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December 2005 Report of Progress

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Edible Biomass/Crop Production

Cary Mitchell

Minimizing ESM for ALS Crop Production

- Gioia Massa and Mercedes Mick conducted a tour of the facilities and answered questions for an elementary school class researching advanced life support on December 1st.
- Yang Yang, Gioia Massa, and Cary Mitchell met with Jerry Shephard in the Central Machine shop to discuss plans for the development of the crop-canopy gas-exchange cuvette system, Minitron III. In addition, discussions were pursued with Al Heber and Connie Li to determine what, if any, gas contaminants may be produced by the LED lighting system. Plans are underway to capture and analyze the cooling air passing through the lightsicles.
- Following an analysis of light output and current levels for both LED lighting systems, a side-by-side cowpea experiment was planted December 20th in the growth chamber comparing intracanopy and overhead LED lighting. In addition, this experiment will be part of a collaboration with systems analyst Jim Russell to examine transpirational burdens under different lighting conditions. The conditions set for this experiment are 30 plants per growth area (0.23 m²) with light levels set to approximately 300 $\mu\text{mol}/\text{m}^2/\text{s}$ at 2.5 cm from the light engines. Plants are being grown for 32 days, with pH and conductivity adjusted every other day. The treatments will be harvested January 20th.
- Lettuce plants were planted Dec. 21st in a side-by-side comparison of manual versus automated hydroponic pH adjustment. This will be the first test with plants of the automated pH control system developed by Moeed Muhktar in George Chiu's lab.
- Harvests of experiments occurred November 29th and December 15th for carrot and December 7th for sweetpotato. Harvested biomass was sent to Lisa Mauer in Food Science for use in antioxidant studies. A second batch of carrots and sweetpotatoes has been replanted for replication.

Caula Beyl

Extending Crop Harvest Index Using Edible Fungi

- **Processing Composted crop residues for analyses**
Final dry weight of paired basil/wheat straw residues composted with *P. ostreatus* 'Grey Dove' was taken and data analyzed. Samples were prepared for analyses of residual lignin content, cellulose and hemicellulose following 80-90 days of fungal colonization.
- **Collaborative activity**
In continuation of our collaborative work with the Food Safety team at AAMU, growth and maintenance of radish and lettuce using a nutrient film technique is on-going. Water samples from the nutrient film including leaf samples are being examined for waterborne food pathogens.



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Food Safety and Processing

Lisa Mauer

Novel Storage and Packaging Operations

- **Radiation effects on oils and antioxidants project:**

Radiation treatment, storage, and analysis of primary lipid and antioxidant samples continuing. Data interpretation is ongoing. An abstract was submitted to the annual IFT meeting on this work.

Replicates of work on oils, including oils with added antioxidants, are underway.

- **Radiation effects on wheat project:**

Work (proximate analysis and basic chemical composition) continued on characterizing Apogee and Perigee and comparing to 3 terrestrial wheat varieties known for quality bread and pasta production.

- **Radiation effects on salad crops/preserved vegetables project:**

Work begun on characterizing different carrot cultivars as well as effects of radiation on preserved/shelf-stable carrots. Other crops will be included in this study at a later date. Selected carrot cultivars are in their second growth cycle in the greenhouse (collaborative activity with Dr. Cary Mitchell and Dr. Gioia Massa).

Leonard Williams

Optimal Food Safety in ALS

- **Manuscript titled "Inactivation of *Salmonella* spp. by a combination of pulsed light sterilization and sanitizers on the surface of whole tomatoes is in preparation for ICES 2006.**

