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ALS-NSCORT 2005 Annual Report

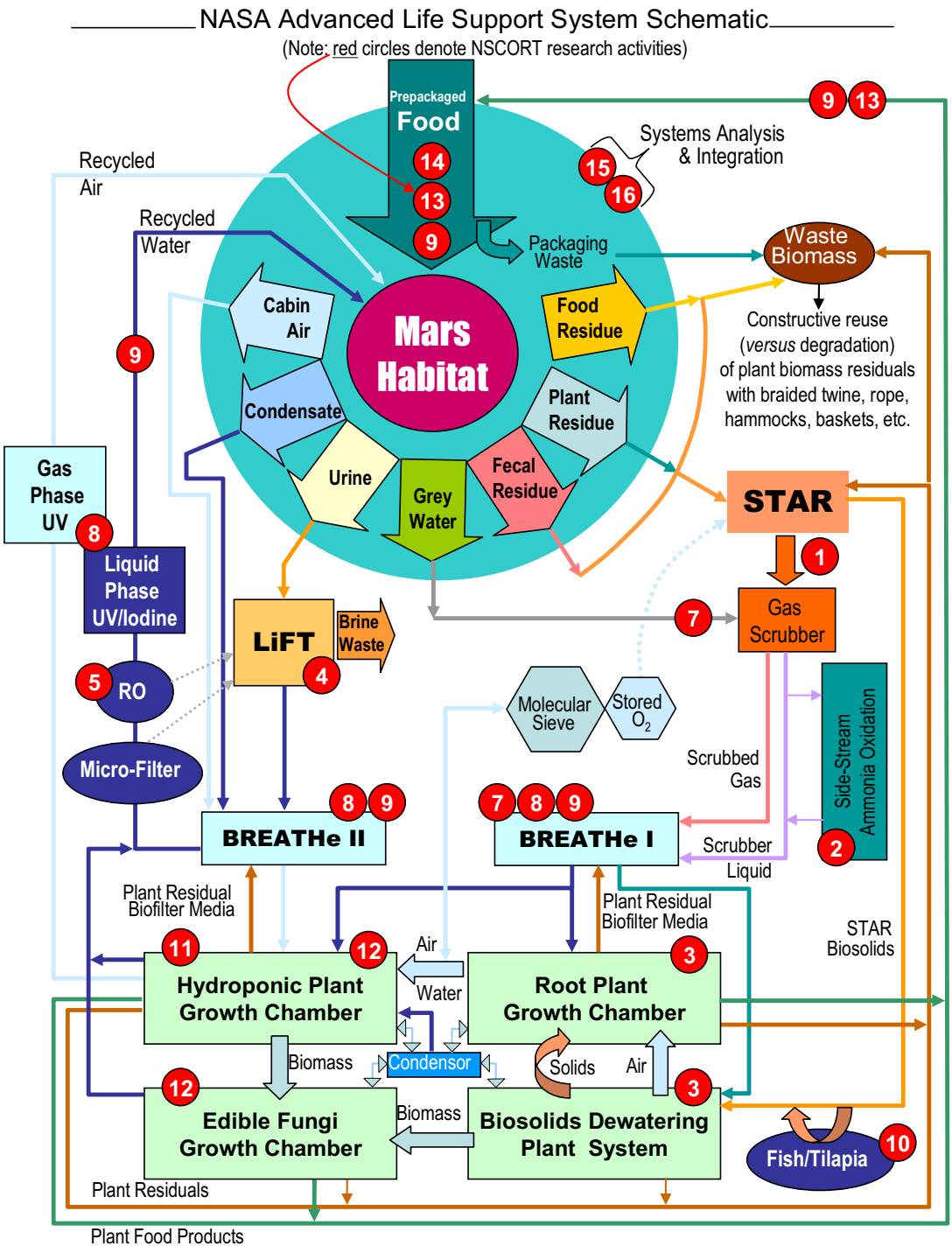
ALS NSCORT

2005 Annual Report

PURDUE UNIVERSITY

ALABAMA A&M UNIVERSITY

HOWARD UNIVERSITY



Note:
This ALS project schematic will be extensively modified in 2006

Table of Contents

From the Director

Dear Colleagues and Friends,

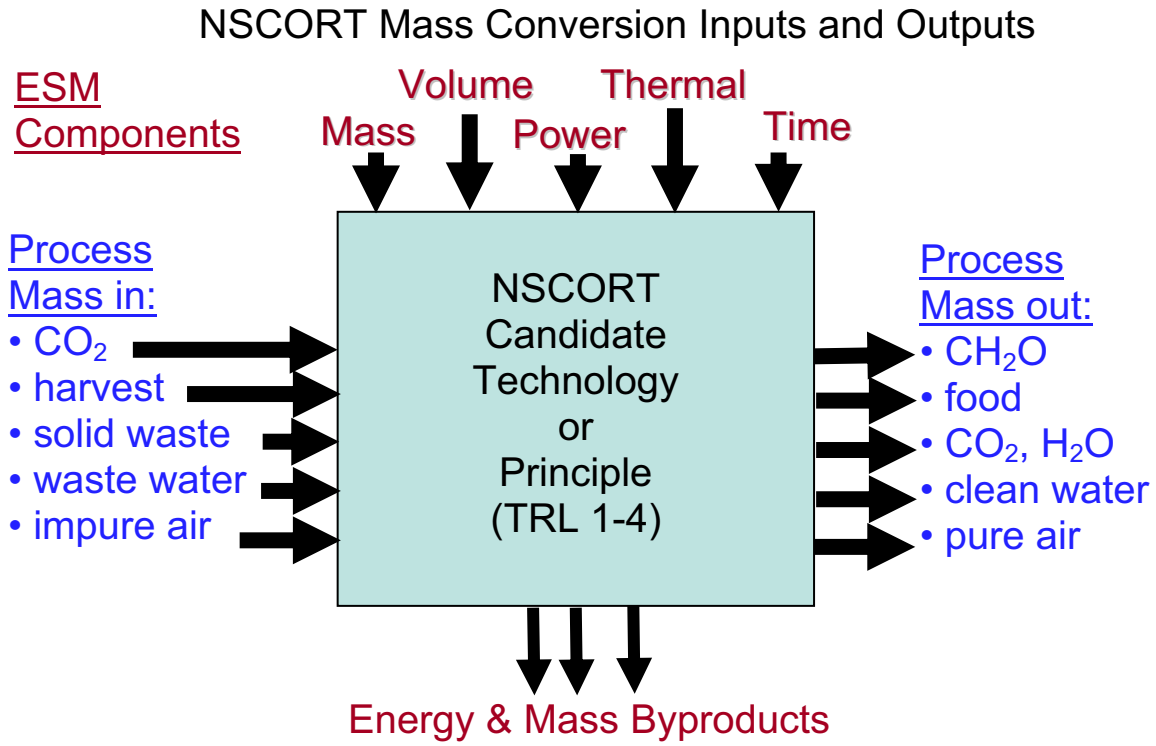
Please find in this volume the third annual report of the NASA Specialized Center of Research and Training in Advanced Life Support. The ALS NSCORT, as it is called, is a consortium of Alabama A & M, Howard, and Purdue Universities involving 16 research projects related to space advanced life support plus a related education project spread over the participating institutions, all of which are funded by a grant from NASA.

NASA's original charge to the NSCORT was to pursue innovative regeneration concepts with potential to significantly lower the "costs" of sustaining a crewed base on Mars without frequent resupply of life-support consumables from Earth. Those costs are folded into a single roll-up metric called "ESM", an acronym for "equivalent system mass". The component terms of ESM include launch mass, volume, the power required to operate a given recycling technology, the power required to reject its waste heat, the crew time required to run the technology, and the duration of a mission that it will operate. Each ESM component except mass *per se* is multiplied by an equivalency factor based upon a counterpart on Space Station, or if that doesn't exist yet, upon studied best estimates. The resulting metric expresses total resource inputs in units of mass equivalency. At about $140,000 to deliver a pound of payload from Earth's surface to Mars surface, it is to NASA's advantage to develop affordable and sustainable life-support systems that could be used indefinitely on Mars. Life-support technologies that can purify waste water and contaminated air efficiently will lower ESM and will help make an advanced life-support system (ALSS) on Mars more affordable. How to provide food for Mars crews over extended missions is one of the biggest challenges facing life-support technologists. Food can be stored, but it eventually runs out without periodic resupply, and many unanswered questions exist regarding the ESM and reliability of long-term food-packaging materials. Because human food has not been effectively chemosynthesized, onsite crop production seems to be the best solution for extended-stay Mars missions involving increasingly large crew sizes. When on-site edible-biomass production becomes energetically feasible through ALS technology development, then carbon, nitrogen, and various other renewable resources will have to be reclaimed from degraded waste biomass and reused to support crop growth. In addition to reducing the actual components of ESM for space life support, mass-equivalency factors specific for regeneration technologies to run a self-sustaining Mars base need to be developed by the ALS Program, and that will require additional R & D.

If the first crewed Mars base needs to be fully operational by 2030, there is not a lot of time to develop and test ESM-lowering candidate technologies in relevant ground and space environments, and

then advance selected ones to a mature technology readiness level (TRL 9). NASA has tasked the NSCORT to provide proof of concept that lower TRL (1-4) candidate processes have sufficient promise to help reduce system ESM at least ten-fold, if developed further for use at a Mars base. Accordingly, the NSCORT focuses on bioregenerative approaches to primary processing in order to leverage the unique activation-energy-lowering capability of enzymes in resource recovery. The suite of NSCORT research projects includes several physico-chemical (PC) approaches not only with potential to lower ESM for secondary or tertiary bioprocessing, but also to negate hazards to human crews posed by toxic by-products of human and microbial metabolism. The ALSS of an extended-stay Mars base almost certainly will be a hybrid system constructed from bioregenerative (BR) as well as PC components, for backup, for redundancy, and for risk mitigation. The NSCORT approach to validating ESM-lowering/hazard-minimizing candidate technologies is compatible with a hybrid BR/PC concept of regenerative life support. NSCORT research projects address important issues of regeneration that are similar to what occurs naturally in Earth's biosphere, except that we try to improve on nature! The energy of light is captured in photosynthesis and assimilated into edible and non-edible plant biomass. The edible biomass portion is processed appropriately for immediate or later consumption. The non-edible crop biomass, the human and food-process solid waste, and impure air and water are converted to renewable resources or purified for reuse in a closed-loop life-support system. The title of the original proposal submitted to NASA by the three partners was "Minimizing Equivalent System Mass for an Advanced Life-Support System by Optimizing Kinetics and Energetics of Major Biotransformations". In order to "optimize" conditions for efficient conversion of mass from one form to another, each NSCORT laboratory has adopted various input and output parameters that serve as metrics for the performance of their candidate technologies under various conditions.

The generic NSCORT experimental approach is as follows:



The box in the middle of the schematic represents something different for each NSCORT research project. It represents a bioreactor, a biofilter, a Tilapia fish, a white-rot fungus, an ultra-filtration membrane system, a UV irradiator, an effluent adsorbent, a hazard-detection system, a food-preservation

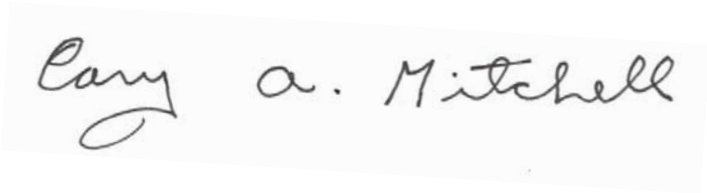
system, a lighting system, or other NSCORT principles under investigation. The metrics used for each different process are specific to the technology under development, but the kind of information coming out of each project is useful for modelers to analyze energy and mass-balance data for different technologies in various life-support scenarios. The horizontal arrows going into the generic "processor" box represent inputs. The horizontal arrows coming out of the box point to products of reactions. NSCORT researchers are interested in the rates of processing under a given set of conditions. In addition to measuring mass throughputs, feedstock disappearance, or useful product accumulation, NSCORT research quantifies the "costs" of improving on nature. The most useful cost metrics for ALS are the ESM units for mass, volume, power, labor, and time. Life-support technologies selected for further development by NASA will be matured in terms of size (i.e., reducing mass and volume) and automation (reducing labor). Key metrics that NSCORT focuses on in doing feasibility studies for candidate processes involve the measurement of reaction rates, measuring or calculating energy requirements for those reactions, and identifying "optimizing" conditions that allow a process to go as completely and as quickly as possible at the lowest input cost. That does not mean creating the highest reaction rate possible by brute force. Rather, it means identifying conditions for achieving the maximum possible bioprocess output for the least ESM inputs, especially those involving power and energy. That might create neither the fastest reduction of solid waste nor the most rapid regeneration of CO_2, but it will identify conditions for the most energy-efficient process in terms of output/input ratios. The physical realities of locating a human habitat on Mars suggest that the power and energy available for life-support functions will be significantly constrained, so time spent measuring appropriate inputs and outputs and optimizing for energy-use efficiency within an acceptable range of reaction rates is time well spent. Optimizing conditions for biological reactions is not simple. Interactions among environmental factors that affect bioprocessing are common, but occur in ways that typically cannot be predicted if more than one variable changes simultaneously. Thus, seeking optimizing conditions for candidate technologies is a work in process for most NSCORT projects. Once a reasonable set of optimizing conditions is defined for each candidate ESM-lowering technology, modeling will be required to determine what tradeoffs will be required to integrate different sub-system technologies into a system with its own optimization requirements.

During year 3 of Center operations, the mix of projects within the NSCORT and the focus within individual projects has evolved so that the Center is going in directions that truly address ALS long-term sub-system and system needs. NSCORT investigators have been encouraged to adjust their experimental approach as needed so that their labs are doing the things that NASA has stated as goals for the Center. Once NASA has validated that the NSCORT is going in the right direction and doing the right things, which will occur at the end of year 3, it will be the goal of Center management that the remaining 2-year run of the Center will be spent doing those things well. Doing things well involves accumulating enough high-quality technology-performance data that the systems-integration group will be able to do fact-based trade studies for various life-support scenarios and allow NASA to set informed priorities for future resource investment. Doing things well means NSCORT projects completing proof-of-concept studies by the end of year 5 so that NASA can make informed decisions regarding subsequent ESM-lowering life-support technology development. Doing things well means that a critical mass of human capital has been trained within the academic setting of the NSCORT and that those trainee graduates (post docs, graduate students) are interested and available to work in future space life-support programs. Doing things well means that the reach of NSCORT education programs has successfully extended to a national scope that NSCORT education programs have been permanently integrated into the K-12 curriculum, and that grassroots support (voting taxpayers) for advanced life support and the space program in general remains strong and is growing.

NASA provides the goals, direction, and inspiration for the space-exploration initiative. Academia provides the free thinking and intellectual environment needed for true innovation to occur. Industry provides the capacity for implementation, scale-up, and for getting things done. During the first 3 years

of the ALS NSCORT, collaborations and partnerships have been forged between academia and industry for this federally sponsored program. It has become increasingly clear that next steps beyond proof of concept, which will require validating ESM-lowering technologies in relevant ground-based and space-based environments, could profit exquisitely from tripartite federal—academic—industrial teams, in numerous different specialty areas, including advanced life support. It will become increasingly important to provide new knowledge on the fly as the need arises, and it will be valuable to have such teams in place to fill information gaps and implement incrementally as NASA programs develop, without losing enormous amounts of time and resources by trying to reconstitute major lost research communities. What we in the NSCORT are discovering works best to meet our goals for NASA could work well on a much grander and even more interdisciplinary scale throughout the space-exploration initiative.

Best wishes,



Cary A. Mitchell
Director, ALS NSCORT

Center Summary 2005

The Center experienced a very dynamic year that included significant personnel changes, collaborative center-wide meetings, seminar series speakers, sponsoring sixteen researchers to ICES in Rome and timely monthly progress reports submitted to the ALS leadership. At the same time, the Center endured financial challenges in the timing of receipt in annual funding that slowed the progress of research especially for our partners at AAMU and Howard Universities.

Financial Report

At the time of annual reporting, the ALS NSCORT has received $1,908,462 or 95% of the $2,000,000 for the third year in the life of the five-year grant. Still outstanding is the remaining $91,538 balance for the year three and the reduction of $500,000 money temporarily de-obligated from the Center's year-two annual budget in July 2004. Both payments are expected to be received from NASA sometime during the life of the grant. Our first installment of year 3 money in the amount of $500,000 was received in March 2005 and the second installment in the amount of $1,000,000 was received in April 2005. The third installment was received in the amount of $408,462 in August 2005, bringing the Center to a total of $1,908,462.

Research and Personnel Changes

Resource Recovery

- Dr. Jim Alleman accepted the position of Civil Engineering Department Head at Iowa State University, which and begins in January 2006 after serving at the Technical University of Crete on a Fulbright fellowship award. Dr. Alleman's STAR project will continue active research through May 2006 and his LiFT project will end in November 2005 after an eight month close-out period.
- Dr. Kathy Banks accepted the position of Interim Department Head of Civil Engineering at Purdue. She will remain active as an individual PI in her BREATHe research project.
- Dr. Al Heber from Agricultural and Biological Engineering has accepted the Focus Area Lead of a combined Air and Water with Solid Waste to form a new Resource Recovery Focus Area. Dr. Heber will continue his research in the NSCORT in Air analysis and purification and BREATHe II. Dr. Heber has also volunteered to begin a new Trace Contaminant Analysis (TCA) Center, supporting the NSCORT with analysis to further our understanding in the inputs and outputs within and between each research project. Dr. Connie Li will assist in the startup of the TCA center.
- Dr. Ron Turco, Agronomy Department, College of Agriculture and Dr. Larry Nies, School of Civil Engineering, College of Engineering will lead a new project that will focus on adaptation and use of aerobic-anaerobic technologies for the treatment of three sources of waste (plant residues, grey water and human waste) on the Mars habitat. (Ref. original proposal to ALS NSCORT Executive Committee)

Systems Group

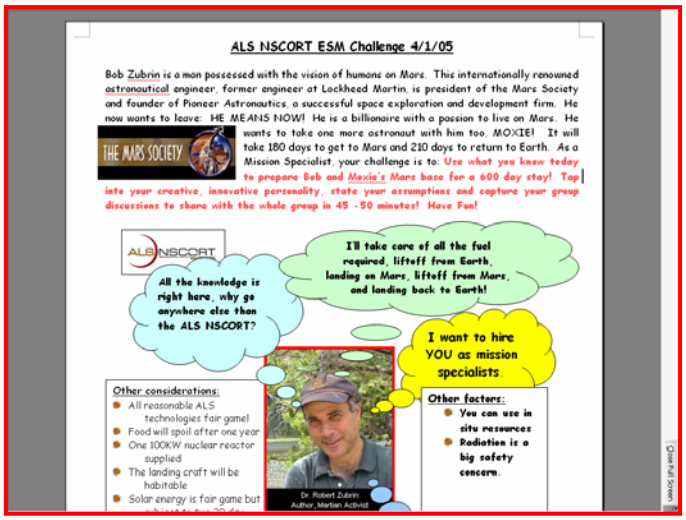
- Dr. John Trimble of Howard University resigned his project in January 2005.

- Dr. Mike Lasinski accepted the position as a Systems Group Post-Doc and began his career in February 2005.
- Dr. Seza Orcun accepted the responsibilities to lead the Systems Group as Guy Gardner assumes teaching responsibilities. Guy Gardner remains on as a consultant to the NSCORT.
- Dr. Jim Russell, from the University of Colorado, accepted the position as a Systems Group Post-Doc and began his career with the NSCORT and at Purdue on September 2005.

Center-Wide Meetings

First Workshop Date and Location:

April 1, 2005 in Rawls Hall, Purdue Campus



Workshop Objective:

Communicate strategy:

Educate the Center members about the criteria required for our NSCORT to be a success:

1. Conducting research that heads in the right direction
2. Doing the right things
3. Doing the right things well

Communicate tactics:

Listing the necessary items in research that will support the success strategy:

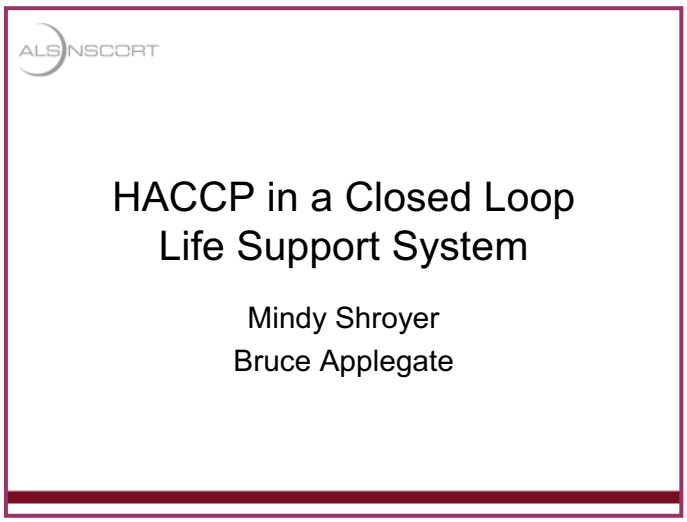
Morning session:

Further educate center members about ESM and the importance of minimizing ESM. Challenge attendees to temporarily leave their area of expertise and think in **new, creative and imaginative ways to reduce ESM**.

Afternoon session:

Educate members about HACCP (Hazard Analysis and Control of Critical Points) and **identify the HACCP applications within to their "new" areas of research.**

Report any discussion points or results that arrive from each focus group.



Workshop Conclusions:

Success strategy and tactics were communicated that stressed safe, synergistic, ESM friendly life-support research that emphasized quantitative measurement and optimization of variable inputs and outputs using bioprocess technologies, with documentation of research findings in peer-reviewed publications.

Switching areas outside of normal research

led some students to report that in theory "thinking outside the box" was beneficial, but in practice, the group dynamics were difficult to initiate and some "misinformation" occurred.

Second Retreat Date and Location:

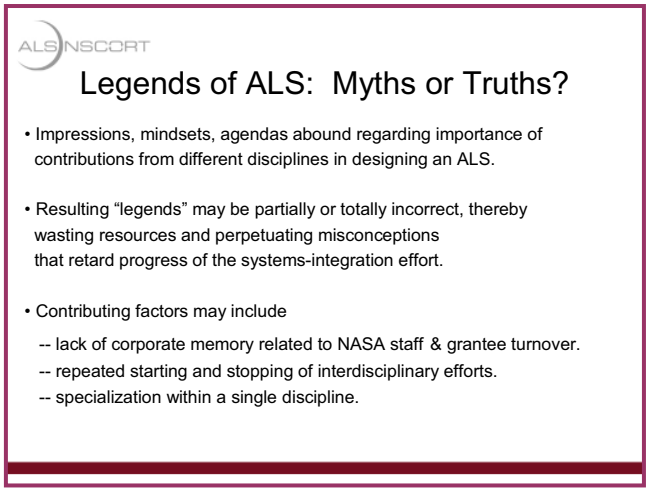
June 24, 2005 in the Wright Forestry Conference Center @ Purdue

Retreat Objective:

Re-emphasize to Center members about the criteria required for our NSCORT to be a success:

1. Conducting research that heads in the right direction
2. Doing the right things
3. Doing the right things well

Communicate "ALS Research Legends: Myths or Truths" to stretch the student's and PI's thinking in potential research collaboration.



