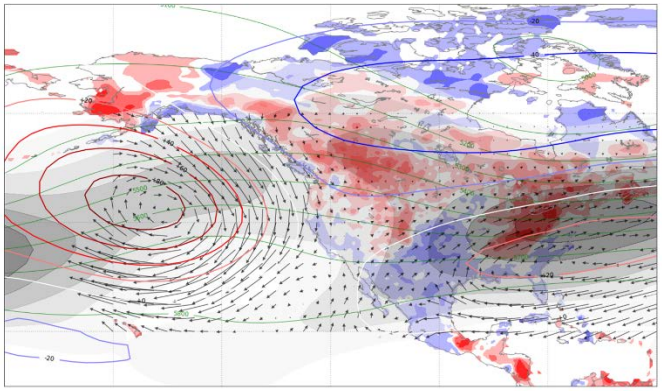
Large scale ocean-atmospheric teleconnections have been used as favorable predictors for climate in local areas. Among such teleconnections, the El Niño–Southern Oscillation (ENSO) is the most influential one which has been proved to impact climate almost all over the world. The ENSO is also responsible for the variance of Great Lakes winter precipitation to a large extent. However, the relation between ENSO and Great Lakes precipitation is not really strong, which could be attributed to, as recent studies have suggested, the interaction between ENSO and other distinct teleconnection patterns simultaneously. To address the problem, we applied a partial least square regression (PLSR) on the December-January-February (DJF) winter precipitation in Great Lakes against the concurrent 500mb geopotential height anomalies over the North America sector, which rewarded us with four distinct pressure patterns that associated with Great Lakes precipitation. By doing this, we made a decomposition of the interaction of distinct teleconnection patterns which impact Great Lakes precipitation, so that the precipitation response to each of the patterns can be studied separately. We inspected the loading vector of each PLSR pattern and calculated the Pearson correlation between PLSR patterns and a series of well-recognized teleconnection patterns. It turns out that the first PLSR pattern we get is highly consistent with the Pacific/North American (PNA) pattern, an ENSO-related atmospheric circulation pattern over the Northern Hemisphere, which means, ENSO is the most dominating signal that controls Great Lakes precipitation, as expected. The mechanism of how ENSO signal propagates from ocean to local precipitation in Great Lakes is well explained in a visualized way in this study. The second

PLSR pattern is highly related to Greenland Blocking pattern, interfering with the main ENSO-precipitation relationship. The third and fourth PLSR patterns cannot be linked to any of the selected teleconnection patterns, but they still have an effect on Great Lakes precipitation. We also explored the predictability of the first ENSO-related PLSR pattern, and found that the DJF precipitation can be well regressed using October sea surface temperature (SST) data and outgoing longwave radiation (OLR, which indicates the convection in global atmosphere system) data both.



Composite map for strong positive PNSR1 pattern. *Precipitation anomalies are displayed as background in terrestrial region – red means wetter than normal and blue means dryer that normal. Actual and anomalous 500mb geopotential heights are shown as green contours and red-blue contours, respectively. Gray shadows visualize the absolute wind speed on 300mb level, which indicates the position and strength of jet stream. Back arrows represent the total column moisture flux anomalies.*

[24A] **Predicting various spring-thaw behaviors on low-volume pavements in New York State**

Paa V. Mung*, David P. Orr, P.E.
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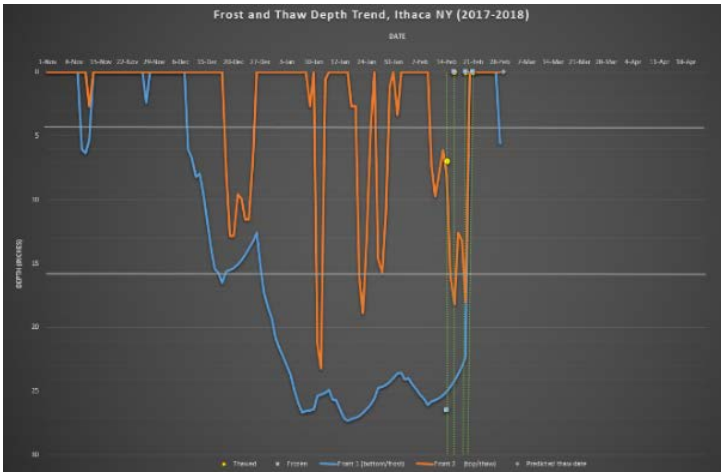
Spring load restrictions (SLR) are critical in maintaining and extending lifespans of low-volume roads. A critical challenge is to predict the spring-thaw period accurately because the phenomenon is dependent not only on the weather, but also on the layer properties of the pavement. The Cornell Pavement Frost Model (CPFM) calculates the spring-thaw seasonal lengths. A spreadsheet tool is being developed that will perform the calculations with inputs that can easily be obtained by highway agencies. The only inputs needed by the agency are materials and thickness of the pavement layers, plus historical and 10-day forecasted daily average temperature. The tool will determine the physical soil properties (thermal conductivity, latent heat, etc.) for the layers and perform the frost and thaw calculations. The user-friendly tool can be used to determine when to post SLR for local roads. This is an improvement over the most common methods for posting highways including: just picking dates, using only weather based values, or waiting until the thaw has already started. It extends the possibility of saving local pavement maintenance expenses and improving design lifespan by restricting heavy loads during the times when the pavement is weak.



Ithaca frost and thaw depths plot generated by the CPFM (2017-2018).