The illnesses and deaths of humans via food poisoning by *Salmonella* has become a growing concern for the food industry including fresh fruits and vegetables. The route that fresh produce takes between the field and the consumer increases the chances of pathogenic microorganisms infecting the produce. Sanitizers or other cleaning agents are routinely used to reduce the microbial loads in the industry as well as in homes for foods and food preparation surfaces. In this study, we examined the efficacy of four sanitizers on their ability to reduce or eliminate the number of *Salmonellae* on the surface of whole tomatoes. Overnight grown cultures of *Salmonella* Senftenberg and *Salmonella* Indiana were pooled together and 1 ml (10^8 cfu/mL) on the top surface of tomatoes. Five dipping trials were conducted over eight days using 4 sanitizer treatments (Prosan®, Peracetic Acid, H_2O_2 , and Chlorine) and two controls (H_2O , no treatment). The tomatoes were rinsed with 1% peptone water and bacteria were enumerated. Prosan® and chlorine caused an average reduction of 2 logs over the eight days. Hydrogen peroxide had an average of 3 to 4 log reductions while peracetic acid had an average reduction of 5 logs over the eight days. Results indicate that peracetic acid was the most effective treatment followed by H_2O_2 over the trial period. Chlorine and Prosan® were among the least effective sanitizers over 8 days of treatment suggesting that peracetic acid when used at low concentrations may be an effective sanitizer for inactivating *Salmonella* on the surface of whole

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tomatoes.

- **Manuscript titled” Efficacy of hydrogen peroxide, chlorine and heat treatment on the reduction of *Listeria monocytogenes* Scott A on carrot seeds during growth and sprouting”.**

Listeria monocytogenes is a Gram-positive bacterium which has a great resistance to low temperatures, heat, and drying. The objectives of this study were to determine the efficacy of chlorine, hydrogen peroxide and heat treatment in killing of *L. monocytogenes* Scott A inoculated onto carrot seeds, the retention of viability of *L. monocytogenes* on dry seeds as affected by storage temperatures of 8°C and 21°C, and the behavior of *L. monocytogenes* on carrot seeds subjected to conditions commercially. The method used for inoculation was suspending the carrot seeds in a solution of 0.1% peptone water and a 24 hour culture of *Listeria monocytogenes* Scott A. The inoculated seeds were divided and held at 8°C for one and six week periods and for a four week period at 8°C and 21°C. The portions were treated with hydrogen peroxide at 0.5%, 5%, and 10% and a chlorine solution at 100, 300, 500, 1000, 2000 ppm (ug). Additionally, inoculated seeds were germinated and samples were plated to determine the incidence of *L. monocytogenes* on carrot sprouts. Samples were extracted from the treatments and spirally plated onto plate count agar. The plates were incubated at 37°C for 48 hours and then colonies were counted with a laser plate reader. The chemical treatments established that the trend reduction of *L. monocytogenes* was greater with the 5% and 10% hydrogen peroxide solutions and the 500,1000, and 2000ppm(ug) chlorine solutions. These results are significant to hazard control of the pre-harvested preliminary stages of food production.

Bruce Applegate Bioamplification Using Phage Display for the Multiplexed Detection of Pathogens in Potable Water and Food

- **Evaluating Bacteriophage Infectivity in a Food Model**

To optimize the assay for minimal food sample preparation, we utilized a previously developed 2 component system of T4 phage bioluminescent *E. coli* (lux) host strain to evaluate the effect of variables such as food components or laboratory media on phage infection. *E. coli* lux cells were serially ten-fold diluted with sterile peptone water, LB broth, and LB mixed with 10% (w/v) ground beef. Samples were analyzed by addition of serial dilutions of T4 phage and reduction of bioluminescence was monitored over time using a Wallac Microbeta scintillation counter. Results showed decreasing bioluminescence for *E. coli* (10^7 - 10^5) cfu/ml in the presence of increasing T4 phage concentrations over time. At approx. 10^7 cfu/ml of *E. coli* lux, a dynamic range of T4 phage concentration was 10^9 - 10^6 pfu/ml with a detection limit of approx. 10^5 pfu/ml in both LB and LB with ground beef with peptone not exhibiting any decrease in luminescence. However, the rate of decrease in bioluminescence was greater in LB than that in LB with ground beef. This study suggests that incorporating LB into the ground beef for



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sample preparation facilitates phage infection and replication.