Communicate the plan to initiate the NSCORT Trace Contaminant Analysis Center.

Present the ALS NSCORT Roadmap "strawman" and construct a deliverable for future management and communications:
As an ALS NSCORT, we are halfway into our five-year mission. The objective for our roadmap is for each of us to open-mindedly look past tomorrow, next week, and next month and set course for cross-disciplinary research for the next two and a half years; begin today with what you would like your research to achieve by November 30, 2007. With the Center's goals as a focal point, entertain collaborative research possibilities that may not have been considered until

today. In turn, take what is known, and as a collective Center, through use of the roadmapping template, document milestones and relationships that will lead to the achievement of ALS and NSCORT research goals.

Retreat Conclusions:

Success strategy and tactics were well received and stressed safe, synergistic, ESM friendly life-support research that emphasized quantitative measurement and optimization of variable inputs and outputs using bioprocess technologies, with documentation of research findings in peer-reviewed publications.

Cary Mitchell's presentation of "ALS Legends: Myths or Truths?" Well received. This presentation engaged the members and spurred much discussion.

Due to upfront planning, clear expectations, training and communicated due dates, the roadmap construction from the morning "strawman" gave to the Center a valuable planning tool and a valuable deliverable that will demonstrate to our sponsor what we expect to deliver at the end of our five year grant.

Third Workshop Date and Location:

Friday, September 30, 2005 in Rawls Hall, Room 3058, Purdue University

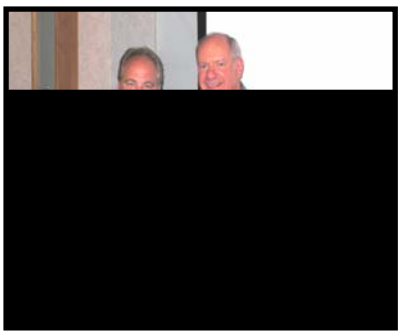
- **Guest speaker:** Ray Wheeler, NASA Plant Physiologist, Kennedy Space Center, (also a megmber of our External Advisory Committee) will address the history of the ALS program, including its precursor program, the CELSS (Controlled-Environment Life Support System) program.
- Lead by Jim Russell and Mike Lasinski, System Group Post-Docs, a new ALS schematic reflecting new research, will be presented and built upon.
- Lead by Seza Orcun, System Group Lead, further development of the Center strategic plan and project roadmaps will be built upon.

Seminar Series Year Three

October 2004

Jay Garland; Chief Scientist, Biological Programs Manager, Dynamac Corporation, Kennedy Space Center, Fla: (*Right*: Jay pictured with Cary Mitchell)

Seminar title: *Plant Production: Design Fulcrum for Water, Carbon, and Nitrogen Processing in Closed Life Support Systems*



April 2005 Karen and Jerry Ross, spoke at the Food Science Club Symposium

Day of Symposiums Highlight Student & Industry Focus

Rockwell Automation Sponsors Traceability Symposium

Over 70 members of the food, pharma and automotive industries attended Purdue's Geneology and Traceability Symposium on April 14. The symposium was hosted by the Food Science Department and Purdue's Office of the Vice President of Industry Research. The purpose of the event was to create an open forum to discuss the reasons, causes, impacts and technological mechanisms of tracking and tracing of ingredients and products. The day's events were underwritten by Rockwell Automation. Purdue President, Dr. Martin Jischke, welcomed the attendees, followed by Dr. John Sullivan, Director of the Center for Advanced Manufacturing. The symposium featured two tracks (depending on type of manufacturing) with presentations from Purdue faculty, administrators, and independent consultants. The featured track on food manufacturing presented the monetary, safety, and regulatory impacts and forecasts to exports/imports as they relate to tracking.

The afternoon session included a case study of Wal-Mart's initiatives with Radio Frequency Identification (RFID). A final session allowed participants to express their views, needs, and ideas about the topic of tracing product.

A reception was held at the end of the day where Purdue alumni Jerry and Karen Ross spoke on NASA's use of tracing manufactured products and foods. Dr. Jerry Ross is an astronaut, holding the record for time in space, while his wife, Karen, works with the foods used during flight missions.

Jerry Ross presented at both symposiums along with his wife, Karen.

Food Science Club Symposium Brings Space to the Classroom

The topic for the 28th annual Food Science Club Symposium held on April 14 this year was "Food in Space." Each year, student members of the food science club select a topic, invite speakers, and organize events surrounding the symposium. This year's space food topic reflected ongoing activities by several faculty members and undergraduate and graduate students in the Food Science Department involved with the NASA Advanced Life Support Specialized Center of Research and Training (or ALS/NSCORT) grant. Speakers at the symposium included Jerry Ross, a NASA astronaut and 1970 Purdue alumnus, Karen Ross, manager of food and product support at the United Space Alliance and 1971 Purdue graduate, and Michele Perchonok, Food Technology Lead at NASA/Johnson Space Center.

More than one hundred people attended the symposium held in the Class of 1950 Lecture Hall. Speakers talked about space flight and what it is like to eat in space, the types of foods used in microgravity environments, and research activities targeted toward providing a variety of high quality, nutritious foods for long duration space missions beyond low Earth orbit. The entire proceedings were videotaped and can be accessed at the www.alsnscort.org website. The Food Science Club members who organized the event should be given praise for the quality of the speakers and program they put together. Thank you to everyone who participated!

Respectfully submitted,

Dave Kotterman

Operations Manager, ALS NSCORT

ALS NSCORT
Center Composition

5 - year grant with NASA

- **1 Dec. 2002 -30 Nov. 2007**
- **$1.89 Million in funding for Year 3 at publication time**
- **$0.2 Million cost share supported by Purdue University for Year 3**
- **16 Projects at 3 universities**

Partnering Universities

- **Alabama A&M University (2 projects)**
- **Howard University (2 projects)**
- **Purdue University (11 projects & Outreach)**

Resource breakdown:

- **50% to resource recovery**
- **20% to systems analysis**
- **10% to food safety & technology**
- **10% to crop production**
- **10% to education and outreach**

Annual Deliverables:

- **Host ALS NSCORT Symposium**
  Involving all PIs, EAC, NASA Observers
- **ALS NSCORT Report to NASA**
- **Support ALS PI Meeting**
  Habitation Conferences

External and Internal Advisory Committees

External Advisory Committee

Morton Barlaz	North Carolina State University
Gary Coulter	Colorado State University
Marc Deschusses	University of California – Riverside
Alan Drysdale	Boeing/NASA Kennedy Space Center
Les Grady	Clemson University
Desmond Mortley	Tuskegee University
Hua Wang	The Ohio State University
Ray Wheeler	NASA Kennedy Space Center

Ex officio NASA:

Dan Barta	NASA Johnson Space Center
Mark Kliss	NASA Ames Research Center

NASA Observers:

Charles Barnes	Lead – NASA Adv. Human Support Tech Program
Jitendra Joshi	NASA Adv. Human Support Tech Program

Executive Committee

Primary Member	Alternate Member	Area Represented
Bruce Applegate	Lisa Mauer	Focus Area Lead, Food Safety/Processing
Caula Beyl	McArthur Floyd	Alabama A&M University
Seza Orcun	Guy Gardner	Discovery Park – ALS NSCORT Systems
Al Heber		Focus Area Lead, Resource Recovery
Charles Rutledge	Pete Dunn	Vice-Provost for Research Office
Joe Pekny	Ned Howell	Discovery Park e-Enterprise
Dale Whittaker	Randy Woodson	College of Agriculture
Edgar Martinez	Klod Kokini	College of Engineering
Kimberly Jones	James Johnson	Howard University
Cary Mitchell		Director, ALS NSCORT

ALS NSCORT Graduate Students by Focus Area for 2005

Thirty-two (32) Total

Focus Area	First Name	Last Name	Lab	Degree Seeking
Education and Outreach	Macon	Beck	Hains - Allen	Masters
Food Safety and Processing	Davida	Alexander	Mauer	Masters
Food Safety and Processing	Tyrico	English	Williams	Masters
Food Safety and Processing	Jake	Gandolph	Mauer	Masters
Food Safety and Processing	Udit	Minocha	Applegate	In Kosovo
Food Safety and Processing	Alecia	Shand	Mauer	Masters
Food Safety and Processing	Mindy	Shroyer	Applegate	Masters
Food Safety and Processing	Adam	Stoklosa	Mauer	Masters
Resource Recovery - Air	Hong	Huang	Heber	Masters
Resource Recovery - Air	Yong	Kim	Banks	Masters
Resource Recovery - Air	Sang-hun	Lee	Heber	Doctorate
Resource Recovery - Solids	Amy	Berg	Volenec	Masters
Resource Recovery - Solids	Kess	Berg	Volenec	Doctorate
Resource Recovery - Solids	Emma	Bruce	Glass	Masters
Resource Recovery - Solids	Reesa	Chee-Wah	Glass	Masters
Resource Recovery - Solids	John	Gonzales	Brown	Doctorate
Resource Recovery - Solids	Shane	Howard	Volenec	Masters
Resource Recovery - Solids	Angela	Nolan	Alleman	Masters
Resource Recovery - Solids	Dawn	Whitaker	Alleman	Doctorate
Resource Recovery - Water	Joshua	Abitoye	Jones	Masters
Resource Recovery - Water	Oluwaseyi	Kayode	Jones	Masters
Resource Recovery - Water	Samantha	LaHee	Jones	Masters
Resource Recovery - Water	Eric	McLamore	Banks	Doctorate
Resource Recovery - Water	Zorana	Naunovic	Blatchley	Doctorate
Resource Recovery - Water	Kelly	Pennell	Blatchley	Doctorate
Resource Recovery - Water	Jeff	Schmidt	Alleman	Doctorate
Resource Recovery - Water	Sybil	Sharvelle	Banks	Doctorate
Systems Group	Chit Hui	Ang	Yih	Masters
Systems Group	Selen	Aydogan	Pekny	Doctorate
Systems Group	Jun	Cai	Chiu	Masters
Systems Group	Tze Chao	Chiam	Yih	Doctorate
Systems Group	Yan-Fu	Kuo	Chiu	Masters

ALS NSCORT Undergraduate Students by Focus Area for 2005

Thirty-one (31) Total

Focus Area	First Name	Last Name	Lab
BioMass Production	Shanwen	Chen	Mitchell
BioMass Production	Rick	Kennedy	Mitchell
BioMass Production	Mercedes	Mick	Mitchell
BioMass Production	Jessica	Rombach	Mitchell
BioMass Production	Craig	Schluttenhofer	Mitchell
Food Safety and Processing	Morgan	Anderson	Williams
Food Safety and Processing	Tony	Bradford	Williams
Food Safety and Processing	Sri	Budiarty	Mauer
Food Safety and Processing	Dedra	Carr	Mauer
Food Safety and Processing	Lenese	Grant	Mauer
Food Safety and Processing	Dina	Romano	Mauer
Food Safety and Processing	Elizabeth	Snuffin	Mauer
Food Safety and Processing	Parisea	Story	Williams
Food Safety and Processing	Tracy	Szefc	Mauer
Resource Recovery - Solids	Ryan	Ellis	Alleman
Resource Recovery - Solids	Kali	Frost	Volenec
Resource Recovery - Solids	Tom	Konopka	Alleman
Resource Recovery - Solids	Suhaili	Muhammad	Alleman
Resource Recovery - Solids	Megan	Rosinski	Brown
Resource Recovery - Solids	Hugh-Berk	Sinclair	Glass
Resource Recovery - Solids	Kurt	Smith	Glass
Resource Recovery - Solids	Brian	Walker	Alleman
Resource Recovery - Water	Michael	Chestnut	Jones
Resource Recovery - Water	Stephan	Clark	Banks
Resource Recovery - Water	Joi	Dunham	Banks
Resource Recovery - Water	Chris	Ghattas	Banks
Resource Recovery - Water	Katherine	Graham	Banks
Resource Recovery - Water	Rebecca	Lattayak	Banks
Resource Recovery - Water	Zenobia	Lewis	Jones
Resource Recovery - Water	Eric	Malony	Banks
Resource Recovery - Water	Elizabeth	Skvarenina	Banks

Investigators, Post Docs and Managers by Focus Area

Thirty-two (32) total

Focus Area	Name	Function	Telephone	e-mail Address
Outreach & Education	Julia Hains-Allen	**Manager**	765.496.6694	hains@purdue.edu
Systems Group	Seza Orcun	**Lead**	765.494.2181	sorcun@purdue.edu
Systems Group	Bin Yao	**Co-I**	765.494.7746	byao@purdue.edu
Systems Group	George T-C Chiu	**Co-I**	765.494.2688	gchiu@purdue.edu
Systems Group	Guy Gardner	**Co-I**	765.494.9258	g2@purdue.edu
Systems Group	Joseph F. Pekny	**Co-I**	765.494.7901	pekny@purdue.edu
Systems Group	Yuehwern Yih	**Co-I**	765.494.8026	yih@purdue.edu
Systems Group	Jim Russell	**Post Doc**	765.494.6307	jfrussel@purdue.edu
Systems Group	Mike Lasinski	**Post Doc**	765.494.4821	lasinski@purdue.edu
Resource Recovery - Air	Albert J. Heber	**Lead**	765.494.1214	heber@purdue.edu
Resource Recovery - Solids	Brad C. Joern	**Co-I**	765.494.9767	bjoern@purdue.edu
Resource Recovery - Solids	Charles C. Glass	**Co-I**	202.806.6571	cglass@howard.edu
Resource Recovery - Solids	James E Alleman	**Co-I**	765.494.7705	alleman@purdue.edu
Resource Recovery - Solids	Jeffrey J. Volenec	**Co-I**	765.494.8071	jvolenec@purdue.edu
Resource Recovery - Solids	Paul B. Brown	**Co-I**	765.494.4968	pb@purdue.edu
Resource Recovery - Water	Ernest R. Blatchley	**Co-I**	765.494.0316	blatch@purdue.edu
Resource Recovery - Water	Kimberly L. Jones	**Co-I**	202.806.4807	kjones@scs.howard.edu
Resource Recovery - Water	M. Katherine Banks	**Co-I**	765.496.3424	kbanks@purdue.edu
Resource Recovery - Water	Ron Turco	**Co-I**	765.494.8077	rturco@purdue.edu
Resource Recovery - Water	Loring (Larry) Nies	**Co-I**	765.494.8327	nies@purdue.edu
Resource Recovery - Air	Congna (Connie) Li	**Post Doc**	765.496.3994	congna@purdue.edu
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Edible Biomass / Crop Production Executive Summary

Three projects within the ALS NSCORT address the feasibility of producing human-edible biomass on Mars or the moon: One project uses lignin-degrading fungi to produce exotic mushrooms; another project examines growth of fresh-water fish on substrates derived from crops and food scraps; and a third is proving that it is energetically feasible to grow crops with electric lighting in space.

The rationale for using heterotrophic fish and fungi in space is both to reduce the solid-waste burden and improve the nutrition and interest of crews in a diet that will be mainly vegetarian once life-support loops are largely closed. The ESM crossover point when fish and mushroom production would begin to compete with human crews for resources such as oxygen will determine the upper limit for using them as alternative food sources or as waste processors.

During the past year, the Center has broadly embraced the concept that resource recovery from bioprocessing must identify potential hazards to crews in a closed life-support system as well as be kinetically and energetically efficient. Whenever processor organisms enzymatically digest organic substrates, such as in a microbial bioreactor, it is legitimate to ask whether the effluents and residues contain toxic substances derived either from breakdown of the waste biomass itself, or as a by-product of microbial metabolism. Alternative food organisms feeding on substrates containing potential toxins need to be analyzed, as do those substrates, before and after bio-processing, to determine the type and degree of processing needed to mitigate risk to crews in a closed system. Similar questions are being asked regarding the use of plants for waste processing.

Emphasis has shifted in the NSCORT toward use of non-human wastes as substrates that make bioreactor effluents and residues potentially safer for subsequent feeding to fish and fungi that, in turn, could be consumed by human crews. The prospect of using white-rot fungi to pre-digest crop residue prior to feeding it to Tilapia is under investigation, either after composting and/or with an intermediate microbial bioreactor step involved. Focus is on meeting the nutritional requirements of fish as well as that of the bioreactor microorganisms they will feed on. In order for fish to obtain essential amino acids from N-deficient ligno-cellulose, bacterial biomass has been identified as a needed component of the bioreactor residue, and nitrogenous and sulfurous volatile effluents from other bioprocesses are to be captured and redirected to enhance bacterial, fungal, and fish nutrition and health. Both alternative food research projects are entering additional collaborations within the NSCORT to better enable fish and fungi to fill dual food and waste-processor roles for an energy-efficient ALS.

The third biomass project is tackling the energy-burden issue of lighting crops with electric sources: One approach is to alter the architecture of crop lighting so that all photosynthetic surfaces within crop stands are equally targeted at all times; a second approach is to use relatively low power so that LED emitters can be positioned close to plant tissues without sacrificing irradiance incident upon those tissues or causing thermal stress; still another approach involves remotely sensing the position of growing plant tissues and selectively switching LED light engines on or off automatically to avoid lighting empty space or non-productive plant tissues; yet another approach uses only the narrow-spectrum radiation of selected LEDs that matches the most efficient absorption of light by crop plants. These approaches are being combined to develop an optimum lighting system with promise to significantly lower the ESM for electric lighting of crops in an ALS.

Cary A. Mitchell
Crop/Biomass Focus Area Lead

Principal Investigator: Cary A. Mitchell, PhD

BACKGROUND

Lighting for crops that space crews could depend on for nutrition and oxygen is problematic. To directly irradiate crops on the moon or Mars with solar photosynthetically active radiation (PAR) would require inflatable surface greenhouses. Such structures would be challenged by needs for transparent films to withstand low external atmospheric pressure, large external temperature swings, strong UV-C fluxes, micrometeorite impacts, solar particle events, cosmic-galactic radiation, as well as the non-reliability of solar PAR to support plant growth. On the moon, the latter relates to extended periods of darkness; on Mars, to global dust storms of unpredictable duration, to diurnal and seasonal light cycles, and to the planet's distance from the sun. To deliver solar PAR to plants growing underground or in containment on the moon or Mars, there not only will be the same solar-availability constraints, but additional losses of transporting light *via* fiber optics or light pipes. Direct or indirect, solar radiation isn't reliable enough to be the sole or primary source of photosynthetic energy for human life support in space.

Electric lamps are a more logical primary source of light to support crop growth in space, but they, too, have their limitations. Much of the input electrical energy to lamps is lost as sensible heat and/or long-wave radiation that must be rejected from a crop-production unit to maintain thermal control. The quantum efficiency of converting broad-band radiation absorbed by leaf tissue to the chemical energy of carbohydrate also is low. When overhead light is incident on crop stands, factors such as plant-to-plant competition and mutual shading of layered leaves also enter the picture, and crop quantum efficiency can be dismal. When crops are young and individual plants are widely spaced, photons falling between plants are wasted and crop quantum efficiencies can be less than 1%. As crop stands mature and leaf canopies close to overhead light penetration, the top layer of leaves has to do all photosynthetic work for the entire canopy. To get the most out of that unsatisfactory situation, over-saturating levels of light typically are applied, which results in greater inefficiency and even more thermal burden. This, in turn, requires the use of lamp types such as high-intensity discharge (HID) lamps, which have very hot PAR-emitting surfaces, requiring a separation of lamps from plants and/or a thermal barrier inserted between them. Thus, the tradeoffs for delivering PAR reliably at a space base creates an energy monster that has scared off NASA from closing the life-support food loop during early exploration missions to planetary destinations

PROJECT GOALS AND OBJECTIVES

- Develop a reliable crop-lighting system for space that avoids the main disadvantages of traditional plant-growth lamps and reduces the ESM for crop production enough that NASA can consider putting plants back into near-term exploration missions.
- Avoid tissue damage by adopting solid-state, light-emitting-diode (LED) arrays that do not have hot light-emitting surfaces and which can be placed very close to plant tissues while maintaining PAR irradiance.
- Reduce the thermal burden of plant-growth lighting by operating the PAR sources at low power.
- Achieve uniform light distribution and maximize PAR absorption within crop canopies by arraying LEDs within or above crop stands so that all photosynthetic tissues are uniformly irradiated.
- Improve the quantum efficiency of absorbed light by using only wavelengths coinciding with maximum light absorption by photosynthetic pigments.
- Selectively target photons to be incident only upon photosynthetic tissue.

RESEARCH PROGRESS

LED Lighting Project: Trials 1-5. Since delivery of the first reconfigurable LED lighting array from Orbitec in mid August, 2004, we have run four hardware tests in the intracanopy (IC) configuration using the dry-bean crop cowpea *(Vigna unguiculata (L.) Walp. IT87D-941-1*). Tests ran for approximately 30 days each, and incremental improvements in crop response were made for each new trial as optimization of input parameters occurred. Plant spacing, plant density, electrical power level, red:blue ratio, reflective curtains, and thigmostimulation were parameters that were varied to evoke marked increases in biomass accumulation with each subsequent trial.

Table 1. Comparison of methods used in trials 1 – 4.

Methods	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**
Number of plants	12	14	14	26
Direct seeding / transplanting	Direct	Transplanted	Transplanted	Transplanted
Spacing	Uniform	Offset	Offset	Offset
Reflective curtains / floor / ceiling	No	No	Yes	Yes
Touch Stimulation	No	No	Plants 1-7	Yes - all
CO_2 Supplementation (ppm)	**500→1000** @ 21 DAP (9 engines)	**500 continuously**	**500→1000** @ 7 DAP (4 engines) **→1200** @ 28 DAP (13 engines)	**500→1000** @ 7 DAP (6 engines) **→1200** @ 21 DAP (14 engines)
Duration	31 days	19 days	32 days	32 days

As the crop stand grew in height during trials 1- 4, an increasing number of light engines were switched on along LED strips from the bottom up. In trials 3 and 4, a reflective false ceiling was installed in the growth compartment and raised incrementally to keep pace with increases in crop height and the number of engines energized. Daily power usage, therefore, increased as the experiment progressed. Trial 5 was performed with the lights reconfigured into an overhead, planar array. The growth conditions for trial 5 were identical to those of trial 4, with the exception of the lighting position and the daily power usage. The average daily power level for trial 4 was calculated, and this was used as the daily power level for trial 5, which corresponded to 55.2% of maximum power or a constant PAR of ~190µmols/m^2/s at 2.5 cm below the lights. Figure 1 shows a comparison of the trials.