[25A] **Correlation between laboratory and field determined resilient modulus (M_R) of pavement subgrade support.**

David P. Orr, PE, PhD [1*]; Sung Soo Park [2];
Tommy Nantung, PhD [3]; Antonio Bobet, PhD [2]
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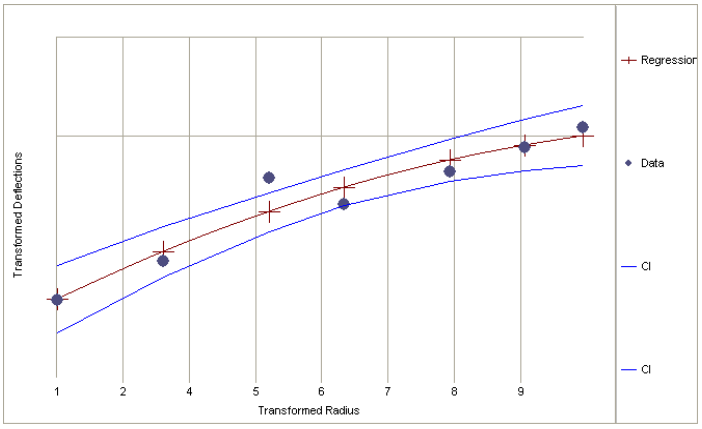
The Indiana Department of Transportation (INDOT) adopted the Mechanistic-Empirical Pavement Design Guide (MEPDG) in 2009. The overall objective of this project is to find a practical solution for INDOT pavement design procedures to effectively determine the subgrade support value, or resilient modulus, M_R. The resilient modulus can be obtained in the laboratory using specialized and expensive equipment or by field testing with a Falling Weight Deflectometer (FWD); which can collect data along the entire project length for the same or less cost. When there is good understanding of the site conditions and layer thicknesses, and there is good quality assurance and quality control (QA/QC), the backcalculation (field) analysis matches closely with the resilient modulus (laboratory) test. In such cases, the backcalculation results would be able to be used to determine the modulus for pavement design.Cornell and Purdue reviewed data from INDOT and made recommendations for improvements to field testing protocols to meet the needs of high-quality QA/QC.



SLIC Anomaly at Indiana SR 37 WB Station 43+05.

[26B] **A multiyear study of soil moisture patterns across agricultural and forested landscapes**

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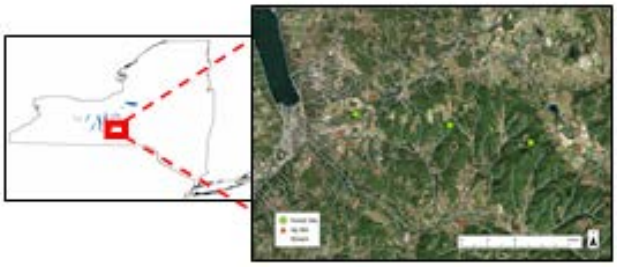
This work is a comparison of varying spatial and temporal soil moisture patterns in wet and dry years between forested and agricultural landscapes. This dataset spans 6 years (2012-2017) of spring, summer, and fall soil moisture measurements across multiple watersheds and land covers in the Finger Lakes region of upstate New York. Due to the long sampling period, we have captured fluctuations in soil moisture dynamics across wetter, drier, and average precipitation years. We can therefore analyze response of a land cover type to precipitation under varying antecedent conditions. A high number of sample points at each of our eight field sites provides the opportunity to address the spatial variability of soil moisture both within individual sites and between sites at the larger landscape scale. Across all years measured, mean soil moisture in forest soils was significantly less than agricultural soils, and exhibited less variation in moisture levels. In the drought year of 2016, soil moisture at all sites was significantly drier than other years. When comparing the effects of land cover and year on soil moisture, land cover was a more significant factor than year, even with the wide differences in annual precipitation observed during the study period. Understanding the difference in landscape soil moisture dynamics between forested and agricultural land will be useful in predicting watershed responses to changing precipitation patterns in future years and in modeling the impact of changing landscapes.



Locations of sampling sites. Red triangles indicate agricultural fields while green squares indicate forest sites.

[27B] **Inactivation of *Ascaris* eggs in human fecal material through *in-situ* production of carboxylic acids**

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New methods for reducing pathogens in human fecal material (HFM) are required to improve worldwide access to sanitation. Here, we investigate the use of *in-situ* biological production of carboxylic acids in HFM. Carboxylic acids (CAs) have been known to inhibit growth of many bacteria. Recent work has also shown that CAs are effective at inactivating eggs of the roundworm *Ascaris suum*, which are often used as indicators for pathogen inactivation due to their extreme resiliency in the environment. Through batch fermentation experiments, we showed that anaerobic microbiomes can produce up to 257 mM *n*-butyric acid and 27 mM *n*-caproic acid using HFM as substrate. We then showed that *A. suum* inactivation was directly controlled by the concentration of the uncharged form of CAs, while the pH and concentration of conjugate base did not have any direct effect on inactivation. We also exposed eggs at 30°C to *n*-butyric acid and *n*-caproic acid individually at different exposure times and concentrations. We fit a two-parameter logistic model to the data and predicted that at 30°C, 257 mM *n*-butyric acid and 27 mM *n*-caproic acid using HFM as substrate. We then showed that *A. suum* inactivation was directly controlled by the concentration of the uncharged form of CAs, while the pH and concentration of conjugate base did not have any direct effect on inactivation. We also exposed eggs at 30°C to *n*-butyric acid and *n*-caproic acid individually at different exposure times and concentrations. We fit a two-parameter logistic model to the data and predicted that at 30°C, 257 mM uncharged *n*-butyric acid can inactivate *A. suum* eggs with an exposure time between 12 and 20 days, and 27 mM uncharged *n*-caproic acid can inactivate *A. suum* eggs with an exposure time between 2 and 6 days.

Ascaris suum *eggs.* *In the uncharged form, carboxylic acids are lipophilic and able to cross the resistant lipid layer of* A. suum *eggs.*

Thank you to all of our 2018 Presenters!