Resource Recovery

Al Heber

Gas-Phase Revitalization Using Biofilters in ALS

- The BREATHe II performance is under further evaluation. Among all the bioreactors operated with different reactor configuration and packing media, the perlite biofilters exhibited the highest overall trace contaminant removal efficiency, followed by the perlite biotrickling filters. However, there has been an obvious decrease in ethylene removal efficiency for the perlite bioreactors as a result of an increase in inlet ammonia concentration and discontinuance of external nitrogen supply in the liquid phase starting from day 74. Nutrients were later added to half of the reactors on day 95, and ammonia loading to each reactor was discontinued on day 109 to observe each reactor's response in ethylene removal and to understand the interactions between ammonia and ethylene removal.
- Profile studies along the height of each bioreactor were conducted on a weekly basis to assess the spatial distribution of each contaminant. Liquid phase nitrogen ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$) concentrations are being analyzed to understand the mechanism of ammonia removal.

The BREATHe II biotrickling filter model is being validated using the experimental data from the polyurethane foam biotrickling filter, and the equations on the gravity effects on multiphase flow in BREATHe II was refined.

Paul Brown

Waste Treatment Using Tilapia

- Acceptance of the paper entitled "Nile Tilapia Oreochromis niloticus as a Food Source in Advanced Life Support Systems: Initial Considerations" by the Journal of Advances in Space Research.
- Submission of the paper entitled "Evaluation of Fish Meal-Free Diets for First Feeding Nile Tilapia, Oreochromis niloticus" to the Journal of Applied Aquaculture.
- Abstract # HLS152 "Effects of Composting on Nile tilapia's Ability to Utilize Nutrients in Potential Advanced Life Support Waste Residues" accepted for presentation at Habitation 2006. Analyses for this study are 75% complete.
- Digestive protein study analysis underway.
- Micro nutrient mass balance study to be completed on 11/25/05.

Dawn Whitaker
Cary Mitchell

Solid-Phase Thermophilic Aerobic Reactor (STAR) Processing of Fecal, Food, and Plant Residues

- Off-gas analysis of the pilot scale reactor has begun for CO_2 , H_2S , and ammonia. The reactor was restarted at 8% solids loading, which had previously been determined to be the optimum solids loading. Off-gases are being monitored throughout the start-up process.



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- Lignin, enzyme, and respirometry testing are in the final stages.

Charles Glass

Nitrogen Cycling in ALS

- **Equipment Needs for Year 3 Experiments**

Due to the limited budget presented to the PI initially for year three only one 5L working volume sequencing batch reactors (SBR) has been ordered from Broadley-James, for a total cost of approximately \$14,000, including the vessel, motor, motor controller, pH and dissolved oxygen probes and their respective meters. Hopefully the equipment will arrive by late June. With the appropriation of a second allotment of funding a second reactor will be ordered within the next week to fulfill the two reactor requirement for the experiments this year.

- **Continued Methodology Development for Year 3**

While equipment orders are in route we will continue to review the literature and plan the experiments in as much detail as possible. The goal of the experiment will be to acclimate a single sludge nitrification-denitrification activated sludge to the highest initial ammonia concentration achievable. We plan to begin with ammonia concentrations found in the local wastewater treatment plant, where the activated sludge is originating from, to hopefully 1,200 mg/L $\text{NH}_4^+\text{-N}$.

Kim Jones

Membrane Processes in ALS

- **Microfiltration Membrane System.** Modified PES membranes were compared to virgin membranes in terms of biofouling (see below) and bacteria rejection. Results showed positive effect of modification (eg. biofouling was reduced); characterization studies are underway to explain behavior.
- Biofouling studies are conducted by relating microbial attachment as a function of number of cells in suspension. Ongoing studies include studying biofouling as a function of membrane surface roughness (via AFM), pore size and hydrophobicity for the virgin and modified membranes
- **Reverse Osmosis/Nanofiltration Membrane System.** RO experiments are ongoing. Concurrent modeling studies are being developed to determine the rate of replacement of the RO membranes as a function of rate of fouling (as quantified by buildup of irreversible fouling layer). Surfactant monomers and trace organics are surrogate foulants for this system.
- **Outreach.** An undergraduate student has been involved in this project since summer 2005.
- **Technology Transfer.** In year three, these results have been presented at four (4) conferences. Two manuscripts are under preparation. Submission



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expected in February 2006.