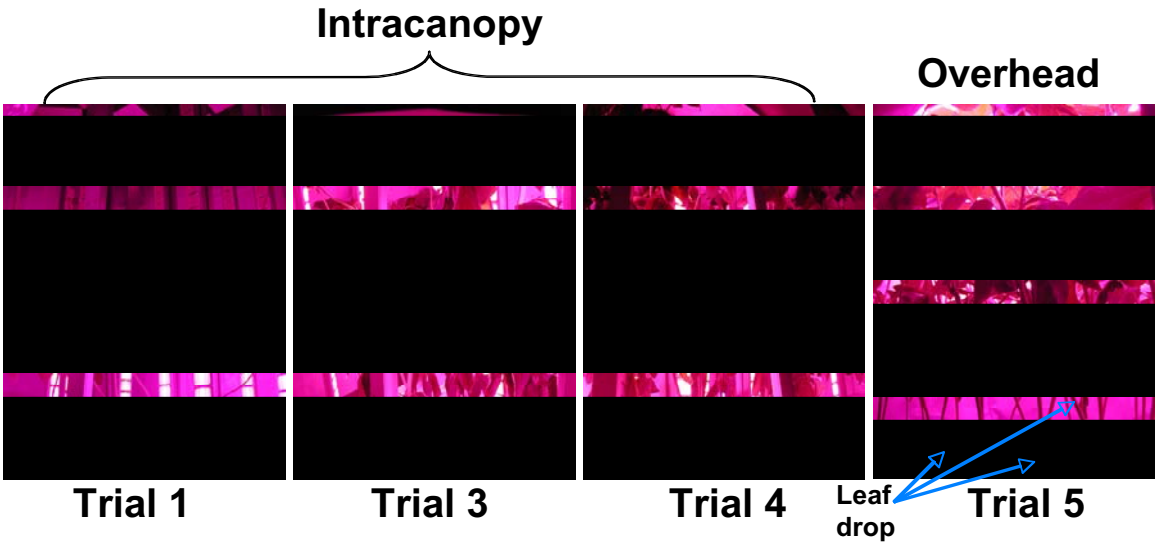


Fig. 1 Crop stands from trials 1, 3, 4, and 5 at harvest. Trial 2 was not shown since it was much shorter in duration, but it looked similar to the photo of trial 1 at harvest.

For all trials, photographic data of plant-growth characteristics, fresh and dry weights of stems, roots, leaves, leaf areas, and stem internode lengths were collected upon harvest. From the photographs it is clear that, in the overhead configuration (trial 5), lower leaf senescence occurred (see fig 1), which does not occur with the lights in an intracanopy configuration. We believe that this leaf drop is due to the low light levels occurring within a closed canopy, as mutual shading occurs from overhead leaves. When PAR levels were measured at the bottom of a mature cowpea canopy in trial 5, light was virtually undetectable (PAR $\leq$ 2µmols/m^2/s). Table 2 shows a summary of the dry weight data comparing the five trials. Trial 5, under the same environmental conditions as trial 4, showed reduced productivity, 10.9 gDW/m^2/day vs. 13.0 g/m^2/day for trial 4. These data for trial five include the dry weight of leaves dropped from the canopy. When these additional leaves are removed (9.1g), indicating edible plant matter (for cowpea), the differences between the two trials are even more marked (9.7g/m^2/day vs. 13.0g/m^2/day). These data indicate that intracanopy lighting successfully applies light only to the plants, and uses more of the plant's photosynthetic capacity. Maintaining lower leaves in the stands grown with IC lighting indicates that those resources invested on the crop are not wasted, as in the overhead lighting configuration that leads to lower-leaf shading and leaf drop.

Table 2. A comparison of the productivity obtained from trials 1 – 5. Numbers in parenthesis in trial 5 indicate edible biomass plus dropped leaves.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Total gDW	6.8	5.5	62.4	96.4	72.0 (81.1)
Average gDW / plant	0.6	0.4	4.5	3.7	2.8 (3.1)
gDW/kW-h	0.2	0.2	0.9	1.0	0.7 (0.8)
gDW/m²	30.9	23.8	271.1	415.4	310.3 (349.4)

gDW/m²/day	1.2	1.3	8.5	13.0	9.7 (10.9)
gDW/m³	60.9	88.2	580.9	718.8	537.9 (605.6)

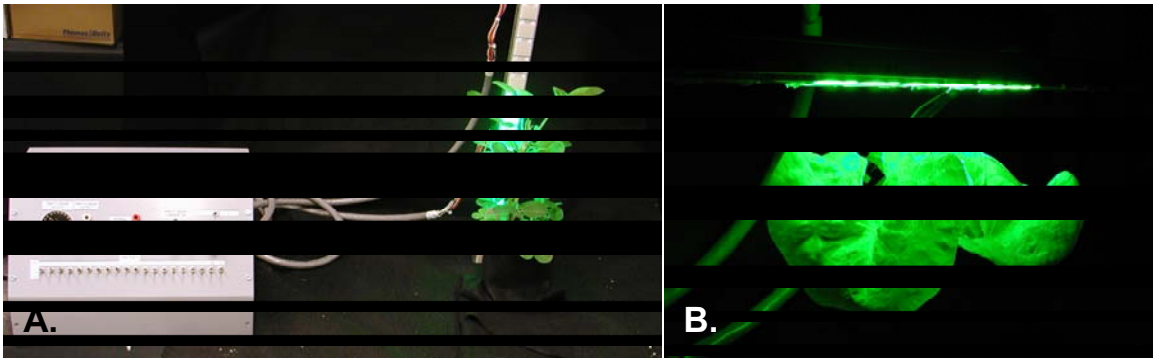
Trial 6 is underway with the lighting array in an overhead configuration. This trial differs from trial five by varying the light level over the duration of the trial. Although all light engines will be energized, the light level will increase as the plants grow in the same manner as in trial 4.

A second reconfigurable lighting array, modified to allow half of the lightsicles to nestle under the others to provide more flexibility in configurations and closer spacing if necessary, will arrive in early October of 2005. Hardware delivery was delayed to allow Orbitec to correct an anomaly found in the first array. Both arrays will therefore be fully functional, with light outputs from a light engine independent of the number of other engines energized. Side-by-side experiments will be conducted comparing simultaneous overhead and intracanopy lighting. These experiments will rigorously test our hypothesis that intracanopy lighting increases crop yield and reduces ESM compared to an overhead lighting configuration for planophile crops.

LED Lighting Project: HELIAC. In January, 2005, Orbitec was awarded a Phase 1 SBIR for the development of "High Efficiency Lighting with Integrated Adaptive Control" or HELIAC, with the Purdue NSCORT as a sub-contractor. A hardware prototype-testing unit was developed at Orbitec in consultation with the crops focus area, to initially apply manual control, and later the option for set programs. The programs include patterns of energizing green LEDs mounted on individual light engines, with reflectance from plants in the growth compartment automatically detected by photodiodes on the unlit engines. The threshold of photodiode sensing can be set to different levels, as well as the power applied to green LEDs that flash. Also, proximity of the detector to the plant is adjustable. When reflectance above threshold is detected by a pair of photodiodes on a light engine, the LEDs on that engine will be energized upon completion of the program, indicating that a reflective object (such as a leaf) was detected in front of that particular location. The prototype was assembled on the framework of a light stick that can be used in either a horizontal or vertical position in the same way as our reconfigurable array elements. One difference for the array test elements is that only green LEDs and photodiodes were populated on the light engines, as opposed to operational engines that have these components as well as red and blue LEDs.

Upon Orbitec's completion of the prototype, it was delivered to Purdue University for plant-based testing. Over several weeks we performed tests with a variety of ALS candidate crop species, including cowpea, soybean, wheat, peanut, sweetpotato, and strawberry. Tests were performed with the test strip suspended vertically in front of a test plant or suspended horizontally overhead at different distances, different thresholds, different light levels, and using three different programmed flashing light patterns. In addition, the HELIAC device was installed in our intracanopy lighting array in place of one lightsicle during the exponential growth phase of a cowpea stand. Repeated testing occurred at different stages of crop development. The data indicated how closely the observed pattern of detection mimicked human observation. Photographic data were also recorded (see figure 2). The final SBIR report submitted to NASA by Orbitec in July, 2005 indicated the results of those trials. Application for a Phase 2 SBIR was subsequently submitted that, will, if funded, allow integration of the detection apparatus into the reconfigurable lighting array to allow IC automation of light-engine switching as well as enable close-canopy (CC) studies selectively switching on light engines in a plane spaced closely above rosette or erectophile crops. CC development requires the automated switching capacity of HELIAC in addition to the independent light-engine switching proposed in the phase 2 to keep pace with plant growth. IC

lighting will also improve dramatically in efficiency if select light engines can be turned off, and if



automation allows precise switching control.
Fig. 2 A. HELIAC prototype with vertical sensing of peanut. **B.** Horizontal sensing of lettuce.

Secondary Projects. Secondary Biomass/Crop Production projects include collaborations with other NSCORT research groups, cultivar selection trials for ALS candidate crops, and manipulation of growth habits that could lead to a reduction in ESM for various candidate crops.

Sweetpotato Cultivation Studies: Single vine training. After a series of greenhouse experiments examining effects of root restriction (container volume) and canopy size (controlled by vine pruning), we determined that sweetpotato (*Ipomea batatas* (Lam.) L. TU-82-155) storage root production was strongly inhibited by shoot pruning, stimulated by root restriction, and influenced by season. Nutritive aspects of the roots, however, were not consistently changed by any of these treatments. We then asked if vine training could be used in lieu of pruning to reduce the volume of space occupied by sweetpotato shoots. By maintaining each plant as a single vine, we were able to coil the vines around vertical cylindrical or conical frames to conserve the area required for vine growth (see figure 3). This reduces the light requirement (per m^2 and per m^3) greatly, without significantly influencing yield. Coupled with earlier studies showing that planting two vines in one pot significantly increased the per-pot yield, we are now planning to further optimize space utilization and light conservation by coiling two vines around a frame in opposite directions, essentially interweaving them. This vertical vine-training system lends itself well to intracanopy lighting strategies for this crop.

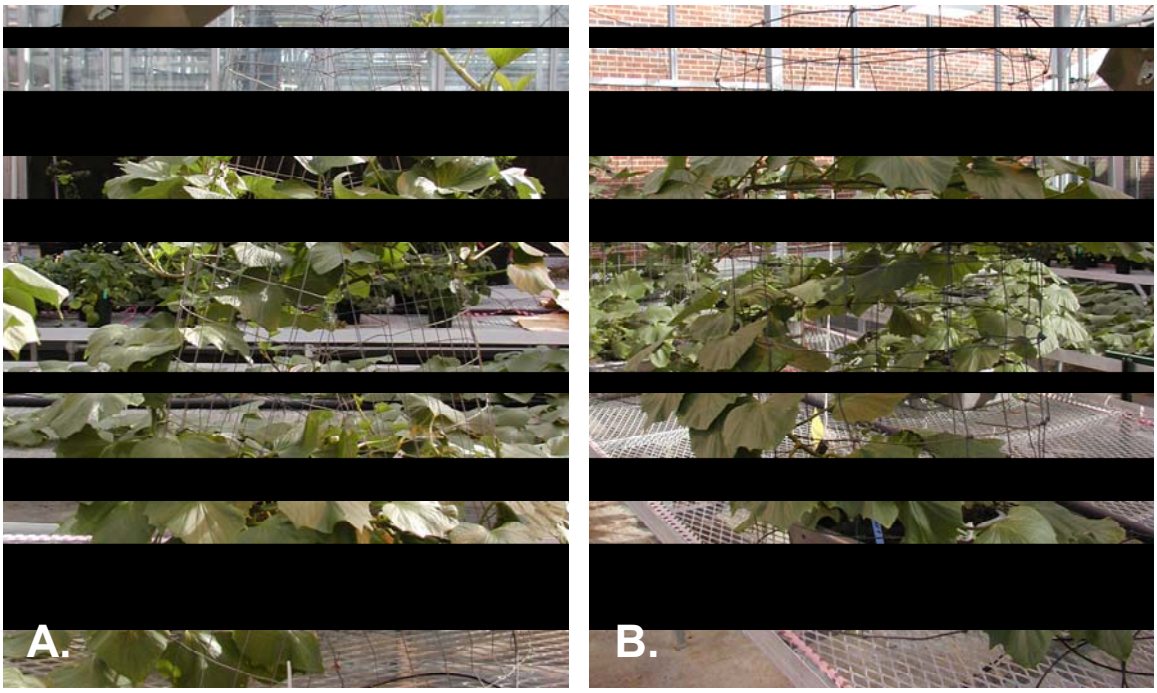


Fig. 3 A sweetpotato vine coiling around **A.** a conical frame and **B.** a cylindrical frame.

Strawberry cultivar selection and pollination: Temperature studies 1.Two plants each of four different day-neutral strawberry (Fragaria sp.L.) cultivars ('Tristar', 'Tribute', 'Seascape', and 'Fern') were grown in three reach-in growth chambers under the following temperature combinations: 15˚C days/10˚C nights, 18˚C days/13˚C nights, and 21˚C days/16˚C nights, as well as the greenhouse, which was set at 26.7˚C days and 23.9˚C nights. This trial ran from 1/26/05 to 5/12/05 and over that time, in general, the highest productivity obtained was from plants in the greenhouse. Plants in the chambers were exposed to approximately 330 µmol/m^2/s of light from fluorescent and incandescent lamps, while plants grown in the greenhouse received generally high, but variable, natural light.

Strawberry cultivar selection and pollination: Temperature studies 2. New strawberry plants were ordered and started (6/2/05) under four different growth conditions. Five cultivars ('Tribute', 'Tristar', 'Seascape', 'Fern', 'Cavendish') were obtained by donation from the Indiana Berry and Plant Company. Plants were planted either in the greenhouse or in one of three reach-in Conviron growth chambers under three different day/night temperature (in ˚C) regimes (14 hr days, 18/10˚, 21/13˚, 24/16˚ day/night temperature, ramping RH 70% day/80% night, with incandescent and fluorescent lighting at 420 µmol/m2/s). Other plants were planted in the greenhouse. Growth chamber plants are maintained separately to prevent insect contamination and the need for pesticide application, so that fruit can be tested for flavor.

Strawberry cultivar selection and pollination: Pollination. Our previous strawberry studies involved no hand pollination and minimal flower shaking only occasionally. Although we had some decent fruit formation, we have decided to test pollination methods and do a crew time vs. yield trade-off study. All plants in the growth chambers are being hand pollinated, with pollen collected and pollination performed each Monday, Wednesday, and Friday. In the greenhouse, we have divided the plants into three groups of ten (two plants of each cultivar per group). One group remains untouched except for fruit harvesting and plant maintenance (removal of runners and dead leaves). A second group is pollinated with a brief (10 sec or less) touch of a vibrating pollination wand to each flower pedicel. The third group is hand

pollinated with pollen collected from the growth-chamber plants. Pollen is not collected from the greenhouse due to pest contamination concerns. We have a low level of insect pests in the greenhouse, and plants there are periodically treated with pesticides at the demands of other facility users. Thus far, there are no pests in the chambers, which means that fruit from the chambers are available for consumption, and other flavor analyses.)

pH control system. In collaboration with Dr. George Chiu, Moeed Mukhtar, a graduate student in Dr. Chiu's lab, has developed a novel pH control system that is reaction invariant. This system has been fully tested without plants, and plant-based-testing is commencing in a recently refurbished EGC walk-in growth chamber.

Outreach.
A tour was given to a group of Indiana artists interested in agriculture in Oct., 2004. This was part of a program organized by Agriculture Communications, and the artists were fascinated with the work being done in the ALS NSCORT. Additionally, a group of students and observers involved in the Biomass Production in Education program visited and toured facilities as part of the BPES symposium in Dec., 2004.

The crops focus-area group also spoke with a group of chemistry education software designers and artists regarding requirements for a self-sustaining life-support system in June, 2005. This consultation pertained to the design of a game to teach chemistry to high school students. The virtual base will be located on earth underground and will mimic a life-support system for the moon or mars.

The ALS NSCORT worked with the Purdue Agriculture Communications Exhibit Shop to develop an exhibit "Mission to Mars" that was located at the Indiana Sate Fair in Aug, 2005. In addition to contributing text, pictures, and layout advice for the exhibit, through a series of meetings and frequent communications, the crops focus area also developed, tested, and delivered an inexpensive hydroponic system as an integral part of the display. The hydroponic system is designed to be built by students, and in the exhibit it was planted with 'Microtina' tomatoes and cuttings of basil. This system was transparent but contained, so that all working components could be viewed easily but not disturbed by the public.

FUTURE RESEARCH DIRECTIONS

- Run simultaneous intracanopy vs. overhead lighting studies.
- Use potato with LED IC lighting systems to compare biomass accumulation with previous BPC studies conducted at the Kennedy Space Center.
- Construct gas-exchange cuvette for intracanopy lighting system – do real-time photosynthesis analyses at different light and CO_2 levels, etc.
- Development of hardware and software capabilities outlined in the HELIAC phase 2 application, especially development of the capacity for independent light-engine switching that will allow for more energy-efficient IC crop production as well as for CC crop growth.
- Collaboration with Dr. Lisa Mauer and her Food Science students to grow and analyze multiple cultivars of several different crops (strawberry, peanut, carrot, tomato) to compare antioxidant levels under different environmental and cultural conditions.
- Compare yield of hydroponic leaf lettuce grown either with automated, self-adjusting pH control or pH control readjusted to set-point either daily, every other day, or every fourth day.
- Conclusion of sweetpotato vine-training exercise – space optimization attempts
- Strawberry pollination conclusions with crew-time analyses
- Peanut cultivation system – test for ever-bearing peanut cultivation practices.

TRAINEES

Postdoc: Gioia Massa

Undergraduate Students: Mercedes Mick, Rick Kennedy, Shanwen Chen, Craig Schluttenhofer, and Jessica Rombach

RESEARCH COLLABORATIONS

- Collaboration with Orbital Technologies Corp, Madison, WI, on NASA SBIR phase I project to collect data on photodiode sensing of plant position to automate light-engine switching of LEDs along HELIAC strips.
- Collaboration with Orbitec to design improvements into the second LED lightsicle array manufactured for NSCORT by Orbitec.
- Collaboration with Desmond Mortley at Tuskegee University and Lisa Mauer in Food Science at Purdue on effects of vine pruning/defoliation/training practices on proximate composition and antioxidant content in sweetpotato tuberous roots.
- Collaboration with Lisa Mauer's Food Science laboratory to grow 'Apogee' and 'Perigee" dwarf wheat varieties for stored-food-irradiation studies.
- Collaboration with Bruce Applegate's Purdue Food Science laboratory to investigate bacteriostatic effects of cowpea root exudates in hydroponic nutrient solutions.
- Collaboration with Caula Beyl's Alabama A & M's laboratory to investigate the ability of white-rot fungi to degrade crop residues while producing exotic edible mushrooms.
- Collaboration with Paul Brown's Purdue Aquaculture laboratory to investigate the digestibility of crop residues by Tilapia fish and the production of edible fish protein.
- Collaboration with Al Heber's Ag & Bioengineering laboratory to explore the suitability of different crop residues as biofilters.
- Collaboration with George Chiu's Purdue Mechanical Engineering laboratory to develop and test a novel automated pH control system for hydroponic crop culture.
- Collaboration with Joe Pekny's Purdue Chemical Engineering laboratory to calculate ESM for biomass production in ALS incorporating NSCORT lighting technology.

PUBLICATIONS

Massa GD, Emmerich JC, Mick ME, Kennedy RJ, Morrow RC and CA Mitchell (2005). "Development and testing of an efficient LED intracanopy lighting design for minimizing equivalent system mass in an advanced life-support system." *Gravitational and Space Biology Bulletin* 18(2): 87-88

Massa GD, Mick ME, Weiss I, Montgomery JA, Mortley DG, Mauer LJ and C A Mitchell (2005) "Effects of root-zone volume, vine pruning, and season on yield, proximate composition, and antioxidant capacity of sweetpotato (Ipomea batatas (Lam.) L. TU-82-155).", *International Conference on Environmental Systems*, Paper #2005-01-2816.

Massa GD, Emmerich JC, Morrow RC and CA Mitchell (2005). "Development of a Reconfigurable LED Plant-growth Lighting System for Equivalent System Mass Reduction in an ALS." *International Conference on Environmental Systems*, Paper #2005-01-2955.

PRESENTATIONS AND RELEVANT ACTIVITIES

- Cary Mitchell presented "Artificial closed ecosystems for human habitation of space." Oct. 30, 2004, University of Seoul, South Korea.

- Gioia Massa presented a poster entitled “Development and testing of an efficient LED intracanopy lighting design for minimizing ESM in an ALS.” American Society for Gravitational and Space Biology, Nov. 11, Brooklyn, NY.
- Cary Mitchell served on the NASA plant peer review panel, Jan. 27-28, Washington, DC.
- Cary Mitchell, Gioia Massa, and Yang Yang presented crops group activities to the annual meeting of controlled environment research, NCR-101, March 12-14, Tucson, AZ.
- Cary Mitchell also served on two National Academy of Science panels:
- Panel D NAS roadmap assessment for NASA Human Health & Support Systems, March 17-18, Washington, DC.
- Future of Biosphere 2 facility workshop on March 22-23, Washington, DC.
- Cary Mitchell gave an invited presentation entitled “Artificial closed ecosystems for human habitation of space” to the University Industry Consortium on April 14, Huntsville, AL.
- Gioia Massa presented a seminar entitled “Development of a reconfigurable LED plant-growth lighting system to reduce equivalent system mass in an ALS” on April 27, Space Life Sciences Laboratory at Kennedy Space Center.
- Cary Mitchell and Gioia Massa spoke with a group of chemistry education software designers and artists regarding the requirements for a self-sustaining life-support system, June 21, Purdue University.
- Cary Mitchell chaired Session 35 – Biomass Production – at the International Conference on Environmental Systems**,** (ICES), July 11, Rome, Italy.
- Gioia Massa presented “Effects of root-zone volume, vine pruning, and season on yield, proximate composition, and antioxidant capacity of sweetpotato (*Ipomea batatas* (Lam.) L. TU-82-155)” at ICES on July 11, Rome, Italy.
- Gioia Massa presented “Development of a Reconfigurable LED Plant-growth Lighting System for Equivalent System Mass Reduction in an ALS“ at ICES on July 11-12, Rome, Italy.
- Cary Mitchell will present a seminar entitled “Strategies for Minimizing the Energetic Penalties of Maintaining Human Presence at an Extended-Stay Mars Base" for the Miami University Biology Seminar, Oct. 12, Miami University, OH.
- Gioia Massa has submitted an abstract for a talk entitled “Crops on the Moon and Mars: An Energy-Saving Approach to Optimizing Biomass Production Using Novel LED Lighting Strategies.” American Society for Gravitational and Space Biology, Nov. 2, Reno, NV.
- Gioia Massa has submitted an abstract for a talk entitled “Development of an efficient, reconfigurable LED lighting system for plant growth in an ALS” for the Habitation 2006 Conference, Feb. 5-8, Orlando, FL.
- Cary Mitchell has submitted an abstract for a talk entitled “Legends of ALS: Myths, Half-Truths, or Truths?” for the Habitation 2006, Feb. 5-8, Orlando, FL.

