UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

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McNair Session - Exploring the Natural World: From Numbers to Nanoparticles and Bats to Bacteria

Session Moderator: Todd Sperry, Office of Minority Affairs & Diversity

287 MGH

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Rheological Characterization of Polymer Solutions with Nanoparticles

Jordan Kennedy, Junior, Mechanical Engineering, Montana State University

McNair Scholar

Mentor: Jennifer Brown, Chemical and Biological Engineering, Montana State University

Xanthan gum (XG), a biopolymer excreted by bacterium *Xanthomanoas campestris*, is used in pharmaceuticals, cosmetics, agricultural products, food products, industrial products, and is used in enhance oil recovery processes because of its properties as a thickening agent, dispersion agent, and stabilizer of emulsions and suspensions. Locust bean gum (LBG), a polymer extracted from the seeds of the carob tree (Ceratonia siliqua), is of interest in the biopharmaceutical field as a medium for oral drug delivery. With the addition of nanoparticles, the material properties of the polymer solutions can be significantly altered. Understanding of polymer-particle interactions and their impact on the material response to shear through rheological measurements is necessary for targeted design of material properties for specific applications. Flow and oscillatory testing was performed on XG and LBG solutions with and without the addition of silica dioxide (SiO_2) nanoparticles. Under constant shear, XG solution shows a shear thinning behavior typical of weak gels. With the addition of nanoparticles, the shear thinning behavior of XG is still present but at an overall higher viscosity. LBG shows shear thinning behavior with a Newtonian region at lower shear rates. The addition of nanoparticles to solution shows a region of shear thickening at lower shear rates and shear thinning behavior at higher shears at a considerable higher overall viscosity than the LBG solution without nanoparticles. When strain is held constand with increasing frequencies, the storage (G') and elastic (G") modulus show that XG has a tendency of more elastic behavior than LBG. The addition of nanoparticles results in more viscous solutions with a higher elastic response. LBG behavior is mroe heavily impacted by the addition of SiO_2 nanoparticles than XG.

The Impact of Nucleotide Ratios on Carbon Flux in *Methylobacterium extorquens* AM

Sandy Nguyen, Senior, Microbiology

Mary Gates Scholar, McNair Scholar

Mentor: Mary Lidstrom, Chemical Engineering

Mentor: Norma Cecilia Martinez-Gomez, Microbiology, Chemical Engineering

Methylotrophic bacteria have long been recognized for their metabolism of reduced single carbon (C_1) compounds. Their potential for biotechnology and the occurrence of C_1 metabolism in all organisms signify their importance on an industrial and fundamental scale. Currently, mechanisms used by methylotrophs to regulate and reset their metabolic network in response to perturbations are not well understood. Previous studies suggest abrupt shifts in nucleotide levels drastically affect growth. This project will examine how nucleotide ratios correlate with carbon flux in *Methylobacterium extorquens* AM1. Once C_1 compounds undergo serial oxidation to formate, carbon flux is partitioned for further oxidation or assimilation. Two reactions involved in C_1 oxidation are known to generate reducing power: (1) the dehydrogenation of methylene-dH_4MPT into methenyl-dH_4MPT catalyzed by MtdA or MtdB and (2) the oxidation of formate into CO_2 catalyzed by Fdh1 or Fdh2. Genetic studies demonstrated differential phenotypes in which *mtdB fdh1* grew similarly to wild type while *mtdB fdh2* grew worse relative to the *mtdB* mutant strain. One possible explanation is a global decrease in NADH production offsets the delicate balance of intracellular nucleotides required for an already reducing power-limited mode of growth. To explore this hypothesis, I will conduct

experiments to: (1) quantify the levels of $NADP^+$, NADPH, NAD^+, and NADH in WT, *mtdB*, *mtdB fdh1*, and *mtdB fdh2* and (2) manipulate nucleotide ratios with overexpression and deletion of the *pntAB* and *udhA* genes. These genes encode for membrane bound (PntAB) and soluble (UdhA) transhydrogenases capable of interconverting between NADPH and NADH. Since mechanisms exist in other bacteria to alter nucleotides pools to meet metabolic demands, a similar mechanism can be predicted to operate in *M. extorquens* AM1. Success in this project will lead to better understanding of how cells balance reducing power and energy for oxidation and growth, ultimately leading to improved biotechnological utilization.