Kathy Banks

Treatment of Grey Water Using Gas Biofilters

- Biofilm traps were added downstream of effluent sampling ports effluent solids capture and estimation. Solids concentrations will allow biofilm shedding model to be developed, aiding in reducing system ESM.
- 2500 ppm of CO₂ was added to the reactors to analyze possible microbial toxicity. No toxicity issues were noted, but air flow rates were adjusted slightly to reduce potential anoxic microenvironments and unsure sustainability of aerobic heterotrophic population(s).
- The multicomponent gas mixture (CO₂, NH₃ and H₂S) is currently being added to the system. NH₃ and H₂S concentrations will initially be relatively low. Biological steady-state removal rates will be reached prior to increasing NH₃ and H₂S concentrations. This will be repeated until full-strength influent conditions are met.
- Analytical analysis of ethylene glycol (EG) by HPLC and BDOXY methods is continuing to determine the biodegradability of EG and its metabolic byproducts. EG may be a potential non-biodegradable product with human toxicity issues.

Chip Blatchley

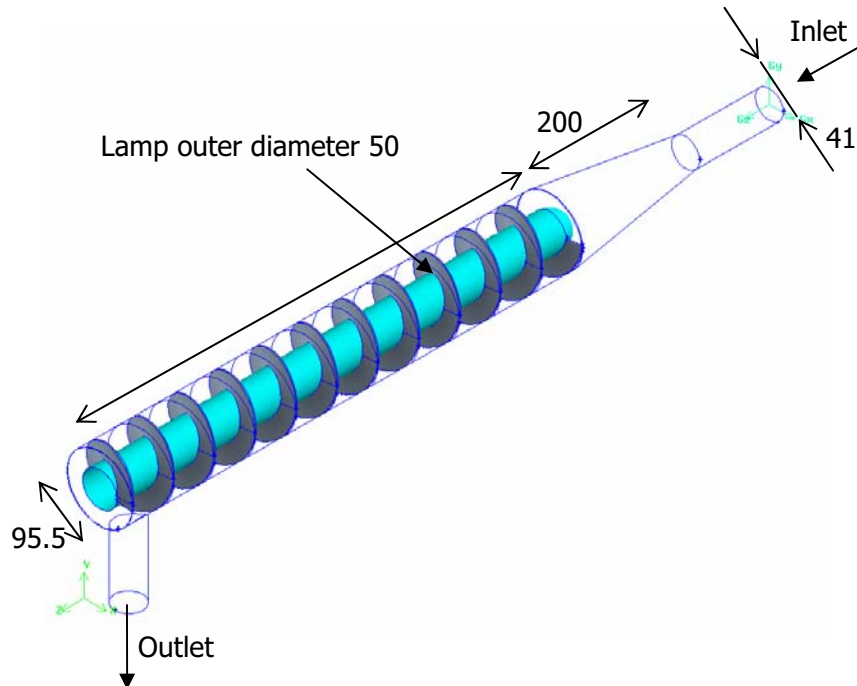
Potable Water Disinfection Subject to Extended Space Travel Constraints

- **Incorporation of the SPACE Intensity Field Model into the Numerical Method for Estimating Process Efficiency**

The enhanced SPACE intensity field model for the XeBr* excimer lamp was incorporated into the design of the reactor to be used for UV water disinfection during long-term space missions by writing a detailed code in FORTRAN, which accounts for radiation received at a point around the lamp through three different radiation pathways: direct radiation pathway, reflection off internal ground electrode and reflection off reactor housing. The design for the UV source and reactor housing was modified to incorporate suggestions by the manufacturer of the excimer lamp which will be used as the radiation source. This reactor design is presented in the figure below, with all the dimensions stated in millimeters. The operational parameters for this reactor were determined by analyzing microbial inactivation efficiencies at a number of operating conditions with the developed numerical method.