EXTENDING CROP HARVEST INDEX USING EDIBLE FUNGI

Principal Investigator Dr. Caula Beyl, PhD., Professor, Department of Soil and Plant Science, Alabama A & M University

Co-Investigators
Dr. L.M. Nyochembeng
Dr. R.P. Pacumbaba

Background

Current goals of space exploration are predicated upon long-term, manned space flights and colonization of planetary habitats such as that on Mars. Long periods in space without payloads of necessary items from Earth require the development of a self-sustaining ecosystem that will allow astronauts to grow their own food and efficiently recycle the waste products, thus minimizing mass and eliminating costly replenishment of supplies. Edible fungal species have been used to degrade lignocellulosic material in plant tissue, converting it directly into fungal protein suitable for human consumption. Lignin, the most recalcitrant component of the plant cell wall to degrade, is susceptible to degradation by manganese peroxidase, laccase, lignin peroxidase including other oxidases, and hydrolytic enzymes produced by edible white rot fungi such as *Pleurotus ostreatus* (Breen and Singleton, 1999; Hatakka, 1994). Optimal and rapid biodegradation of lignocellulosic material of crop residues by candidate edible white rot fungal strains is paramount in the use of these organisms to achieve effective biomass recycling in an advanced life support system (ALS). Incorporating organic N into the substrate, and use of effective composting/cropping methods under favorable environmental conditions, may enhance growth and fruiting of the edible fungi thereby increasing the rate of biodegradation of inedible crop residues and biomass recycling.

Project Goals

Our goal is to achieve effective low cost biomass degradation and recycling using edible white rot fungi with ultimate production of an edible product (mushrooms) that will add diversity to the restrictive diet of the astronaut. Edible mushrooms therefore are a vital component of sustainability within the Advanced Life Support (ALS) system configuration.

Objectives

The objectives of our effort are to optimize biodegradation of inedible crop residues and production of mushrooms, to evaluate and select most efficient species and strains of edible white rot fungi, determine most efficient cropping patterns, nutrient requirements, amount of light, temperature, CO_2, O_2 and humidity required for optimal fungal colonization and fruiting.

Research Progress

Pairing crop residue to enhance fungal growth and biodegradation of recalcitrant lignocellulosic waste

Previously, it was shown that pairing sweet potato or basil with wheat or rice straw improved mycelial growth and degradation of both sweet potato and basil. The goal of this study was to optimize white rot fungal growth and biodegradation of sweet potato and basil crop residues through pairing with wheat or rice straw. Our objective was to determine the best ratios in the crop pairing that would support optimal edible fungal growth and maximize degradation of the difficult crop residue in the mixture. Edible fungal strains of two species - *P. ostreatus* ('Pohu', 'Grey Dover') and *P. eryngii,* maintained as mycelial spawns on culture media in Petri dishes, at room temperature (23±2°C) in the laboratory were used. Rice straw, wheat straw, sweet potato, and basil were milled to pass through a 2mm sieve. The milled residues were weighed, placed in 750 ml food containers and thoroughly mixed by stirring with a glass rod. Sweet potato residue was combined with wheat or rice straw at various ratios (0:100, 20:80, 40:60, 60:40, 80:20, 100:0 [w/w]), and basil with rice straw at similar ratios to obtain a final weight of 80g per container. The substrate combinations were sufficiently moistened by adding 125ml distilled deionized water to each container. All cultures were autoclaved at 121°C and 1.1kgm^{-2} for 30 min. The sweet potato/rice and sweet potato/wheat residues were seeded with 3x3mm agar blocks of pure cultures of *Pleurotus eryngii* or *P. ostreatus* ('Grey Dover') while the basil/rice was inoculated with *P. ostreatus* ('Pohu'). Inoculated cultures were capped and incubated in the dark for up to 21 days.

In the sweet potato/rice straw or sweet potato/wheat straw combination, a 20:80 ratio was most favorable for growth and degradation of sweet potato by *P. eryngii* (Fig.1). However, when *P. ostreatus* strain 'Grey Dover' was used, up to 60% sweet potato could be incorporated into the mixture with rice straw or 40% with wheat straw (Fig. 2) and still obtain substantial degradation. 'Grey Dover' is more efficient and more tolerant of sweet potato than other species of edible white-rot fungi (EWRF) we have tested. Pairing sweet potato or basil with wheat or rice straw helped to diminish the impact of the inhibitory compounds present in the residues to allow fungal growth and colonization of the residues. Similarly, mycelial growth and colonization of *P. ostreatus* 'Pohu' was better in a 20:80 or 40:60 combination of basil with rice straw at 14 days after inoculation (Fig. 3). Mycelial growth and colonization of recalcitrant crop residue by EWRF can be enhanced by combining the residues with wheat or rice straw at defined percentages of incorporation.

Both rice straw and wheat straw residues had promotive effects on fungal growth while alleviating the inhibitory effect of sweet potato or basil in the different substrate mixtures.

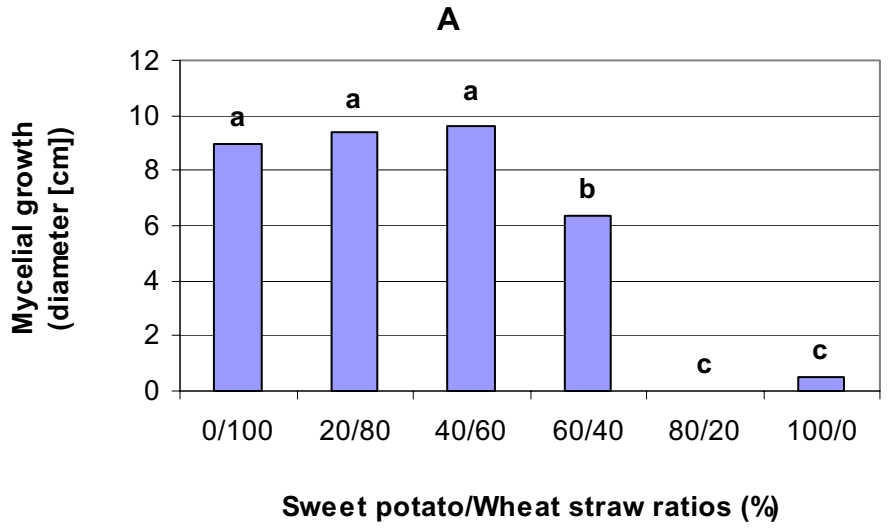


Figure 1. Mycelial growth of *P. ostreatus* 'Grey Dover' at 14days after inoculation (DAI) in sweet potato paired with wheat straw at various ratios

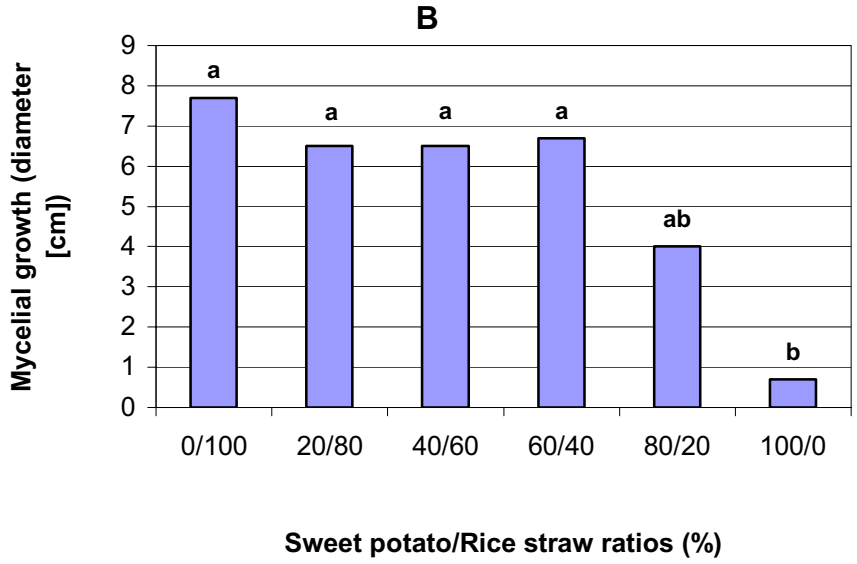


Figure 2. Mycelial growth of *P.* ostreatus 'Grey Dover' at 14 DAI in sweet potato paired with rice straw at various ratios

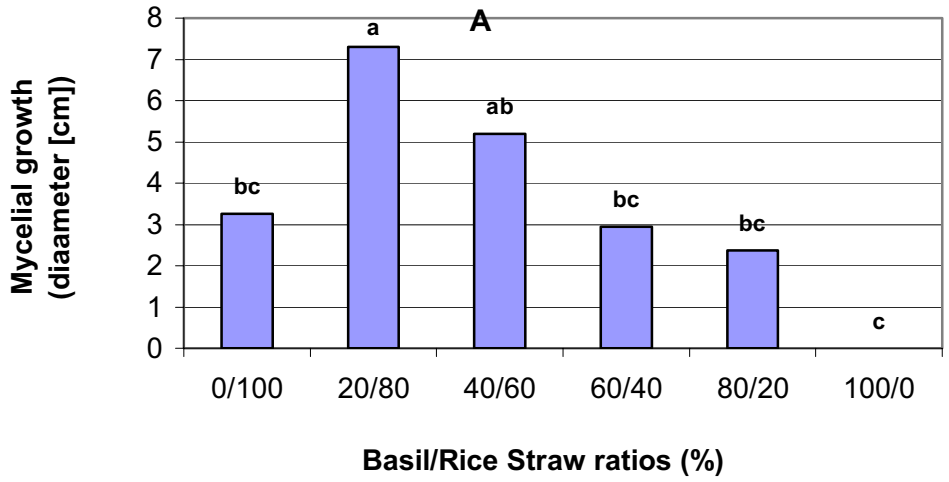


Figure 3. Mycelial growth of *P.* ostreatus 'Pohu' at 14 DAI in basil paired with rice straw at various ratios

Optimizing edible fungal growth and degradation of inedible crop residues using various cropping methods

The objective of this study was to determine the most efficient cropping pattern that supports fungal growth, and biodegradation of crop residues. Three *Pleurotus* species- *P. ostreatus* ('Grey Dover'), *P. pulmonarius,* and *P. eryngii* and two shiitake mushroom (*Lentinula edodes*) strains LE001 (ATCC #20546) and LE002 (ATCC #20635) were used in the study. Tomato residue was obtained from greenhouse grown tomatoes while straw was obtained from field-grown rice and wheat and stored air-dried in the greenhouse. Lettuce residue was obtained from growth chamber grown plants at Kennedy Space Center, FL. Cowpea, soybean, sweet potato, and basil inedible residues were provided by Dr. Cary Mitchell's lab at Purdue University. The dry residues were milled and passed through a 2mm sieve to obtain fine particle size substrates.

A. Mixed cropping

Processed crop residues (wheat, rice, soybean, cowpea, tomato, lettuce, sweet potato) were weighed and 11.5g of each crop residue were combined in single Plantcon® tissue culture containers (Sigma, St. Louis, MO) to make 80.5g of residue per container. The residues were thoroughly mixed, moistened, and autoclaved. They were seeded with three species of *Pleurotus* (*P. ostreatus* ['Grey Dover' and 'Pohu'], *P. eryngii*, *P. pulmonarius*) and four strains of *L. edodes* (LE2, LE3, LE001, LE002) and incubated in the dark at 22±2°C.

P. ostreatus strains 'Pohu' and 'Grey Dover' were the most prolific strains for mycelial growth and colonization in mixed residues (Table 1). Mixing crop residue is advantageous in that degradation can be more effective using only a few efficient strains therefore eliminating the need to screen large numbers of strains to select specific ones that degrade only certain crop residues. Furthermore, mixing crop residues helps dilute the secondary metabolites of some crop residue such as sweet potato and basil, which may be inhibitory to fungal growth, while gaining additional C/N from the cereals/legumes residues for growth. Oyster mushroom strains were better than shiitake strains under mixed cropping patterns.

Table 1. Mycelial growth and colonization of mixed crop residues by edible white rot fungal species

Species/Strain	Mycelial growth and colonization	
	Diameter of surface mycelial growth (cm)	Depth of growth in substrate (cm)
P. eryngii	3.42 c	0.45 d
P. pulmonarius	6.20 b	2.50 b
P. ostreatus 'Grey Dover'	6.76 ab	3.36 a
P. ostreatus 'Pohu'	7.35 a	3.25 a
L. edodes		
LE002	6.38 b	2.0 c
LE001	2.80 cd	0.0 e
LE2	2.31 d	0.0 e
LE3	2.40 d	0.0 e

B. Co-Culture

Fungal co-culture was used to verify if there were synergistic colonization and degradation of crop residue, or antagonistic activity by two prolific species of *Pleurotus*. *Pleurotus ostreatus* 'Grey Dover' and *P. pulmonarius* were cultured on wheat straw amended with food waste at 0, 20, 40 60, 80 and 100% (v/v). Agar blocks containing the pure fungi were placed side by side at the center of the substrate. The diameter of the circle formed by outgrowing mycelia from both strains was used to determine growth, while recognizable basidiocarps were counted as fruit bodies.

Co-culturing *P. ostreatus* 'Grey Dover' and *P. pulmonarius* enhanced mycelial growth and colonization, and basidiocarp production, compared to composting with single species (Table 2). However, the rate of degradation as measured by degradation efficiency index (DEI), depended on the concentration of organic nutrients in the compost and not the strains used. Food waste amendment did not improve degradation of wheat straw (Table 3). Food waste concentration of 40% and above significantly reduced degradation capability of the oyster mushroom species. Co-cropping fungal species had a synergistic effect on fungal growth and fruiting. Use of efficient cropping methods may enhance fungal growth and fruiting, thus increase the rate of biodegradation of the substrates and efficiency of biomass recycling.

Table 2. Mycelial growth and basidiocarp production in single and co-cropped species of *Pleurotus* on food waste amended wheat straw

Species	Number of basidiocarps	Radial mycelial growth (diameter, cm)
P. ostreatus 'Grey Dover	5.0 b	4.75 b
P. pulmonarius	29.5 a	4.5 b
P. ostreatus + *P. pulmonarius*	25.2 a	5.5 a

Table 3. Effect of concentration of food waste amendment in wheat straw on biodegradation of the crop residue colonized by two oyster mushroom species for 60 days

Food waste (%)	Dry weight residual (g)	DEI [*] (%)
0	34.4 c	42.6
20	36.4 bc	39.3
40	39.5 b	34.1
60	45.2 a	24.6
80	41.0 ab	31.6
100	44.7 a	25.5

[*] The difference between the initial and final dry weights of residue, divided by the initial dry weight, multiplied by 100.

Nitrogen amendment enhances edible white-rot fungal growth and biodegradation of containerized inedible crop residues

The objective of this study was to evaluate the contribution of nitrogen amendment to mycelial growth, fruiting and degradation efficiency of strains of white rot fungi. Furthermore, to increase fruiting and

expedite fungal colonization and biodegradation of crop residues, wheat straw was amended with food waste as a source of nitrogen. Food waste was obtained from Dr. Jim Alleman's laboratory at Purdue University, and kept at room temperature until use. Edible fungal strains of three species - *P. ostreatus* ('Grey Dover' 'Blue Dolphin'), *P. eryngii*, and *P. pulmonarius*, chosen based on their ability to grow and rapidly colonize several substrates such as wheat, rice, etc. were cultured in YMVSA amended with food waste at 0, 20, 30, 40 and 50 % (v/v), to screen for species tolerating high concentrations of food waste. Two species that exhibited rapid growth in media containing 50% food waste were used further to study mycelial growth, fruiting and degradation of wheat straw amended with various food waste concentrations. Food waste slurry was diluted with distilled, deionized water to 6 concentrations [0, 20, 40, 60, 80 and 100% (v/v)] that were each added to milled wheat straw at 94ml per 60g wheat residue per container. Each treatment concentration was replicated five times. The mixed residue was steam sterilized at 121°C and 1.1kgm^{-2} for 40 min. Cultures were seeded with 3x3mm agar blocks of pure cultures of either *Pleurotus ostreatus* (Grey Dover), or *Pleurotus pulmonarius* under aseptic conditions. The inoculated cultures were placed at 22±2°C and 8 hours light for growth and fruiting.

Of the three oyster mushroom species *P. ostreatus* 'Grey Dover', *P. pulmonarius*, and *P. eryngii*, *P. ostreatus* and *P. pulmonarius* had similar mycelial growth rates which were significantly greater than that of *P. eryngii* (Fig. 4). Growth of all species decreased with increased concentration of food waste supplemented in the YMVSA medium (Fig. 5). This decrease in growth could be caused by the increase in pH of the amended media to about 7.4. The optimum pH range for *Pleurotus* is about 5.5-6.5.

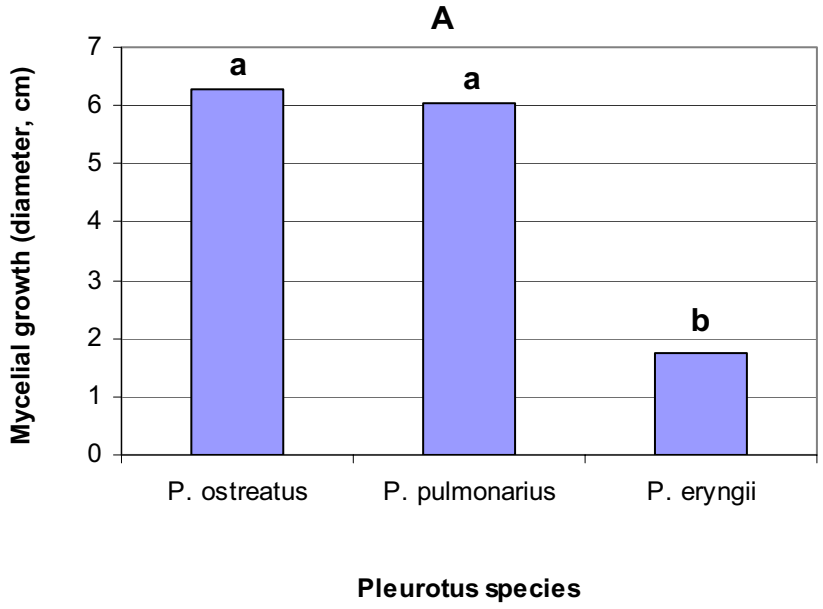


Figure 4. Mycelial growth of three oyster mushroom species in food waste amended YMVSA culture media at 7 days after inoculation (DAI)

When cultured individually, *Pleurotus* species were most efficient in biodegradation when food waste was supplemented at 80% (v/v) in wheat straw. However, in the dual cultures, addition of food waste to wheat straw did not improve degradation efficiency (Table 4.).

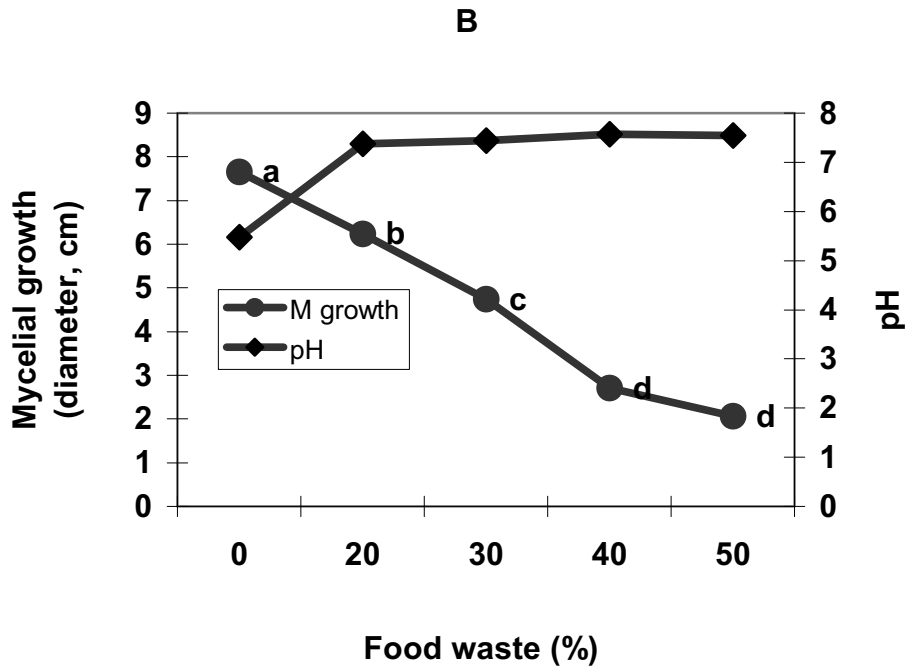


Figure 5. pH of food waste amended YMVSA culture media and mycelial growth of three *Pleurotus* species at different food waste concentrations

Similarly, food waste amendment did not significantly improve mycelial growth in both strains compared to the control. It is thought that lignocellulosic wastes contain low amount of available N (Silva et al., 2005). Therefore, N addition may be necessary to increase fungal growth and degradation of the substrate. However, N addition is not always beneficial, for example, Buswell et al. (1995) found that N suppressed Mn-dependent peroxidase (MnP) production in *L. edodes*.

Table. 4. Performance of two species of oyster mushroom on biodegradation of food waste amended wheat straw

Food waste (%)	Dry weight (DW [g]) and degradation efficiency index (DEI [%])					
	P. ostreatus (PO)		*P. pulmonarius* (PP)		PO + PP	
	DW	DEI	DW	DEI	DW	DEI
0	38.2 bc	36.3	44.2 ab	26.3	34.4 c	42.6
20	44.1 ab	26.5	48.2 a	19.6	36.4 bc	39.3
40	42.4 abc	29.3	45.1 ab	24.8	39.5 b	34.1
60	43.0 ab	28.3	39.8 ab	33.6	45.2 a	24.6
80	35.7 c	40.5	39.2 b	34.6	41.0 ab	31.6
100	45.7 a	23.8	42.1 ab	29.8	44.7 a	25.5

Future Research Directions

Determination of the effect of light duration and temperature on a) growth of mycelium, b) appearance of fruiting initials, c) development of edible mushrooms, and d) degradation efficiency for Pleurotus and the other species will be the next most immediate focus.

Research Collaboration

Collaborations with Dr. Paul Brown- of Purdue University, is ongoing. We furnish Dr. Paul Brown with fungal predigested composted inedible plant biomass including pure cultures of *Pleurotus spp*. for his fish project.

Collaboration was also initiated with Drs. Barrett Vaughn and Desmond Mortley, of CFESH at Tuskegee University on the use of the OXY-MAX composting equipment for measurement of respiration (CO_2 monitoring) and water vapor dynamics for various strains of white rot fungi grown on different substrates and/or combinations.

We have also interacted with the Space and Rocket Center in Huntsville in a) design and development of their new space agriculture display area and b) in education of 4-H instructors and students on the role of edible white rot fungi in advanced life support systems.

Publications and Presentations To-Date

Nyochembeng, L.M. and C.A. Beyl. (2004). “Growth Response of Edible Fungi on Processed Crop Biomass and Food Waste Amended Rice Straw”. Oral presentation. ALS/NSCORT Summer Research Symposium, Purdue University, August 3, 2004.

Nyochembeng, L.M., C.A. Beyl and R.P. Pacumbaba. (2004). “Enhancing edible white rot fungal degradation and recycling of solid wastes by incorporation of urea and STAR effluent”. NASA ALS/NSCORT EAC meeting, Howard University Nov. 18, 2004

Nyochembeng, L.M., C.A. Beyl and R.P. Pacumbaba. (2005). “Factors essential for optimizing solid waste degradation and recycling using edible white rot fungi”. SAE Transactions (accepted)

Nyochembeng, L.M., C.A. Beyl and R.P. Pacumbaba. (2005). “Edible fungal growth and fruiting on composted containerized inedible crop biomass”. Poster presentation at ASHS annual meeting held in Las Vegas, NV, July 18-21, 2005.