Comparative Lipid Synthesis and Acyl Saturation of Psychrophilic and Psychrotrophic *Geomyces* Fungi

Hannah Blair, Recent Graduate, Wildlife Ecology & Management, Arkansas State University

McNair Scholar

Mentor: Thomas Risch, Biological Sciences, Arkansas State University

Geomyces destructans is a psychrophilic (cold-loving) fungus that causes cutaneous infections in cave dwelling bats and high mortality in North American populations. *Geomyces pannorum* is a closely related psychrotrophic (cold-tolerant) species that is a rare skin pathogen of vertebrates. Cold-adapted organisms adjust lipid synthesis to lower membrane viscosity and thus survive unfavorable habitats. Lipid profiles, or lipid class composition, may partially explain ecological niche and *G. destructans* pathogenicity to bats. Additionally, profiles are species specific and may be utilized to differentiate closely related species and detect disease. We incubated *Geomyces* at 5, 8, 15, 18, and 22 C and isolated fungal lipid content. Broad lipid classes were determined to be primarily sterols, free fatty acyls (FFAs), and triacylglycerides (TAGs). *Geomyces destructans* produced higher proportions of unsaturated 18 carbon TAGs than G. pannorum. *Geomyces* produced more 18:3 (18 carbon, 3 double bonds) TAGs at five degrees than at higher temperatures. *Geomyces destructans* made higher proportions of TAGs at its growth limits, suggesting alterations in lipid synthesis to decrease cellular toxicity and reproductive effort. Furthermore, these results indicate *Geomyces* alter lipid structure to survive cold temperatures by increasing lipid unsaturation. Future studies should focus on temperature optima of enzymes involved in TAG synthesis and disruption of lipogenic metabolic processes. Lipid profiles among multiple *Geomyces* species should be further investigated as a method of disease detection.

Degradation of Bat Wings by *Geomyces destructans*

Cheyenne Gerdes, Senior, Wildlife Ecology and Management, Arkansas State University

McNair Scholar

Mentor: Thomas Risch, Biological Sciences, Arkansas State University

White-nose Syndrome (WNS) is a wildlife disease caused by the pathogenic fungus *Geomyces destructans* that has resulted in the mass mortalities of North American cave bats. One clinical sign of WNS is wing necrosis. *Geomyces destructans* may secrete proteases that degrade tissue, thus reducing wing strength and elasticity. We isolated *Geomyces destructans* extracellular enzymes from an in vitro system and applied enzyme solution to bat wing tissue. The toughness, strength, and elasticity of tissues was assessed with tensile testing. Protease activity was assessed with SDS-PAGE and peptide mass fingerprinting by MALDI-TOF MS. Protein profiles generated by SDS-PAGE indicate higher solubilized protein in treated samples. Major bands were identified as integumentary proteins by MS. Tensile testing did not detect damage, but *Geomyces destructans* proteases may cleave host integument.

Statistical Designs of Experiment: Construction of Generalized Minimum Aberration Designs of Size 32

Laura White, Senior, Mathematics, Arkansas State University

McNair Scholar

Mentor: Hong Zhou, Mathematics and Statistics, Arkansas State University

Statistical designs are a way to layout experiments before conducting your experiment. A well-chosen experimental design allows one to get more information out of the experiment. Regular fractional factorial designs are types of experimental designs that are used for studying effects of two or more constraints simultaneously, but leave large gaps in run size. Run sizes are the amount of experiments an experimenter has to conduct. Non-regular fractional factorial designs can be constructed for every run size that is a multiple of four, which allows run size flexibility and economy. My research focuses on construction of optimal designs of size 32 runs using graphic processing unit (GPU) technology. I have been working on creating a design table of non-regular fractional factorial of size 32 runs. Creating design tables make it possible for engineers and scientist to plan experiments for any combination of runs and number of variables to be studied.