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Ron Turco/
 Larry Nies

PAABLO and WAABLO in ALS

- **Plant-based Anaerobic-aerobic Bioreactor Linked Operation (PAABLO).** The objective is to develop and optimize anaerobic-aerobic bioreactor system to allow the production of methane from plant-based waste materials and greywater. This will allow reduction in plant waste biomass and remediation of greywater and the formation of a possible source of energy.
- **PAABLO biosolids study.** Methods for the optimization of the PABLO biosolids for use in fungal and plant cultivation will be investigated. This will require an evaluation of the makeup the aerobic reactor's microbial population to maximize the formation of soluble carbon for use in the anaerobic portion of PABLO and the need to develop a biosolids recovery system.
- **Waste-based Anaerobic-aerobic Bioreactor Linked Operation (WAABLO).** The objective is use a bioreactor system to optimize production of methane from human waste materials and to provide a means of drying and storing the material.

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- **WAABLO biosolids study.** This will require an evaluation of the makeup the aerobic reactor's microbial population to maximize the formation of soluble carbon for use in the anaerobic portion of WABLO and minimize the formation of residual biosolids. For biosolids that are formed, we will optimize the procedures for sterilization and compactions.
- **Progress to date.**
- We have constructed a work plan for the project that will be implemented as soon as our post-doc is on board.
- We have met with the systems group to discuss their information needs and to give them a better understanding of what we are trying to accomplish.
- We have written and circulated an advertisement for a post-doc. The advertisement has resulted in many responses but 4 individuals are being evaluated (outside letters etc) for a campus interview.
- Major equipment to be used for the assessment of bioreactor diversity has been ordered.

Integrated Systems and Analysis Group

J. Pekny, G. Chiu, Systems Modeling of ALS with Dr. Seza Orcun, Focus Area Lead
Y. Yih

- A simulation was built to collect data for analysis of possible state transitions for the Markov model that reflects various states of the system.
 - An agent-based software was used for this simulation.
 - Breeds of agents included in this simulation include plants (tomatoes, lettuce), crewmembers, CO₂ and O₂ tanks that can release or withdraw gases to or from the environment to balance out excess or shortage of CO₂ or O₂.
 - CO₂ and O₂ are defined as properties of the "patches" that make up the environment in this study.
 - Crewmembers metabolic rates and plants photosynthesis data used in this simulation were calculated based on BVAD (2004).
- Water and energy balance of crops are performed
- Mass and energy analysis of PAABLO & WAABLO are performed.
- ALS NSCORT Process Map has been updated.
- The proto-type study in collaboration with Envision Center of Purdue University on scientific visualization of evolution of ALS system is completed.
- Chit-Hui Ang (Advisor: Dr. Yih) successfully completed her study in the ALS NSCORT program and fulfilled her M. Sc. requirements in Industrial



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Engineering.

- Chit-Hui Ang presented her graduate work titled "A Simulation Approach to Minimize Water Supply, Water
- Storage Capacity, and Water Treatment Capacity Requirements in an Advanced Life Support System for Mars Missions" on Dec 15, 2005 at SIMA telecon.

ALS NSCORT Education

Julia Hains-Allen

Education

- ALS/NSCORT in collaboration with ESMD Education supported a booth at NSTA Southern Area Conference in Nashville, TN December 1 - 3, 2005.
- December 1st a campus tour was given to 15 seventh graders from The Orchard School of Indianapolis. The students were doing research to complete their semester long project of developing a representation of a Mars habitat.
- The pilot program in Valparaiso, IN was finished. It is currently being evaluated on its effectiveness and to find where improvements can be made.
- The previous contacts that were made in Montana, New Jersey, and Iowa were followed up. There has been one extension educator training in Montana planned for March 21 – 23. There have been two extension educator trainings in New Jersey planned for March 7-8 and May 4-5.