Nyochembeng, L.M. and C.A. Beyl. (2005). “Use of edible white rot fungi to enhance cellulose, hemicellulose and lignin degradation of crop residues”. Seminar given to faculty and students of the College of Agricultural, Natural and Environmental Science at Tuskegee University, March 30-31, 2005.

Nyochembeng, L.M. and C.A. Beyl. (2005). “Using edible fungi to break down inedible crop residues on long-term space missions.” Oral presentation at the University and Industry Consortium (UIC) 2005 spring meeting held in Huntsville, AL, on April 13-14 2005.

References

Breen, A. and F.L. Singleton. 1999. Fungi in lignocellulose breakdown and biopulping. Current Opinion in Biotechnology 10:252-258

Buswell, J.A., Y.J. Cai, S.T. Chang, J.F. Peberdy, S.Y. Fu, and H.S. Yu. 1996. Lignocellulolytic enzyme profiles of edible mushroom fungi. World Journal of Microbiology and Biotechnology 12:537-542.

Buswell, J.A. Cai, Y, and S. Chang. 1995. Effect of nutrient and manganese on manganese peroxidase and laccase production by *Lentinula edodes*. Fems Microbiol Lett. 128:81-88

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi:production and role in lignin degradation. Fems Microbiol Rev. 13:125-135

Pacumbaba, R. P., Beyl, Caula A., and Pacumbaba, R. O., Jr. 1999. Shiitake mycelial leachate suppresses growth of some bacterial species and symptoms of bacterial wilt of tomato and lima bean in vitro. Plant Disease 83:20-23.

Sarikaya, A. and M.R. Ladisch. 1997. An unstructured mathematical model for growth of *Pleurotus ostreatus* on lignocellulose material in solid state fermentation systems. Applied Biochem. Biotechnol. 62:71-85.

Silva, E.M., A. Machuca, and A.M. F. Milagres. 2005. Effect of cereal brans on *Lentinula edodes* growth and enzyme activities during cultivation on forestry waste. Lett. Applied Microbiol 40:283-288.

Executive Summary – Food Safety and Processing

The food subsystem is an integral part of ALS. Purdue and Alabama A&M Universities are continuing to address key components of food processing, food safety, energy requirements, and the effect of long term exposure of food to radiation. It is also important to consider that the research, although space based, has terrestrial benefits as well. Research to understand the affects of radiation has begun using various food stuffs. Experiments have shown that there is a dose dependant relationship in the decrease of products of primary lipid oxidation and an increase in the secondary products. Preliminary results also showed negative affects on the antioxidant capacity ascorbic acid and the wheat cultivars Apogee and Perigee while terrestrial cultivars were not affected. Packaging scenarios have also been investigate to examine potentials for reduction of waste including biodegradable packaging demonstrating areas in which significant mass could be eliminated. To ensure food safety and shelf life from microbial contamination of potential salad crops research is being performed to examine the effect of various forms of physical and chemical control strategies and combinations. Synergistic effects using the combination of chemical and physical methods showed the greatest reduction in a majority of the combinations. Research is also continuing to identify potential areas of contamination and control strategies using an integrated HACCP (hazard analysis and critical control points) expanded to evaluate the ALS system as a whole for identification of key parameters and possible points of contamination from the various waste processing regimes, hydroponics, and tilapia waste water. Phage based pathogen detection has progressed from proof in principle experiments utilizing M13 bacteriophage. Significant progress is being made on the recombinant bacteriophage for pathogen detection. Recent collaboration with industry has also provided a potential significant alternative to the integrated pathogen detection food packaging format.

Bruce Applegate
Focus Area Group Leader – Food Safety and Processing

BIOAMPLIFICATION USING PHAGE DISPLAY FOR THE MULTIPLEXED DETECTION OF PATHOGENS IN POTABLE WATER AND FOOD

Principal Investigator **Dr. Bruce Applegate, PhD., Associate Professor, Department of Food Science, Purdue University**

Co-Investigators **Dr. Michael Ladisch**

Background

The research will generate and purify phages that are designed to selectively detect water and foodborne pathogens. Using specially designed markers that are inserted into the DNA of the phage, the expression of the phage can be tuned to reflect the presence (or absence) of the target cell as well as give a measure of concentration and type of cells in a given sample. Proteins displayed on the outside of the phages can be used as "handles" to distinguish one type of phage from another, much like a license plate will identify one car from another, even if the cars are alike in every way except for the owner. Since the protein is displayed on the outer surface of the phage and since it reflects a special characteristic of the DNA contained inside the phage particle, this is referred to as phage display. The proposed project combines the knowledge and experiences of fundamental molecular biology of phages and applies this knowledge to existing technologies in both the molecular construction and detection of the modified bacteriophages. The assay will provide an alternative to current culturing methodology and nucleic acid amplification technology which are time, labor, and equipment intensive. This technology platform can be integrated with antibody-based assays coupled with impedance based spectroscopy, biochips, fluorescence microscopy, and enzyme-linked immuno-assays (ELISA's) which address the diverse earthbound needs of detection methods for food and waterborne pathogens. However these technologies can require significant equipment expense and mass. Therefore a test strip format which will provide low mass and multiplexed detection of a variety of pathogens could be developed to be utilized for direct testing of potable water and food for both coliforms and pathogens. This assay could be utilized as a stand-alone kit to test potable water or in an integrated food packaging format, which will allow the testing of foods prior to their removal from the package. The potential integration of the phage bioamplification step with ELISA's will provide a sensitive and low mass approach to both water and food safety.

Project Goals and Objectives

The overall goal of this research is to harness the power of bacterial phage display to develop a biological amplifier for the detection of small numbers of pathogenic organisms in potable water and foods. The fundamental hypothesis of this research is: Bacteriophage can be genetically modified using recombinant DNA technology to produce an antigenic peptide which is only expressed in progeny phage after infection of a specific viable pathogenic organism. The hypothesis will be tested by addressing the following objectives:

1. Genetically modify and propagate modified bacteriophages for the detection of viable pathogens.
2. Recover and purify modified bacteriophages using affinity chromatography.
3. Develop an assay using the modified bacteriophages to detect pathogenic organisms in potable water and food using an ELISA format.
4. Integrate the developed bacteriophage assay into novel food packaging materials.

Cumulative Research Progress to Date

***Salmonella spp bacteriophage*:** To facilitate the construction of a modified P22 bacteriophage for *Salmonella spp.* it was necessary to construct a recombination vector for insertion of a modified tail spike protein. The vector was modified from a previously constructed vector pTP369 (Casjens et al). Plasmid pTP369 contains a 2558 bp region of the P22 genome corresponding to the region containing gene 23 (antitermination protein), gene 13 (lysis protein), gene 19 (lysozyme) gene 15 (lysis control), orf 201 (unknown protein) and orf 80 (unknown protein). The recombination vector was constructed by removing approximately 1 kb of the P22 genome in the region of orf 201 and orf 80 to allow the insertion of DNA for recombination. A multicloning site and a TA cloning site were inserted to facilitate rapid insertion of modified DNA to construct the appropriate epitope. Primers were utilized to amplify the tailspike protein from P22 with the appropriate His modifications. We are currently adding the appropriate promoter configurations to allow repressed expression of the His modified tailspike protein in the preparative host strain. The preparative host strain repressor gene cassette was constructed for insertion into the preparative host strain genome. Work is continuing on inserting the modified tailspike gene in the previously constructed P22 recombination vector. Appropriate promoter and terminator configurations were added to the modified tailspike protein to allow repression of expression of the His modified tailspike protein in the preparative host strain. Preparative host strain containing the *lacI* repressor gene cassette was constructed.

E. coli* O157:H7 bacteriophage*: Phage based detection of *E. coli* O157:H7 will be accomplished using bacteriophage ϕV10. *Bacteriophage* ϕV10 was originally isolated by R. Khakhria and has been shown to specifically infect many strains of *E.coli* O157:H7. It has a genome of approximately 42 kb and is classified as a temperate phage (can form lysogens). The phage was obtained from Dr. Rafiq Ahmed at the National Laboratory for Enteric Pathogens in Winnipeg Canada. To determine the specificity of ϕV10, a previously characterized library from pathogenic *E. coli* outbreaks was screened for ϕV10 susceptibility. Environmental isolates were also screened to provide evaluation of false positives. The assay consists of a simple plaque assay using a previously prepared phage suspension with a titer of approximately 2 x 10^{-3} plaque forming units per mL. Using this assay, 187 strains of *E. coli* were tested. Of these samples, 106 were known to be isolates of *E. coli* O157:H7 and 81 wereknown to be non-O157:H7. Results showed 93 positives and 81 negatives. The data correlated with the previous identifications. However 13 of the previously identified positives did not form plaques and were investigated further and found to be contaminants. No false positives were detected.

The complete geneome of phiV10 was sequenced to provide data which will allow a more deliberate construction of the recombinant phage avoiding the use of transposon mutagenesis. Sequence analysis determined the ΦV10 genome to be 39,104 bp long with a G+C content of 49.0%. It contains 56 proposed open reading frames (ORFs). Functions were proposed for 19 of the 56 predicted proteins. The genomic organization and hypothetical proteomic composition of ΦV10 reveal a probable common ancestry with the *Salmonella enterica* Group E1 phage ε15. Forty-one hypothetical ΦV10 proteins have homologs in ε15, thirty of which are more similar to their ε15 homologs than to any other protein. The serotype conversion genes of ε15 are absent from ΦV10. A putative acyltransferase gene occupies a position in the ΦV10 genome where serotype conversion genes are located in ε15. A 2425 bp cluster of seven ORFs located between the putative DNA replication and terminase genes has considerable sequence identity with DNA from *E. coli* O157:H7phage VT2-Sa. No known bacterial virulence genes were found in the ΦV10 genome. The apparent host specificity of ϕV10, its ability to lysogenically convert without transducing host genes, and its lack of identifiable virulence determinants suggest that ϕV10 should be useful in biotechnology and that its use in biotechnology should not constitute any undue hazard, making it a good candidate for detection assays.

A similar recombination system for insertion of foreign DNA into the phage genomes as described for P22 above was developed for the modification of the *E. coli* O157:H7 bacteriophage ΦV10. This was accomplished by isolating a ΦV10 lysogen of *E. coli* O157:H7. The lysogen was further characterized and shown to be inducible for the lytic phenotype. The strain was subsequently transformed with pKD46,which contains an arabinose inducible recombinase. This system allows the electroporation of linear DNA containing foreign genes flanked by DNA sequences from regions of the phage genome facilitating insertion of reporter genes into the phage genome. Initial work has identified regions for modification, allowing insertion of reporter genes which do not affect the phage life cycle. We are also utilizing pKD46 for analysis of the unknown open reading frames found in the genome of bacteriophage ΦV10. Key parameters were determined for optimization of the recombination system for reporter gene insertion and gene inactivation. Primers were designed to begin the systematic evaluation of the unknown open reading frames. Amplicons were constructed for the systematic disruption of identified open reading frames in *phi* V10. We are continuing the recombination and lysogen rescue experiments.

***Listeria monocytogenes* bacteriophage:** Preciaus Heard (Summer Minority Fellowship Student) began initial screening of a *Listeria monocytogenes* library to isolate a lysogenic bacteriophage. Preliminary results suggest she was successful and we are currently further evaluating the isolated bacteriophage and continuing to screen the library. Preciaus Heard has rejoined the lab and will continue the *Listeria* work for her Masters project beginning in January 2006.

Sample Preparation: Another important aspect in the long term goals of this research is the application of the multiplexed phage assay in a food packaging format. We have begun to examine key parameters to allow integration of the assay into a package format in which minimal sample preparation is essential. We used a previously developed two-component system consisting of T4 bacteriophage and bioluminescent *Escherichia coli* lux cells to evaluate the effect of food components (ground beef) or laboratory media (0.1% peptone water and LB) on phage infectivity. *E. coli* lux cells serially diluted using 0.1% peptone water, LB broth, and LB with 10% (w/v) ground beef were mixed with varying concentrations (0-8.4 log pfu/ml) of T4 bacteriophags, and phage infectivity was monitored over time using a luminometer. Bioluminescence intensities from *E. coli* lux cells (8.7 log cfu/ml) decreased over time in the presence of increasing concentrations of T4 phages with a dynamic range of 3.4-8.4 log pfu/ml in LB broth, while peptone did not exhibit any differences of bioluminescence intensities between samples. We also found that phage infectivity occurred in LB with 10% ground beef without any significant influence of food components or natural flora in ground beef. In addition, it was confirmed that 0.5% sodium chloride supplemented in LB broth was a key component affecting phage infectivity. These initial results are being pursued to develop the assay media to be incorporated into the self contained food package assay.

Packaging:

As previously mentioned above the long term goal of the project is to incorporate the phage detection assay in food packaging. The initial proposed product would contain a lateral flow assay for the multiplexed determination of multiple pathogens. We have begun collaboration with Embedded Concepts, which has patented technology which allows the embedding of antibodies into plastics. This innovation would greatly enhance the potential for integration of the phage based assay into food packaging material without the need foe lateral flow simplifying development of stand alone kits involving sample preparation and detection all in one. To evaluate the embedding process we are currently exploiting an inducible recombinant M13 with a streptavidin-binding epitope which was previously developed to demonstrate the proof in principle of the assay. Phage samples were highly

purified using detergents and dialyzed with water to reduce the presence of background particulates during imaging. Phage samples were pipetted onto commercially-available streptavidin-coated microscope slides and allowed to adhere at room temperature for 15 min. The sample was then pipetted away and the slide was washed with water to remove nonbinding phage. Samples were then analyzed for binding using Atomic Force Microscopy (AFM). . As the AFM images show, there is a large difference in the number of phage binding to the streptavidin-coated slide depending upon whether the phage expressed the modified copy of the surface protein or not. The wild-type negative control sample did not bind to the streptavidin-coated slide (Figure 1A) while the recombinant phage not expressing the modified copy of the protein (gIII) (uninduced) showed minimal binding (Fig. 1B) compared to that of the phage expressing the modified copy (Fig. 1C). AFM was utilized to provide visualization of the binding to determine the protocols for sample preparation for the analysis of the embedded streptavidin. Our goal is to use this M13 phage system in conjunction with AFM to examine both the surface density and binding activity of plastics embedded with streptavidin to provide proof in principle experiments for the assay in this format..

Future Research Directions

We are continuing the development of the various bacteriophage and are beginning initial work on lateral flow assays for the development of the kit based system for pathogen detection. However, the recent collaboration with Embedded Concepts might change the focus from a lateral flow assay to indicator spots, reducing the complexity of the assays.

Trainees:

Udit MInocha , Department of Food Science, Doctor of Philosophy (Currently deployed in Kosovo)
Melinda Shroyer; Department of Food Science, Doctor of Philosophy (expected graduation Dec. 2005).
Preciaus Heard (begins Jan. 2006)
E. Igboegwu (undergraduate researcher)

Research Collaboration:

External collaborations : Dr. Applegate in collaboration with Dr. Rafiq Ahmed at the National Laboratory for Enteric Pathogens (Winnipeg, CA) sequenced the genome of the *E. coli* O157:H7 bacteriophage *phiV10*. Project has been completed and a manuscript has been submitted.

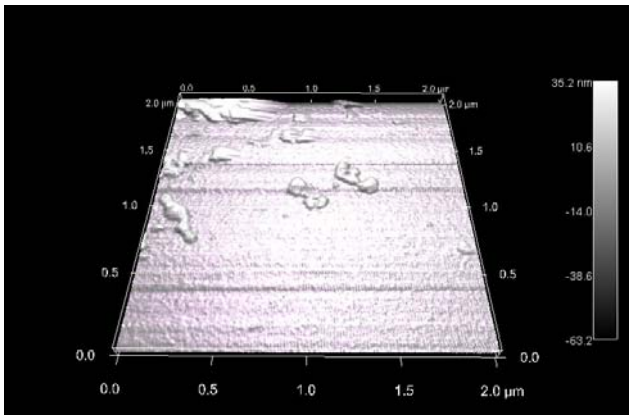
Private Industry collaborations: Dr Applegate is collaborating with Embedded Concepts on embedding antibodies in plastic films to develop an integrated food package with pathogen detection capabilities.

Internal collaboration: Dr. Applegate is also collaborating with Dr. Mauer and Dr. Williams to develop a general HACCP plan for the integrated system to identify key control points related to potential bacterial contamination in the waste/water processing system. Dr. Applegate is collaborating with Dr. David Nivens in the use of AFM to examine phage binding to embedded antibodies. . Dr. Applegate is also working with Dr. Williams providing bioluminescent pathogens for studies of control interventions for pathogen elimination.

Leveraged funding:

Dr. Applegate received a grant from the Purdue University TRASK fund to support the development of a food based assay utilizing phage based detection the synergy between the ALS NSCORT project and this effort should allow the reduction to practice and the filing of a Utility patent on the technology platform being developed in this effort.

A



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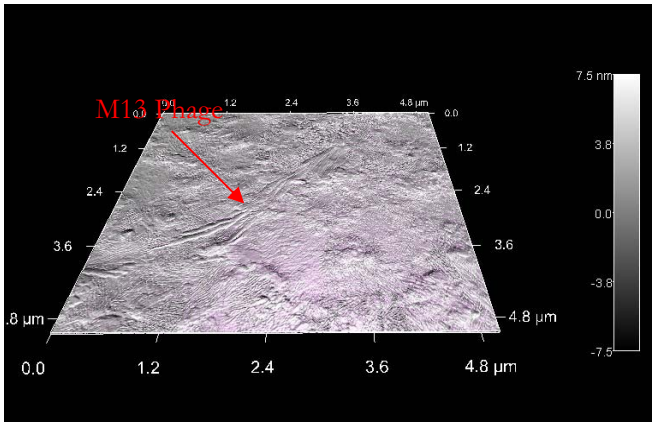

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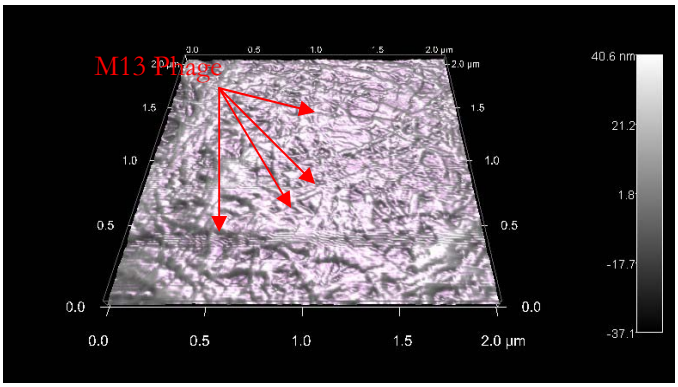


Figure 1. A) AFM image of negative control consisting of wild type M13 subjected to binding assay on the surface of streptavidin coated microscope slides. B) Modified M13 containing second copy of gIII protein which has been modified to contain a streptavidin binding epitope under the control of the lac promoter (uninduced). (Note due to the low number of bound phage the area examined is appx. 2 fold larger than the other images) C) Modified M13 expressing the second copy of gIII protein which has been modified to contain a streptavidin binding epitope.

Presentations To-Date:

1. Shroyer, M., U. Minocha, M. Ladisch, and B. Applegate. 2004.Bioamplification using phage display for the detection of pathogens. American Society for Microbiology Conference on the New Phage Biology. Key Biscayne, FL.
2. Minocha, U., R. Jennings, A. Bhunia, and B. Applegate. 2004. Genome sequence analysis and evaluation of strain specificity of *E. coli* O157:H7 bacteriophage phi V10. American Society for Microbiology, New Orleans, LA.
3. Shroyer, M., U. Minocha, N. Bright, L. Perry, and B. Applegate. 2004. Development of a recombination system for rapid construction of *E. coli* O157:H7 reporter bacteriophage. American Society for Microbiology, New Orleans, LA.
4. Minocha, U., N. Bright, L. Perry, and B. Applegate. 2004. Detection of the foodborne pathogen *Escherchia coli* O157:H7 using an AINS recombinant phiV10 bacteriophage based bioluminescent reporter system. American Society for Microbiology Biodefense meeting, Baltimore, MD.
5. S. Kim, E. E. Igboegwu, A. I. Terekhov and B. M. Applegate. 2005. Bioluminescent Assay for Evaluating Bacteriophage Infectivity in a Food Model. American Society for Microbiology, Atlanta, GA.

Publications:

1. Perry, L., M. Shroyer, U. Minocha, L. Farris and B. Applegate. 2005. Journal of Bacteriology. In review.
Genomic analysis of *Escherichia coli* O157:H7 bacteriophage ΦV10

Patents:

1. *Phage Detection of Pathogens (PDP System)* Bruce Applegate and Michael Ladisch. (Provisional patent filed February 2004).

Pending Research Milestones and Benchmarks

Completion of Recombinant Bacteriophage

Development of lateral flow assays and their integration into a food packaging format.

Principal Investigator: Lisa J. Mauer

BACKGROUND

Food must be safe, nutritious, and acceptable throughout a long duration mission to maintain the health, well-being, and productivity of the astronauts. In addition to a pre-packaged food supply, the ALS NSCORT proposes to utilize crops to provide food and oxygen for astronauts. Research is required to better understand the ability to convert edible biomass into safe, nutritious, and acceptable food products in a closed system with many restrictions (mass, volume, power, crew time, etc.). An understanding of how storage conditions encountered in a long-term space mission will impact food quality is also needed. High levels of radiation encountered on a mission to Mars will provide a threat not only to human health but to the stability of the food, hence radiation is one of the top three concerns for a Mars mission (Wald, 2003). The focus of this project is to provide the highest quality food possible for the duration of a mission by combining shelf-stable extended shelf-life foods and bulk ingredients with crops grown in space, to characterize the impact of space-relevant radiation doses on food quality and antioxidant capacity, and to provide quantitative measures of ESM components related to the food system when possible.

PROJECT GOALS AND OBJECTIVES

1. *Optimization of Food Quality:* To optimize food quality by determining what factors will limit shelf-life, what countermeasures are available to minimize the impact of these factors, what foods and ingredients will have a shelf-life less than the duration of a mission, and how quality traits and composition of foods and crops are affected by storage conditions.
2. *Integrated Package Safety Indicator System:* To ensure the safety of a food prior to consumption, this collaborative effort with Dr. B. Applegate will integrate a pathogen detection system into a food package to facilitate pathogen detection.
3. *Equivalent System Mass:* To provide equivalent system mass parameters for food processing unit operations and packaging scenarios.

RESEARCH PROGRESS

Research efforts for 2004-2005 focused on: 1) characterizing the effects of space-relevant radiation doses of gamma-radiation on pure oils and antioxidants as well as wheat (crop and/or bulk ingredient) and identifying threshold radiation levels for quality changes, 2) characterizing the impact plant cultivars and growth conditions have on nutrient profiles, antioxidant capacity, functionality, and acceptability of foods (starting with sweet potato and wheat, adding strawberry, carrot, tomato, and peanut beginning Sept. 2005 in collaboration with Dr. Cary Mitchell), and 3) estimating packaging waste generated by different food storage scenarios.

Radiation Dosimetry

Verification of absorbed radiation dose is an integral part of the experimental design for our studies. A Fricke dosimeter was used to verify differences in radiation treatment dose from the Gammacell 220 used to expose samples to varying levels of radiation. An aqueous ferrous sulfate solution was irradiated at different levels, and absorbance was read spectrophotometrically at 304nm. Significant differences (α=0.05) were present between all doses tested (0, 3, 10, 50, and 100Gy).

Radiation Effects on Oils

Radiation can initiate a process known as autoxidation, where an initiator species reacts with the lipid, removes a hydrogen, and produces a lipid free radical. This lipid free radical can then react with other fatty acids and produce an accelerating chain reaction of similar events during propagation. Products of this oxidation of fatty acids include peroxides, alcohols, aldehydes, and carbonyls. Further reactions with these products lead to off odors and flavors accustomed to rancid fats. Additionally, essential fatty acids may be oxidized, and this could compromise the long-term health status of the astronauts depending on the magnitude of the essential fatty acid loss. Results from a study in which soybean oil was irradiated at 0, 3, 10, 100, and 1000Gy and stored at 65ºC, showed an increase in both primary (Figure 1) and secondary (Figure 2) products of oxidation up to 100Gy and a drop between 100Gy and 1000Gy. Soybean oil samples run concurrently but stored at 25ºC showed no significant changes in oxidation levels. Based on literature values, it is estimated that one day of storage at 65ºC equates to one month of storage at 25ºC. Current research is designed to further explore the oxidation response between 100 and 1000Gy using soybean and peanut oils irradiated at 0, 3, 10, 100, 200, 400, 600, 800, and 1000Gy stored at 65ºC, as well as to extrapolate accelerated study results to estimate shelf-life of oils following exposure to varying radiation levels.

Figure 1 – Conjugated diene values from soybean oil irradiated at stated dose then stored for 21 days at 65ºC.

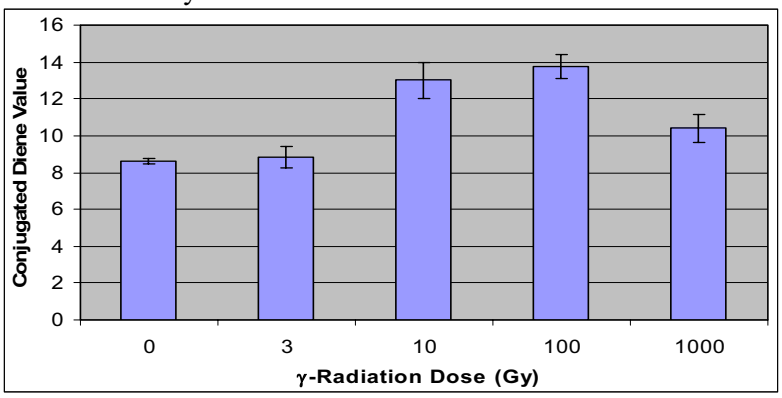
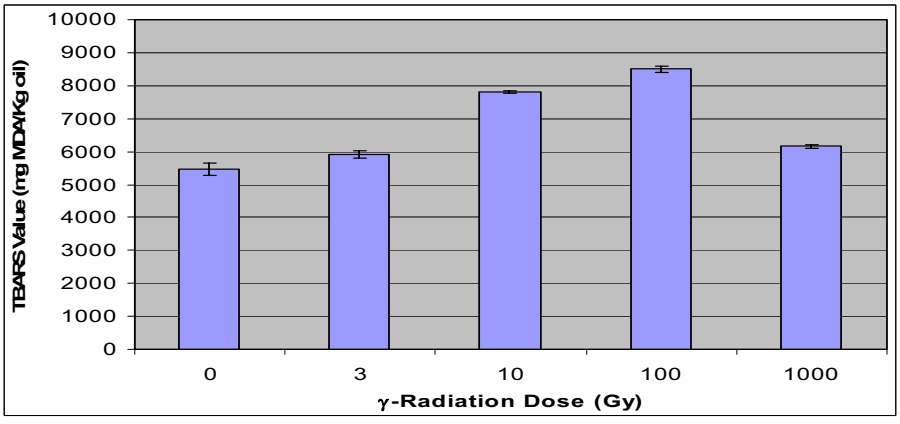


Figure 2 – TBARS values from soybean oil irradiated at stated dose then stored for 21 days at 65ºC.



A study using menhaden oil irradiated at levels between 0 and 100 Gy followed by storage at 65ºC for up to 21 days showed a dose dependent correlation between decrease in primary products of lipid xidation (conjugated triene) and formation/increase of secondary products (TBARS), data not shown.

Radiation Effects on Antioxidants

Radiation doses during transit and on the surface of Mars are expected to be as high as 3 sieverts, excluding solar events. Studies have shown that antioxidants may provide long term health protection from oxidative stress caused by radiation exposure; therefore, to counteract the impact of elevated radiation exposure on astronaut health, consumption of antioxidants will be important. In addition to vitamin supplements, antioxidants within foods will also provide protection. It is important to identify how storage conditions and elevated radiation will impact the antioxidant capacity and stability in both nutritional supplements and foods. Treatment of 500□M Trolox (water soluble vitamin E analog) solutions with 0, 3 ,10, 50, or 100Gy gamma radiation dose showed significant difference (□=0.05) between the Trolox solutions irradiated at the three lowest doses (0, 3, and 10Gy) and the highest dose of 100Gy using the ferric reducing antioxidant power (FRAP) assay (Figure 3). Results from a study using ascorbic acid exposed to 0 or 10 Gy radiation (Table 1) show that the 10Gy radiation treatment decreased the power of the ascorbic acid to act as a reducing agent in the FRAP assay. Antioxidant capacity preliminary results for wheat cultivars exposed to select radiation levels (Figure 4) show that the antioxidant capacity of Apogee and Perigee were affected by the radiation while terrestrial cultivars were not significantly impacted. This work is currently being replicated. Continued work with model systems of Trolox, ascorbic acid, and other antioxidants as well as with crops and dietary supplements is ongoing in an effort to relate decay of antioxidant power and impact of radiation dose to stability and antioxidant capacity of antioxidants provided by dietary supplementation and in foods.

Figure 3 – Effect of radiation dose on the reducing power (FRAP assay absorbance at 593 nm) of 500□M Trolox solutions, monitored over time

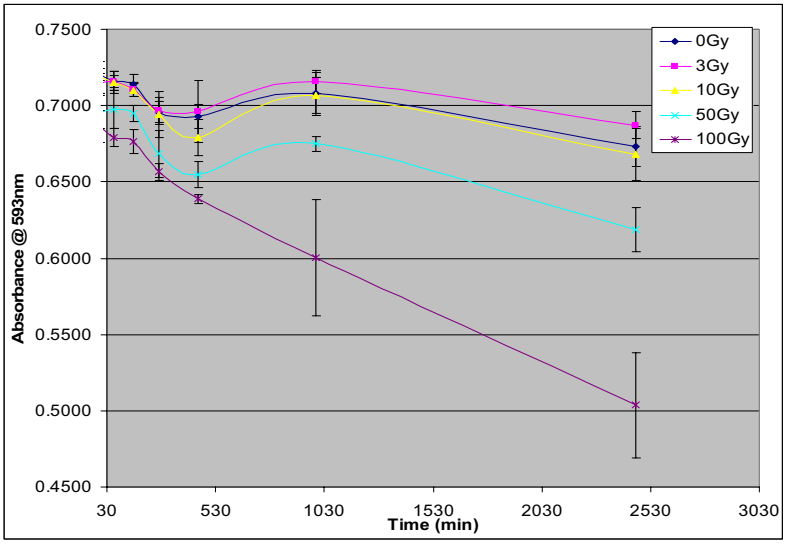
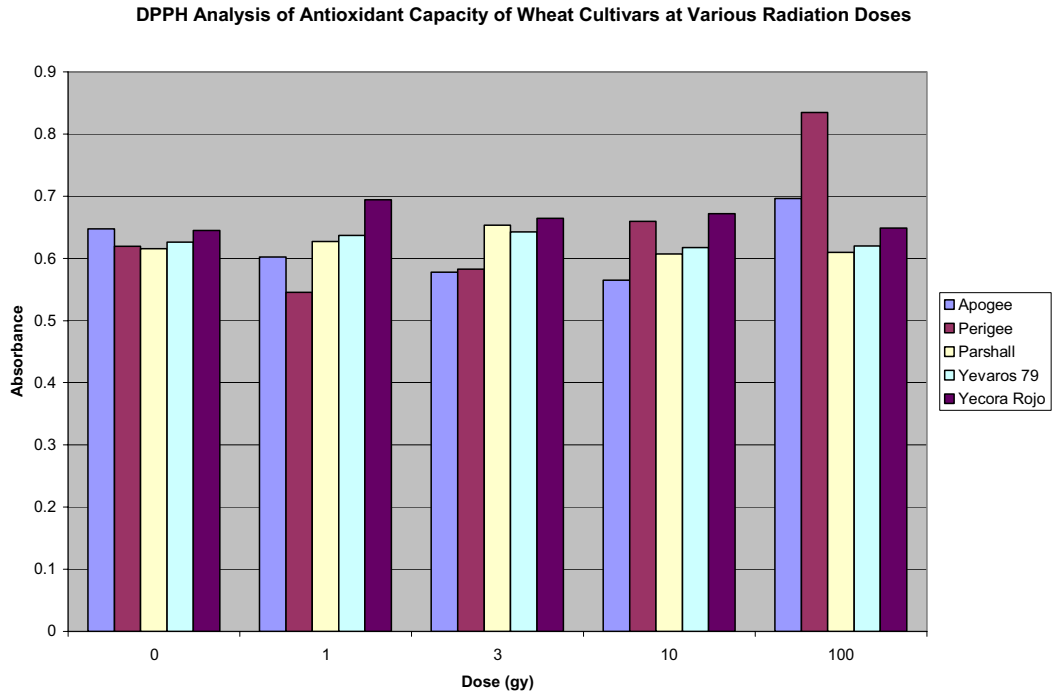


Table 1 – Time (min) taken to reach 25, 50, & 75% decay of reducing power in 4 concentrations of ascorbic acid treated with 0 and 10Gy □-radiation

% Decay	**25%**		**50%**		**75%**	
Conc. (□M)/ Dose	**0Gy**	**10Gy**	**0Gy**	**10Gy**	**0Gy**	**10Gy**
100	57.45	47.53	112.23	93.09	205.91	170.97
250	47.03	72.43	94.73	151.93	176.27	287.84
500	153.26	99.19	356.00	215.04	702.57	413.08
1000	291.12	279.55	741.64	685.01	1511.80	1378.16

Figure 4.



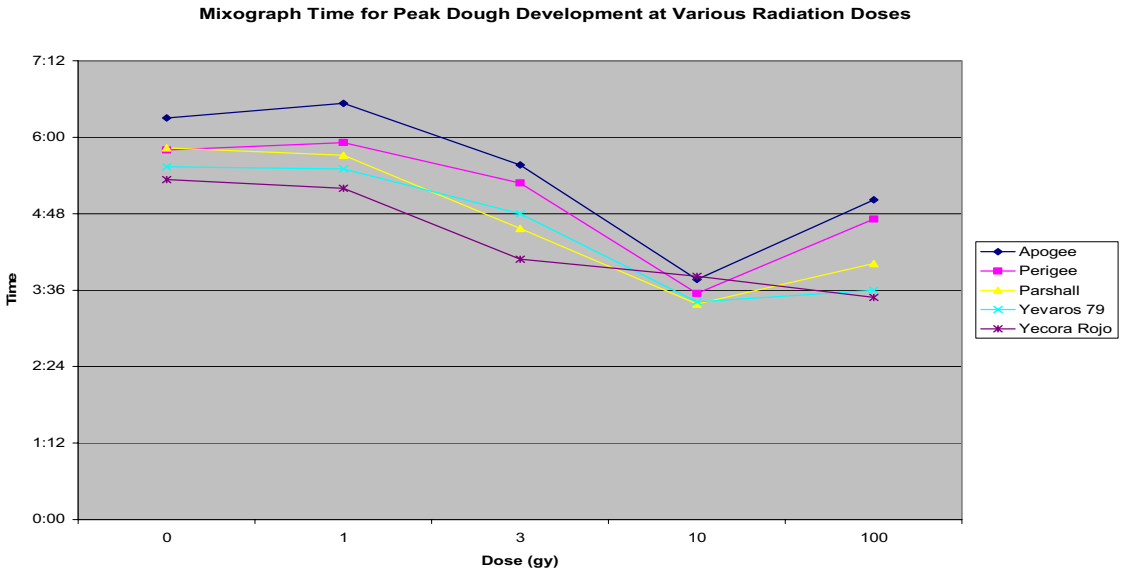
Radiation Effects on Wheat

Wheat is the most studied candidate crop and has the highest priority. Apogee and Perigee cultivars of wheat were developed at Utah State University to yield high amounts of wheat berries with a minimum amount of inedible crop waste. While growth conditions have been characterized, further analysis of these cultivars must be conducted to characterize the protein, lipid, and starch functionalities related to food quality, as well as antioxidant capacities, and to compare the food-functionality of these cultivars to common cultivars used in the food industry. Ilan Weiss, M.S. student who graduated in 2003, conducted initial experiments to compare Apogee and Perigee (field and hydroponically grown) to commercial wheat cultivars; however, due to limited sample size and no replicates, Adam Stoklosa, current M.S. student, is continuing this work and growing both Apogee and Perigee himself in a greenhouse at Purdue. In addition to this work, because wheat can be shipped as a bulk ingredient and/or grown in space, studies must be conducted to determine the effects of radiation exposure on the quality and functionality of wheat.

Wheat Protein Characterization.

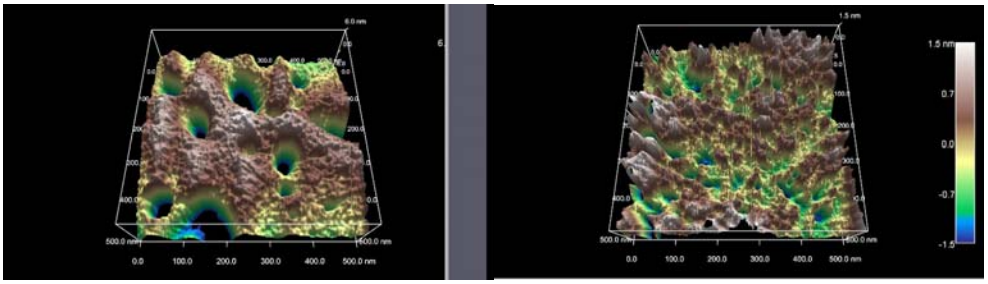
Wheat gluten (protein) functionality can be indirectly measured using a mixograph. In a mixograph, the plasticity and mobility of dough subjected to a prolonged, relatively gentle mixing at a constant temperature is measured. The amount of force required to mix the dough indicates the dough strength and dough stability, which in turn indicates the amount of glutenin/gliadin interactions which have occurred. Following exposure to low doses of radiation (0 to 10 Gy), peak mixing times decreased and gluten interactions improved (Figure 5). Testing at higher doses indicated longer mixing times may be caused by protein degradation. Apogee and Perigee cultivars were found to absorb 25% more water than terrestrial cultivars and also required the most time to develop.

Figure 5.



Preliminary results found no differences in banding patterns of wheat proteins from all cultivars used in this study on SDS-PAGE, indicating a similarity in protein composition, although relative amounts of the proteins may have varied between cultivars based on the intensity differences observed (data not shown). Further work is being done to characterize the protein structures. In an initial study investigating the impact of radiation on protein physical structure, AFM images captured differences between control and irradiated samples (Figure 6). The surface of gliadin in its native state is hydrophobic. When exposed to a hydrophilic surface such as the diamond used in this study, the gliadin aggregates with itself and repels from the surface. Upon exposure to radiation, increased availability of hydrophilic amino acids occurs (Matloubi 2004). This apparently occurs after gliadin has been exposed to 10 kGy radiation, and the gliadin protein is attracted instead of repulsed by the diamond surface, and a more compact adhesion occurs.

Figure 6. Atomic force microscopy images of wheat gliadin proteins before (left image) and after exposure to 10 kGy radiation (right image).

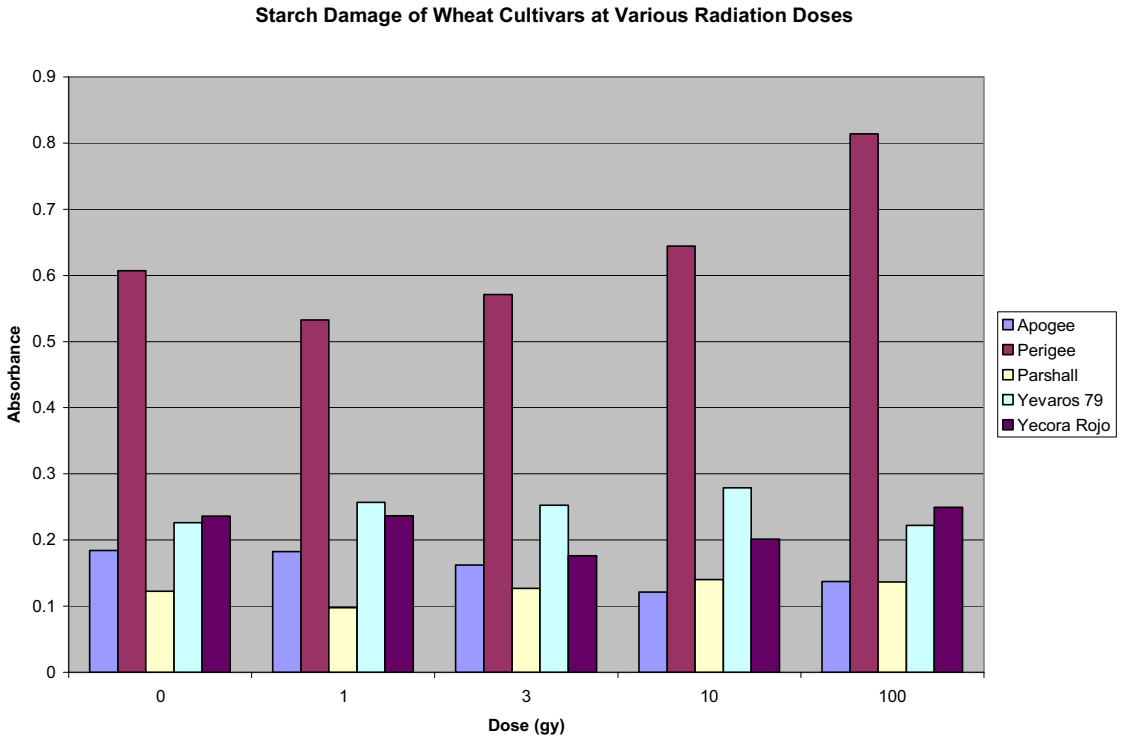


Wheat Starch Characterization

The starch content of wheat appears to be inversely related to the protein content of the cultivar. Most wheat cultivars fall into the range of 63-72% with hard and soft wheat having a starch content of

approximately 64% and 69% respectively. Starch acts as a temperature activated water sink in baked cereal products. As temperature increases and starch gelatinizes, the starch competes with other components for the available water in the system. Therefore, starch often determines the structure of the baked product. The extent of starch pasting is a function of the availability of water to the starch granules. The final state of the starch contributes to the textural attributes of the baked product. Both starch concentration and structure contribute to starch functionality. Results from a study that exposed wheat to various radiation doses indicate that starch granules from the Pergiee cultivar are the most susceptible to mechanical and radiation damage (Figure 7). The other cultivars, including Apogee, appear to resist significant damage.

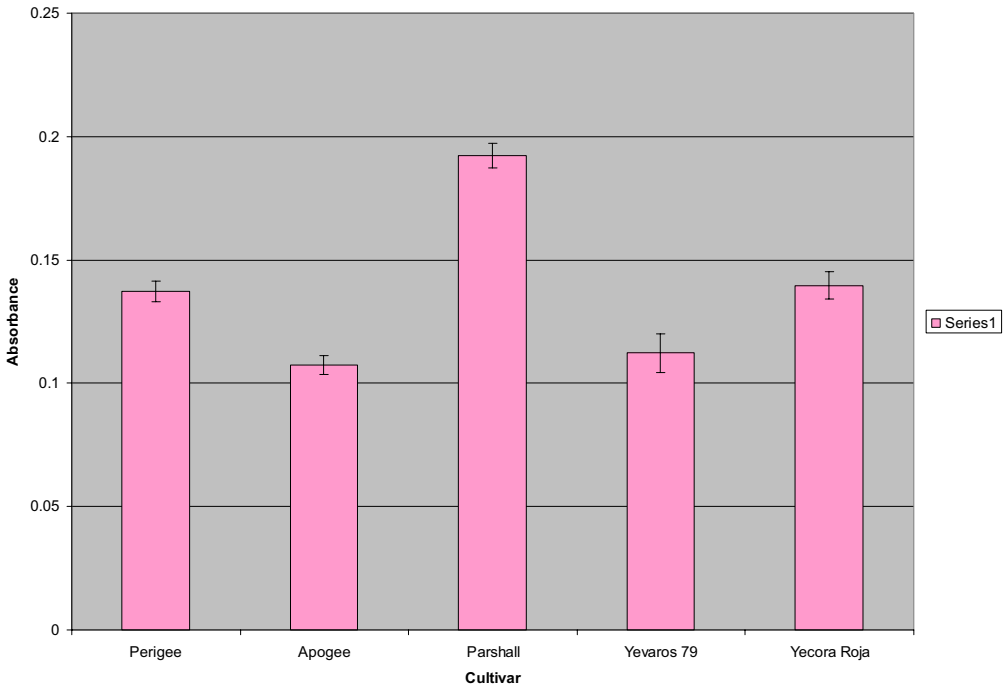
Figure 7.



Wheat Lipid Characterization

Wheat contains 1.5-3% lipid depending on the cultivar and growing conditions. The majority of lipids in wheat are contained within the aleurone layer of the endosperm and the germ. Linoleic acid, oleic acid, and linolenic acid constitute the majority of fatty acids found in wheat. These lipids provide numerous health benefits and linoleic and linolenic are essential fatty acids, but due to their number of unsaturated bonds, they also are susceptible to oxidative rancidity. The products of oxidative rancidity reactions have a significant impact on flavor stability and texture during food processing and can impact overall product acceptability. In a preliminary comparison of lipid oxidation between wheat cultivars measured using the TBARS assay (Figure 8), the Parshall cultivar does have elevated levels of oxidation.

Figure 8. TBARS Analysis of Lipid Oxidation of Various Cultivars



Food Packaging Waste Scenarios

The goal of an Advanced Life Support (ALS) system on a planetary outpost is to be bioregenerative. The packaging materials currently used will accumulate as unusable waste. Because handling unusable waste in an enclosed, bioregenerative life support environment will be difficult, it is imperative to investigate alternate packaging scenarios that will both minimize waste and maximize product quality (barrier properties are essential). Using the BVAD waste assumptions, package waste mass for the 1080 day mission to Mars would be between 1555 kg and 1750 kg. All comparisons of other packaging options obtained from a survey of literature/commercial products were made to reference values from Levri et al. (2001), adapted and shown in Table 2.

Table 2. Packaging waste estimates for a mission to Mars.

Packaging Type	Mass per clean package (g)	Number of packages consumed per person per day	Waste (package mass, g) per day for 6 crew members	Package waste (kg) for 1080 day mission with 6 crew members
Rehydratable	16.61	6.29	627	677
Thermostabilized (1-serving)	8.61	3.71	192	207
Beverages (+ straw)	14.56	3.43	300	324
Irradiated	8.00	0.43	21	23
Natural form/IMF	6.93	3.57	149	161
Fresh foods (tortillas)	10.50	1.57	99	107
			Total package waste (kg) =	1499 (some estimates up to 1750 kg)

Based on these estimates, spreadsheets were developed to predict packaging waste generated for a variety of food storage and consumption scenarios, and this data was presented at the 2005 Institute of Food Technologists' Annual Meeting. To summarize conclusions, removing the rehydratable foods following the 240 day outbound flight has the greatest single impact on package waste, reducing the waste by ~17% if irradiated, IMF, and/or thermostabilized foods are substituted. Removing 90% of single-serve beverages during the 600 day surface stay reduced package waste by ~11% Biodegradable packaging may not be a viable option for space foods due to barrier property limitations and strict control of food quality and safety. Impact of biodegradable packages on solid waste handling systems is unknown. Depending on amount of mass reduction over current package formats, lighter-weight high-barrier package materials that meet NASA constraints could reduce package waste (with an average 1 gram per package reduction, the total package waste would be reduced by ~8%).

FUTURE RESEARCH DIRECTIONS

- Complete replicates of wheat experiments for composition, structure, and functionality
- Identify radiation dose levels that impact structure/function of wheat, including bread quality
- Complete replicates of radiation dose effects on stability of pure oils and antioxidants and expand to include dietary supplements to determine threshold radiation doses that impact quality traits and impacts of space-relevant radiation doses on predicted shelf-life of oils and antioxidants
- Investigate countermeasures (packaging, oil stabilizers, modified atmosphere conditions) to reduce radiation effects on oils and identify shelf-life of oils
- Investigate the effects of radiation and storage on oil quality in peanuts (bulk ingredient and crop)
- Characterize the effects of common food processes, space food storage conditions, and cultivar selection on the nutrient profiles and quality traits of strawberries, tomatoes, and carrots in order to recommend cultivars, preservation methods, and storage conditions to optimize food quality, sensory acceptability, and nutrient profile
- Pursue integrated package safety indicator system with Dr. Bruce Applegate

TRAINEES

MS Students:	Ilan Weiss (graduated 5/03), Jake Gandolph, Adam Stoklosa, Davida Alexander (started 8/05), Alecia Shand (started 8/05)
Undergraduate Students:	Davida Alexander, Sri Budiarty, Deidra Carr, Jake Gandolph, Lenese Grant, Dina Romano, Elizabeth Snuffin, Tracy Szefc

RESEARCH COLLABORATION

- Ongoing research activities with Dr. Cary Mitchell and Dr. Gioia Massa to characterize the effects of growth conditions and cultivar selection on composition and function of select crops (wheat, strawberry, tomato, carrot).
- Ongoing research activities with Dr. Michele Perchonok at Johnson Space Center related to space food quality. She is a member of the committees for two M.S. students currently working on this project.
- Ongoing research discussion with Dr. Bruce Applegate regarding integration of a safety indicator system into a food package.

- Assisted Dr. Lester Wilson at Iowa State University with radiation of his soybean samples during summer of 2005.
- A NASA SBIR Phase I grant on modeling of extrusion to adapt an extruder for use in space conditions was funded in 2005 in collaboration with Triple F and researchers at Purdue (Drs. Osvaldo Campanella and Martin Okos in the Agricultural and Biological Engineering Department at Purdue and Dr. Carlos Corvalan in Food Science). A NASA SBIR Phase II grant has been submitted to continue the support and development of this equipment.
- A NRA grant for ground-based studies for radiation biology (focus on radiation effects on food quality and shelf-life) was submitted in November, 2004, in collaboration with Drs. Michele Perchonok, Steve French, and Lester Wilson. This was not funded.
- Co-chaired the "Food Processing" session for the 2004 Society of Automotive Engineers (SAE) International Conference on Environmental Systems (ICES) meeting with Dr. Michele Perchonok. ICES 2004. July 16-19, Colorado Springs, CO.
- Visited Dr. Tony Pometto and the NASA Food Technology Commercial Space Center at Iowa State University in June, 2004.
- Participated in a sweetpotato study with Dr. Cary Mitchell and Dr. Desmond Mortley at Tuskeegee. We investigated the effects of growth conditions on yield and composition of TU-82155 sweet potatoes. This work was presented at ICES in 2005.
- Obtained wheat samples from Dr. Bruce Bugbee in 2002 to support research of Ilan Weiss to characterize compositional differences between cultivars. This work is ongoing by Adam Stoklosa.
- Participant in the NASA-NSCORT Executive Committee meetings 2003-.
- Participated in the Advanced Food Technology Workshop held at JSC in April of 2002.

PUBLICATIONS

1. Weiss, I. B.F. Ozen, M. Perchonok, K.D. Hayes, and L.J. Mauer. 2003. Comparison of Equivalent System Mass (ESM) of Yeast and Flat Bread Systems. Proceedings of the SAE International Meeting, Vancouver, BC, Canada July 7-10. Paper # 2003-02-2618.
2. Gandolph, J., M.G. El-Abiad, M. Perchonok, L.J. Mauer. 2004. Equivalent System Mass (ESM) estimates for commercially available, small-scale food processing equipment. Proceedings of the SAE International Meeting, Colorado Springs, CO, July 19-22. Paper # 2004-01-2526.
3. Gandolph, J., M.G. El-Abiad, L.J. Mauer, and M. Perchonok. 2004. Equivalent System Mass (ESM) estimates for commercially available, small-scale food processing equipment. *SAE Transactions Journal of Aerospace*. 1:1189-1206. (accepted from proceedings shown in 2.)
4. Weiss, I., M. Perchonok, K.D. Hayes, and L.J. Mauer. 2004. ESM of producing yeast and flat breads from wheat grains: a comparison of grain mill type. Proceedings of the SAE International Meeting, Colorado Springs, CO, July 19-22. Paper # 2004-01-2525.
5. Weiss, I., K.D. Hayes, L.J. Mauer, and M. Perchonok. 2004. ESM of producing yeast and flat breads from wheat grains: a comparison of grain mill type. *SAE Transactions Journal of Aerospace*. 1:1177-1188. (accepted from proceedings shown in 4.)

Popular Press Article:

1. "Developing good eats for space missions: space scientists looking for ways to make food better, longer lasting". Dec. 20, 2004. Written by Amanda Onion at ABC News Internet Ventures. Featured on abcnews.go.com/Technology/print?id=339634.

PRESENTATIONS

Oral Presentations at National/International Meetings:

1. Weiss, I. B.F. Ozen, M. Perchonok, K.D. Hayes, and L.J. Mauer. 2003. Comparison of equivalent system mass (ESM) of yeast and flat bread systems. Proceedings of the SAE International Meeting, Vancouver, BC, Canada.
2. Gandolph, J., M.G. El-Abiad, M. Perchonok, and L.J. Mauer. 2004. Equivalent System Mass (ESM) estimates for commercially available, small-scale food processing equipment. Proceedings of the SAE International Meeting, Colorado Springs, CO.
3. Weiss, I., M. Perchonok, K.D. Hayes, and L.J. Mauer. 2004. ESM of producing yeast and flat breads from wheat grains: a comparison of grain mill type. Proceedings of the SAE International Meeting, Colorado Springs, CO.
4. Massa, G.D., M. E. Mick, I. Weiss, J.A. Montgomery, L.J. Mauer, D. G. Mortley, and C. A. Mitchell. 2005. Effects of root-zone volume, vine training, and root/shoot ratio on yield, proximate composition, and anti-oxidant capacity of sweetpotato (*Ipomea batatas* (Lam.) L. TU-82-155). Proceedings of the SAE International Meeting, Rome, Italy.

Poster Presentations at National Meetings:

5. I. Weiss, K.D. Hayes, M. Perchonok, and L.J. Mauer. 2003. Comparison of equivalent system mass (ESM) and food metric value (FMV) of yeast and flat bread systems. Institute of Food Technologists' Annual Meeting and Food Expo. July 12-16, Chicago, IL. (poster)
6. M. Shroyer, U. Minocha, I. Weiss, L. Williams, L.J. Mauer, B.M. Applegate. 2004. NSCORT food system and safety design for a NASA mission to Mars. Habitation 2004. Jan. 4-7, Orlando, FL. (poster)
7. I. Weiss, K.D. Hayes, B. Bugbee, M. Perchonok, L.J. Mauer. 2004. Characterization of wheat cultivars intended for growth during long-term space missions and comparison to select common terrestrial cultivars. Institute of Food Technologists' Annual Meeting and Food Expo. July, Las Vegas, NV. (poster)
8. J. Gandolph, J. Burgess, M. Perchonok, B. Watkins, and L.J. Mauer. 2005. Effects of gamma-radiation on the reducing power of antioxidants. Institute of Food Technologists' Annual Meeting and Food Expo. New Orleans, LA.
9. J. Gandolph, J. Burgess, M. Perchonok, B. Watkins, and L.J. Mauer. 2005. Effects of gamma-radiation on lipid oxidation and fatty acid composition. Institute of Food Technologists' Annual Meeting and Food Expo. New Orleans, LA.
10. L. Snuffin, M.H. Perchonok, and L.J. Mauer. 2005. Food packaging waste scenarios for a mission to Mars. Institute of Food Technologists' Annual Meeting and Food Expo. New Orleans, LA.
11. A. Stoklosa, D. Nivens, and L.J. Mauer. 2005. Characterizing effects of Gamma Radiation on Wheat Proteins and Starches using Atomic Force Microscopy. Institute of Food Technologists' Annual Meeting and Food Expo. New Orleans, LA.

Presentations at Advanced Food Technology (AFT) Telecons in 2004-2005:

12. L.J. Mauer. 2005 (Sept. 20). "Research update for NASA-NSCORT food science activities". AFT Telecon.
13. L.J. Mauer, A. Stoklosa, J. Gandolph. 2005 (May). "Research update for NASA-NSCORT food science activities. AFT Telecon.

Presentations at NSCORT Meetings in 2004-2005:

14. Gandolph, J., I. Weiss, L.J. Mauer. 2004. Food Processing and Packaging. NASA Specialized Center of Research & Training in Advanced Life Support - External Advisory Committee Meeting. 2004. Howard University, Washington, DC.

Invited Talks:

15. L.J. Mauer and B.M. Applegate. 2002. Space Needs, Earth Applications. Industrial Associates Semi-Annual Meeting. Purdue University, West Lafayette, IN. May 8-9. (invited talk)
16. L.J. Mauer and B.M. Applegate. 2002. Space Needs, Earth Applications. Indiana Section Institute of Food Technologists Meeting. Lafayette, IN. October 16. (invited talk)
17. L.J. Mauer. 2003. "Mars, Food, and Agriculture". Council for Agricultural Science and Technology VIP Day, Purdue University, West Lafayette, IN, January 17. (invited talk, keynote speaker)
18. L.J. Mauer. J. Gandolph, A. Stoklosa. 2004. "Food for space travel". Wabash Area Lifetime Learning Association, Inc. Continuing Education Program, West Lafayette, IN. October 20.

Professors in the Classroom:

19. L.J. Mauer, I. Weiss. "NASA Space Foods" for 2 advanced physics classes Dec. 10, 2003. Lafayette Jefferson High School
20. L.J. Mauer, J. Gandolph, A. Stoklosa. "Space Foods" for 2 $4^{th}/5^{th}$ grade classes. Feb. 22, 2005. Burnett Creek Elementary School.
21. L.J. Mauer. "Space Foods" for one 5^{th} grade class. Oct. 5, 2005. West Lafayette New Community School

Other Education-Related Presentations:

22. L.J. Mauer, J. Gandolph. One lecture entitled "Food for Long-Term Space Missions" for the CE597R course developed by Dr. J. Alleman and given Spring semester of 2004.
23. L.J. Mauer, J. Gandolph, A. Stoklosa. August 5, 2004. NASA Key Learning Camp. "Space Foods": two hands-on laboratories for high school students.

Outreach Activities at the Indiana State Fair:

24. L.J. Mauer, I. Weiss. "Space Foods Interactive Display Booth". August 6, 2002. Purdue Day at the Indiana State Fair.
25. Contributed to the Center-wide "NASA-NSCORT" display exhibited at the Indiana State Fair Our Land Pavilion in August, 2005.

Principal Investigator **Dr. Leonard Williams, PhD., Research Assistant Professor - Food Microbiology and Safety; Immunochemistry, Alabama A & M University**

Co-Investigator: Lloyd Walker, Professor of Food Science
Department of Food and Animal Sciences

Significance of Project to ALS
Foodborne diseases are estimated to cause ~76 million illnesses, 350,000 hospitalizations, and 5,000 deaths annually in the United States (Mead et al., 1999). Therefore, foods contaminated with pathogenic microorganisms, such as *Salmonella* spp., *Staphyloccocus aureus*, *Campylobacter jejuni*, *E. coli* O157:H7 and *Listeria monocytogenes* remain a major health concern for both NASA and the food industry.

Currently, several technologies and intervention steps have been evaluated for inactivation of these pathogens in salad crops (lettuce, onion, carrots, etc.), such as heat treatment, sanitizers, disinfectants, irradiation, microwave radiation and pulsed electric field to name a few. These technologies in combination with the implementation of Hazard Analysis Critical Control Points (HACCP) plans for NASA's Advanced Life Support (ALS) is important in reducing or eliminating potential microbial threats possibly present in hydroponically grown and packaged food products.

Project Goals and Objectives
The primary goal of this project is to develop a HACCP system which can be used for the validation and testing of food products for ALS mission and determine the critical points in processing and production of packaged products provided from plants produced by Purdue and AAMU researchers, and develop a HACCP and food safety system to monitor and validate the effectiveness of reducing any hazards in these packaged products.

Research Progress:
Sampling plans for both whole tomatoes has been completed. Currently, experiments are underway to detect *E. coli*, *Salmonella*, and selected coliforms from hydroponically and conventionally grown salad crops, including mushrooms. The efficacy of selected sanitizers and combination with pulsed light sterilization as a corrective action for reducing or eliminating pathogenic microorganisms on surface of whole tomatoes is being investigated. Also, preliminary studies were conducted to determine the recovery of selected mesophiles and psychrophiles (*Citrobacter*, Pseudomonads, *E. coli*, and coliforms) from a hydroponic system growing salad crops.

A. *Inactivation of Salmonella by pulsed light sterilization and sanitizers*

To determine the role of sanitizing agents and pulsed light sterilization on reduction of a high inoculum of *Salmonella* spp. on the surface of tomatoes, we selected several sanitizers, based on a previous study, which was shown to be highly effective in reducing *Salmonella* on the surface of tomatoes. Three sanitizers, peracetic acid, hydrogen peroxide and Prosan®, a commercially available product were all chosen based on a previous studies (English and Williams, 2004) and its ability to leave behind limited amounts of chemical residues. Two stain mixtures of *Salmonella* was obtained from the AAMU Food Microbiology Laboratory Culture Collection to determine the efficacy of sanitizers and pulsed light sterilization on whole tomatoes stored for 8 days at 25°C. Cells were grown in 150 ml of tryptic soy

broth (Difco, Decton Dickson, Sparks, Md.) at 37°C for 24 h, and harvested by centrifugation at 3,300 x g for 25 min at 4°C.

Pulsed UV-light treatment. Pulsed UV-light treatment was conducted with a laboratory scale, batch-pulsed light sterilization system (SteriPulse-XL 3000; Xenon Corporation). This unit generated approximately 5.6 J/cm2 per pulse on the strobe surface, for an input voltage of 3,800 V and with three pulses per second, as per manufacturer's instructions. The output of the UV-pulsed light sterilizer followed a sinusoidal wave pattern, with 5.6 J/cm2 per pulse being the peak value of the pulse. The power values of the treatments were based on the peak value. The duration (pulse width) was 360 µs. The inoculated whole tomatoes treated with various sanitizers were treated under pulsed UV light for 60 s at a distance of 2.5 cm from the UV strobe. Similarly, untreated (no sanitizer treatment) were treated under pulsed UV light for 60 s at a distance of 2.5 cm from the UV strobe. After pulsed UV-light sterilization, tomatoes were stored for a period of 8 days at 25°C in a temperature controlled incubator. At day 0, 2 and 8 days, samples were randomly selected from each treatment group and microbiological analysis was conducted.

Microbial analysis: For inoculated tomatoes, untreated samples and samples immediately after pulsed UV-light treatment (day 0) were analyzed for surviving populations of *Salmonella*. Briefly, a 1 ml sample from tomato rinse solution was serially diluted with 0.1 M phosphate buffer. This was followed by spiral plating of a 0.1 ml sample onto plate count agar. After incubation at 37 C for 24h, the colonies were enumerated and log reduction was calculated by subtracting the log value of control from that of treated sample. Each experiment was repeated four times.

Data can be summarized as follows: To demonstrate the effectiveness of pulsed light and sanitizer treatment on surface of whole tomatoes, *Salmonella* cells were inoculated onto the surface of whole tomatoes, treated with selected sanitizers and pulsed UV-light up to 60 s at a distance 2.5 cm from the UV strobe. The corresponding power of pulsed UV light at the surface was 1,008 J/cm2/s for 60 s treatment time. The viability of *Salmonella* on the surface of whole tomatoes was significantly reduced in both 1% hydrogen peroxide and peracetic acid treatment when used in combination with pulsed UV-light sterilization. At day 0, approximately log 6 cfu/tomato of Salmonella was recovered from both water and no treatment group, respectively. However, 1% prosan in combination with pulsed light sterilization resulted in approximately log 5 cfu/tomato of *Salmonella* survival. At both 2 and 8 days of storage 1% peracetic acid with pulsed UV-light sterilization was the most effective in reducing the viability of *Salmonella* on the surface of the tomatoes (Table 1 and Figure 1). A complete inactivation of *Salmonella* on tomatoes dipped in peracetic acid (1%) and treated with pulsed UV-light, resulted in a 6 log reduction (Table 2 and Figure 2). Our results indicated that 1% hydrogen peroxide in combination with pulsed light treatment resulted in a significant ($p \leq 0.05$) reduction of *Salmonella* on the surface of the tomatoes when compared to water (control) and pulsed light treatment used alone, that the least effective treatment was 1% prosan and pulsed UV-light treatment at both 0 and 8 days of storage.

Table 1. Influence of pulse-light on the viability of *Salmonella* on surface of whole tomatoes.

Treatment	Log10 CFU/Tomato Storage Time (d) 0	2	8
No treatment	6.04[a]	4.30 [a,b,c]	5.16 [a]
Water	5.56[a]	5.0 [a,b]	4.00 [a]
H202 + PLS	1.89 [b,c]	2.02 [b,c]	4.54 [a]
Prosan + PLS	4.21 [a,b]	5.76 [a]	4.82 [a]
Peracetic acid + PLS	ND [c]	0.80 [c]	2.10 [a]
PLS	4.69 [a]	6.11 [a]	4.02 [a]

[a] Measured as $\log_{10}$ CFU per plate, where counts are averages of four replicate trials. Values followed by the same letter do not differ at the $P \leq 0.05$ level, whereas values followed by different letters differ at the $P \leq 0.05$ level.

[b] ND, Not detected

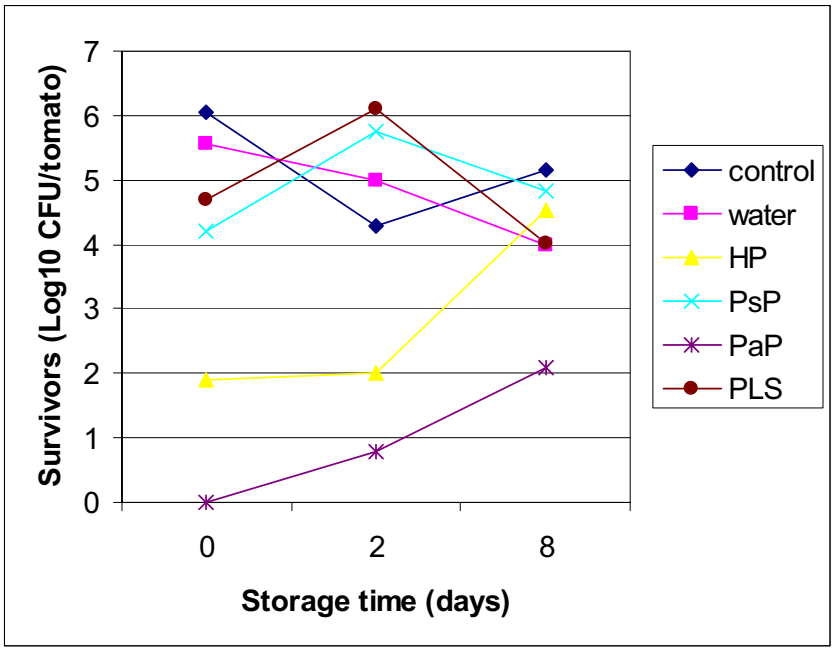


Figure 1. Survival of *Salmonella* on tomatoes during storage for 8 days treated with selected sanitizers and pulsed-UV light sterilization.

Table 2. Log reductions of *Salmonella* after pulsed light sterilization on whole tomatoes.

Treatment	Log Reduction per tomato Day 0	Day 8
No treatment	6.04 [a]	5.16 [a]
Water	0.48 [c]	1.16 [b]
H202 + PLS	4.15 [a]	0.62 [c]
Prosan + PLS	1.83 [b]	0.34 [c]
Peracetic acid + PLS	6.05 [a]	3.06 [a,b]
PLS	1.35 [b]	1.14 [b]

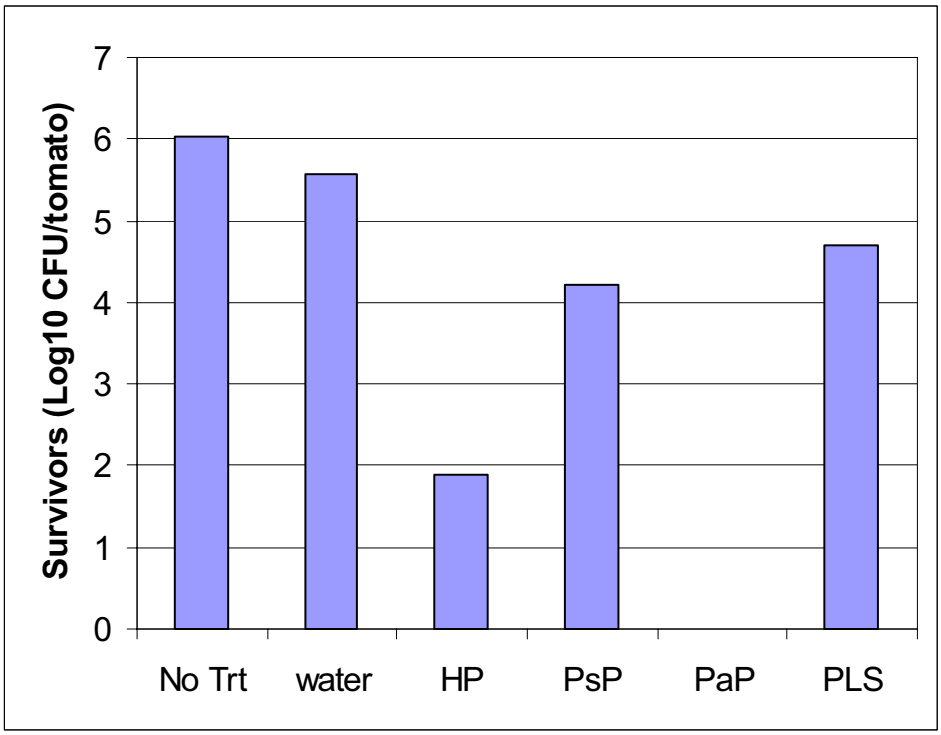


Figure 2. *Salmonella* survivors on whole tomatoes following exposure to pulsed-UV light sterilization and sanitizers.

B. *Preliminary study*- **Microbial ecology of water from hydroponic distribution system**
The development of bacterial communities in hydroponic water distribution systems leads to a food chain which supports the growth of microorganisms incompatible with water quality requirements and esthetics.

Nevertheless, very few studies have examined the microbial communities in hydroponic water distribution systems and their trophic relationships. A preliminary study was conducted to examine the microbial ecology water in a hydroponics distribution system and it role in salad crops. In this study, our hypothesis was that most plant or waterborne microorganisms can thrive in a hydroponic system during growth of salad crops, due to the abundant of nutrients present in the water and generally created by the plants. The objectives of this study was to determine the microbial ecology of mesophilic and psychrophilic microorganisms in a hydroponic distribution system growing salad crops (lettuce, sweet potatoes, beets and carrots). Enumeration of bacterial populations included mesophilic aerobic bacteria, psychrotrophic aerobic bacteria, coliforms, generic *E. coli* and pseudomonads. For determination of total plate counts (TPC), appropriate dilutions were plated in duplicates on plate count agar. For enumeration of total coliforms and generic *E. coli* populations, 3M petrifilm coliform/*E. coli* Count Plates and Violet Red Bile Agar was used. For enumeration of citrate producing bacteria and Pseudomonads, Citrobacter isolation agar and Pseudomonas isolation agar were used, respectively. All plates were incubated at 37°C and enumerated according to the manufacturer's directions, except for the psychrotrophic aerobic plates that were incubated at 20°C. The mesophilic aerobic TVC and coliform counts were determine after 24 h of incubation, the *E. coli* counts taken after 48 h, and the psychrotrophic TPC counts were taken at 3 days. Figure 1 and 2 depict the microbial profiles of hydroponic water distribution system over a 12-week period. Figure 1 represent total Pseudomonads, *Citrobacter* and generic *E. coli* detected. There was a significant decline ($p \leq 0.05$) in the numbers of mesophilic microorganisms in the hydroponic system over 12 weeks of of the experiment.. The reduction was sustained after 7 weeks. After seven weeks, Pseudomonads were the only bacteria detected by traditional plating methods. Figure 2 illustrates the psychotropic and total plate counts from hydroponic water. The total viable counts in the hydroponic system did not significantly ($p \geq 0.05$) decline until week 11. The number of psychotropic microorganisms followed a similar trend to the Citrobacter and E. coli enumerated under mesophilic conditions (Figure 1 and 2).

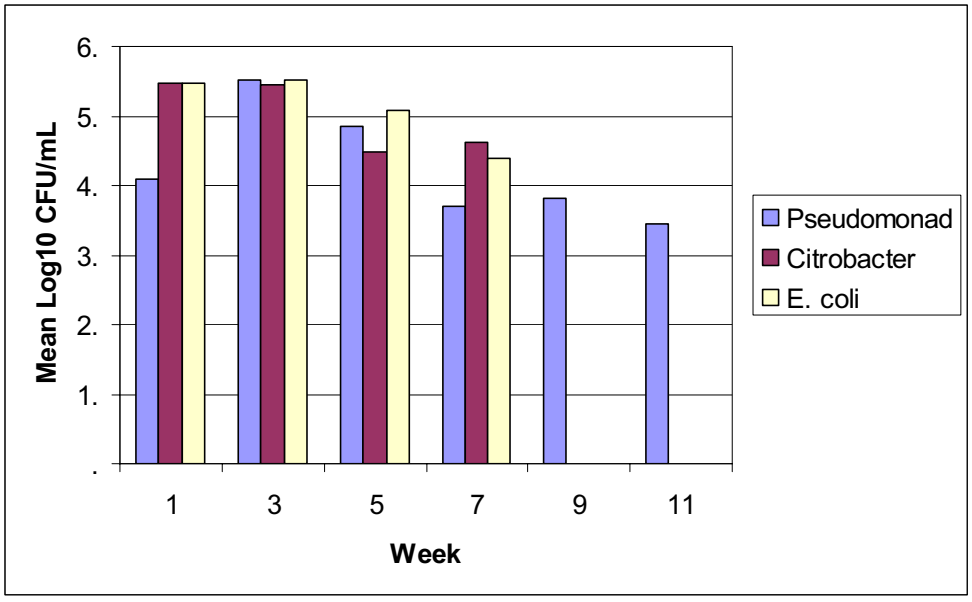


Figure 1. Mesophilic and isolation of selected microorganisms from hydroponic water system.

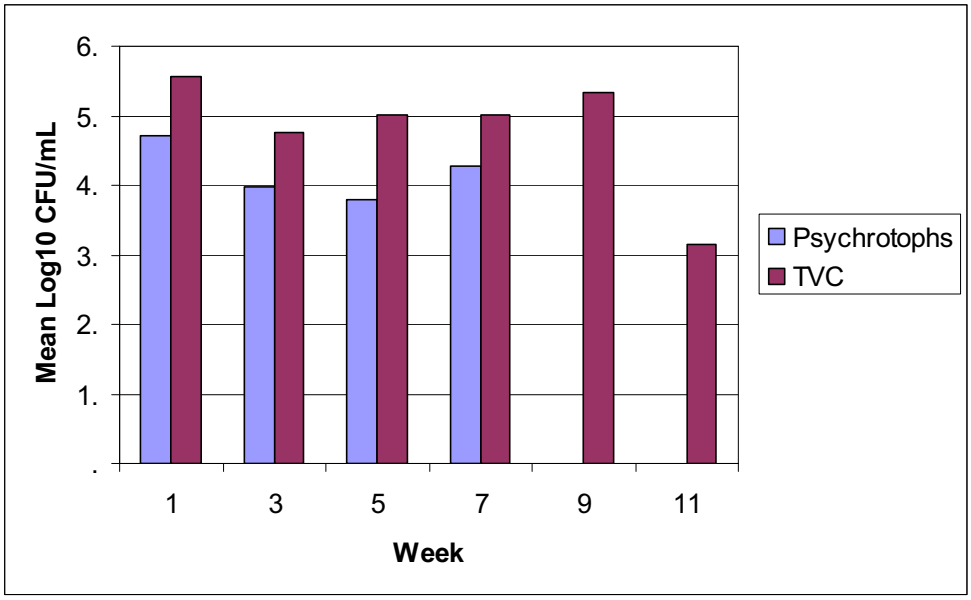


Figure 2. The total viable and psychrotrophic counts from hydroponic water system.

Future Direction of Research: 1. Development of sampling protocols for detecting and enumerating microorganisms and its by-products retained in hydroponically grown salad crops. 2. Determine role of biofilms on salad crops using bioluminescence strains developed in Bruce Applegate Lab (Purdue). 3. Determine the effects of irradiation on color changes, sprouting and germination of salad crop seedlings.

Trainees: M.S. Candidate- Tyrico English

Ph.D. Candidates- N'Jere Austin (not funded)
Krishuan Caldwell (not funded)

B.S. Candidates- Tony Bradford
Morgan Anderson
Parisea Story

Research Collaboration: This project has several collaborative aspects. First, Prosan® sanitizer was provided by Dr. Lopes of Microcide, Inc. in collaboration with NASA Food Technology Commercial Space Center at Iowa State University. Drs. Carla Beyl and Leopold Nyochembeng, will continue hydroponic study and examine the microbial ecology of mushrooms grown using non-edible waste, including human waste.

Publications and Presentation to date

English, T. and L.L. Williams. 2004. Efficacy of selected sanitizers on reduction of *Salmonella* spp. on whole tomatoes. Alabama Industry and Research Cluster, November 2004.

Williams, L.L. "How safe is space food?" Monrovia Middle School, December 2004.

English, T., N. Austin and L.L. Williams. 2005. Inactivation of *Salmonella* spp. by pulsed light sterilization. *In press*. J. Food Prot.

English, T., N. Austin and L.L. Williams. 2005. Efficacy of a commercial sanitizers of reduction of Salmonella on whole tomatoes. *In press*. J. Food Prot.

Pending Milestones and Benchmarks

Preparation of abstracts for upcoming Habitation 2006; IFT and International Association for Food Protection Annual Meeting. Prepare manuscript in collaboration with Drs. Beyl and Nyochembeng.

References

English, T. and L.L. Williams. 2004. Efficacy of selected sanitizers on reduction of *Salmonella* spp. on whole tomatoes. Alabama Industry and Research Cluster, November 2004

Mead, P.S., L. Slutsker, and V. Dietz. 1999. Food related illness and death in the United States. Emer. Infect. Dis. 5:607-625.

Resource Recovery (R^2) Executive Summary

The air/water and solids focus groups of the NSCORT were merged into a single resource-recovery (R^2) focus area during year 3 of Center operations, because many R^2 projects are now coming together across disciplines. Two research projects came to a close during the past year, a new project started up, and a center-wide analytical effort was initiated within the R^2 group. In addition, sensitivity has spread across the Center regarding the need for all research projects to identify steps or points in bioprocessing where hazards to human crews might occur. Potential hazards resulting from biological approaches to bioprocessing include microbial contamination, toxic metabolites in waste feedstocks and/or bioreactor products, and noxious gases released from metabolic or physico-chemical degradation of organic substances in air, water, or solid phases. Absorption, inhalation, or ingestion of hazardous organisms or substances by crews are of particular concern in closed systems.

A new NSCORT approach to degrading crop and food waste (Plant-Based Anaerobic-Aerobic Bioreactor-Linked Operation, or PAABLO) biologically pretreats grey water, reduces solid waste loads, and produces methane for subsequent energy reclamation, without producing copious waste heat. An analogous bio-reactor (Waste-AABLO, or WAABLO) reduces human solid waste in a separate process stream. PAABLO recalcitrant residues are directed to a fungal composter for partial hydrolysis of ligno-cellulose, the hydrolysates are returned to the anaerobic reactor to maximize methanogenesis, energy reclamation, and solids reduction, and the pre-treated grey water is directed to BREATHe I for aerobic co-treatment of air and water. BREATHe I also can remove NH_4^+ from aquarium water and urea from urine in separate process streams. Micro-, nano-, and RO-membranes filter out trace organics and desalt inorganics from BREATHe effluents, and iodine and UV_c provide final polishing and fail-safe quality control. Noxious gases emitted from bioreactors and incinerators are processed by BREATHe II biofilters and/or concentrated onto zeolite adsorbent particles. Nitrogen and sulfur-rich adsorbents or eluates are added to fungal composters and microbial bioreactors to stimulate the growth of reactor organisms feeding on N- and S-deficient ligno-cellulose and enhance their hydrolysis of feedstock polymers. Consumption by Tilapia fish of microbial biomass containing amino acids essential for fish development enables those fish to indirectly reduce the load of recalcitrant crop waste while supplementing crew diets with small amounts of animal protein.

By using products of one waste treatment as substrates for another, by focusing on bioregenerative approaches to primary processing and limiting physico-chemical approaches to secondary processing and polishing functions, and by analyzing for bio-hazards at critical process points, the NSCORT R^2 group is significantly reducing ESM for resource recovery while minimizing potential risks in closed systems.

Cary Mitchell for Al Heber, R^2 Focus Area Lead

SOLID-PHASE THERMOPHILIC AEROBIC REACTOR (STAR) PROCESSING OF FECAL, FOOD AND PLANT RESIDUES

Principal Investigator **Dr. James E. Alleman, Purdue University**
Dr. Cary Mitchell, Purdue University

Co-Investigators **None**

Background

The Solids Thermophilic Aerobic Reactor (STAR) is under investigation for the initial treatment of all biodegradable solid wastes that would be generated in a crewed mission, including inedible plant wastes, paper, fecal matter, and food wastes. STAR operates similarly to the autothermal thermophilic aerobic digester (ATAD) currently in use in a number of wastewater treatment plants. The projected advantages of the STAR system include a reduced retention time, increased rapid pathogen inactivation, lower reactor volume requirements, and ease of automation as compared to other biological waste treatment systems.

The system operates under thermophilic, microaerobic high moisture conditions. Temperatures are maintained at 55-65°C. These temperatures allow rapid inactivation of pathogens, meeting EPA CFR 503 regulations for Class A biosolids in a matter of hours (USEPA 1993). It is desirable to maintain the temperature just above 55°C rather than achieving higher temperatures for two principle reasons: ESM considerations, and microbial population diversity. The STAR system includes the EVAC toilet, which is plumbed to a storage tank where waste is transported via vacuum. The reactor is batch fed from the storage tank once per day, and consists of a stainless steel tank with a heating band, an air diaphragm pump to circulate the sludge, piping for mixing and aeration, and a scrubber system. A visual sight gauge as well as pressure sensors allow monitoring of volume, and online monitoring includes pH, ORP, CO_2, temperature, and O_2.

Operational parameters will be evaluated to optimize solids degradation and resource recovery while minimizing ESM within the system. Recycling of effluent supernatant will be evaluated, studying the effects of recycling of supernatant and the affiliated enzymes and thermophiles both to increase degradation and reduce the amount of water added to the influent to increase moisture levels. Lignin degradation will be evaluated in STAR for optimization, as this organic component is typically the most recalcitrant. The knowledge gained in the completion of the work contained herein will benefit other downstream technologies and ALS systems, and will continue progress on the path to a regenerative long-term habitat.

Project Goals and Objectives

The primary goal of this project is to develop a novel high-temperature solids digestion system for processing biodegradable wastes within a sustainable closed-loop ecosystem. The pathogen-free residuals streams generated by this process would be amenable to direct water and nutrient recovery. The

design is loosely modeled after the successful, commercial Autothermal Thermophilic Aerobic Digestion (ATAD) wastewater sludge treatment process (1). Associated objectives are:

1- To create a thermophilic reactor system mechanically able to suitable mix and aerate high input solids levels at an expected range of 6-10% solids,
2- To maximize the positive ESM-enhancing attributes in terms of gas transfer rates to maximize performance, solids shear to reduce particle size, solids and organic destruction performance to reduce waste volume and enable further recovery of water, nutrients and carbon, and pathogen pasteurization to prepare the product for further use or storage (2,3),
3- To minimize ESM-degrading attributes, in regard to mass and volume, crewtime, noise generation, vibration, energy consumption, and heat loss,
4- To evaluate the system's performance utilizing a long-term mission waste stream including human fecal, paper, food residuals, and plant materials, and
5- To evaluate the complementary use and operational performance of a vacuum waste collection system, particularly in regard to high-solids waste transfer.

Research Progress

1. *Initial pH adjustment and loading study, Dawn Whitaker:* The treatment of raw wastes by the STAR system is a novel application of autothermal thermophilic aerobic digestion (ATAD). In the first experimental phase, a reactor was designed scaled for 1-3 crew members to provide proof of concept. HRT was varied from 9-18 days, and solids concentrations of the influent varied from 4-10%. Total and volatile solids destruction was evaluated, and pH and ORP utilized as performance monitors to determine optimal solids loading.

In the fall of 2004, it was determined that the initial pH levels present in the first few hours of start-up were critical for the success of the start-up. Due to a change in feedstock formulation, the influent pH was lowered. This lower initial pH prevented the successful start-up of the reactor. Conditions remained hampered in the system, reflecting acid hydrolysis rather than the desired micro-aerobic digestion. Degradation rates were lower, achieving only ~50-55% total solids degradation, and pH levels remained around 4, which would negatively impact downstream uses of the effluent for projected plant growth. Early in 2005, an in-depth study of the effects of pH and solids loading began.

The reactor was maintained at 55-60°C during the study. Aeration was provided using a stainless steel diffuser submerged in the reactor tank with airflow of 2.5 L/min. An air diaphragm pump provided circulation and mixing. The reactor was batch fed on a daily basis. HRT for the study was maintained close to 10 days. The one exception to this was a trial at 8% solids loading with an HRT of 18 days as well as 10 days to determine the effect of HRT on the solids loading deemed most likely to succeed. Successful start-up of the system was determined to be dependent on the utilization of pH control (using NaOH) for the first several hours of the run. As shown in the representative graph (2% solids loading) in Figure 1, the system was able to become self-regulating after the first 8-12 hours, after which time no further addition of NaOH was needed. This result was found to be consistent at all solids loadings evaluated (data not presented).

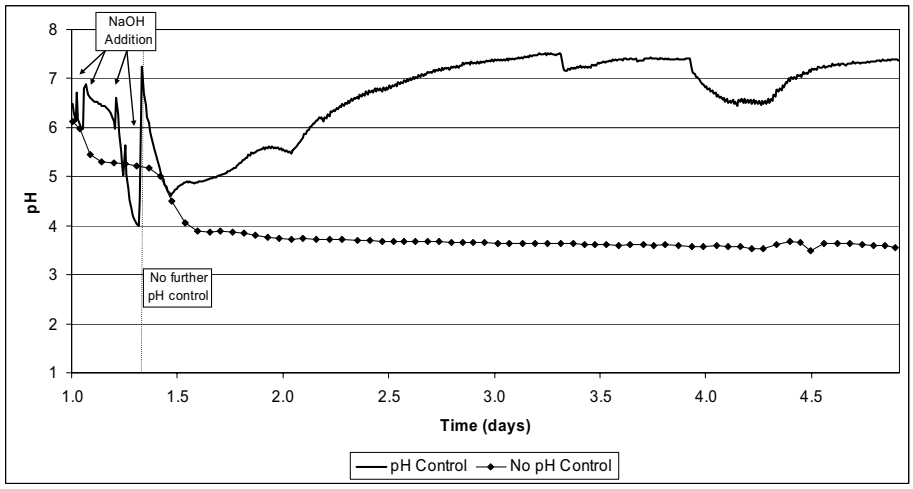


Figure 1: Start-up pH response

The other crucial parameter evaluated was solids loading. Both aeration and mixing are dependent upon the influent solids levels. Due to pump constraints, it was hypothesized that the optimal solids influent level would be 10%. Optimization of the influent solids loading showed the highest degradation (75%) at an influent solids loading of 6%, as shown in Table 3. However, there was only a minimal loss of efficiency at 8% loading (71-73%). Degradation was decreased to 60% total solids loss at the 10% influent loading.

Table 3: Solids Degradation Results

Influent Solids %	HRT	Average Total Solids Loss (%)
4	10	74
6	13	75
8	18	73
8	9	71
10	11	60

Successful start-up will likely require the addition of base to maintain pH levels until the system becomes self-regulating. The optimal influent solids loading level was determined to be 8%, with a HRT of 9 days. In order to optimize the system, mass of both the reactor and contents must be reduced, and the addition of water should be minimized. By increasing the solids loading from 6 to 8%, 700 mL of water is conserved per crew member per day. Over the course of a long-term mission this reduction is substantial. Further water conservation could be achieved by increasing the solids loading to the 10% range; however, these preliminary studies at 10% solids loading showed relatively unreliable performance of the system as well as a significant reduction in degradation. Finalization of results at the 10% loading is ongoing at the time of this writing, and will be completed in early October.

2. *Composting study, Angela Nolan*: Based on the inedible plant biomass generated by the all-crop diet, 6.7 kg of inedible plant biomass would be generated per person per day (4). This is an enormous quantity of plant matter, and presents extreme difficulty with regards to pumping in the STAR system. It was therefore decided to add an additional chamber specifically for the treatment of inedible plant matter

beyond that of the salad machine crops for this type of scenario. Day-to-day operation of the STAR reactor would include human fecal matter, toilet paper, food residuals, and the inedible biomass generated by the salad machine crops. The additional chamber would only be utilized for treatment of large quantities of crop biomass.

Initial research focused on finding an optimal method to treat the plants prior to composting. A preliminary study was to evaluate whether the size of soybean plants affected their ability to degrade during composting or not. It is a common notion that smaller particle size enhances degradation as opposed to larger sizes, but the question is "how small is small?" The time it takes to cut the plants increases as the particle size needed decreases. By testing the ability of different sized soybean plants to degrade, we hoped to use the results to develop a pre-processing method that would incorporate optimal degradation conditions for the plant and lower work requirements to help reduce ESM calculations.

Soybean stems and leaves were obtained and cut into 0.5, 1.0, and 1.5-2.0 centimeter squares using scissors. A mass of each size was placed in a mini-composter that consisted of an air-tight plastic container with a mesh screen in the middle. The soybeans were placed on top of the screen (to allow liquid to fall through), and three valves allowed for the addition of air, removal of air, and the removal of excess liquids. The composters were then placed in a temperature-controlled vessel containing approximately three inches of water and a submersible heater that maintained temperatures at 48°C. Each day, the gases in the composter headspace were evaluated for oxygen and CO_2 using a Rosemount Analytical O_2 and CO_2 Analyzer. The carbon dioxide and oxygen peaks were used to evaluate activity within the composter based on the fact that oxygen is consumed and carbon dioxide is produced during composting.

This experiment was only conducted for one month, and little data was obtained during that time as the focus was on improving the physical set-up. Although there is not adequate data to show a correlation between particle size and degradation capacity, it was concluded that the experimental setup has the capacity to produce such data when the experiment is allowed to run throughout its duration.

3. *Bench-scale filtrate recycling and enzyme activity study, Dawn Whitaker:* An evaluation of the potential use of STAR effluent filtrate to enhance degradation and reduce the amount of clean water required by the system to adjust influent solids levels is currently being completed. It is hypothesized that degradation within the STAR system will be increased by recycling filtrate from the treated sludge effluent back into the influent stream. An additional benefit will be the reduction in the amount of clean water required for operation, which will benefit the ESM of the system. The premise of the study is to compare the effects of hot water, effluent inoculum and filtrate inoculum on the degradation of both STAR feedstock and newsprint. The lignin content in the feedstock will be varied by the use of a lower lignin content paper along with newsprint. Unadulterated STAR effluent will be evaluated in the system to determine any further degradation potential and CO_2 generation.

STAR effluent is filtered sequentially through a 10- and 1-micron filter to separate solids. The analyses utilize a Columbus Instruments respirometer, which monitors and controls CO_2 and O_2 levels within the headspace of the 1-L vessels. The system is currently equipped to run 3 samples in each run. Samples are well-mixed and maintained at 55°C. A factorial experimental matrix will be tested, evaluating each of the components separately (filtrate, solids, feedstock, effluent) and all combinations of the components. Additionally, enzyme activity will be investigated in the individual solid and liquid components of STAR effluent. A time-scale study will be completed on the evolution of enzyme activity over the daily cycle of the system.

4. *Lignin Degradation Study, Dawn Whitaker:* Degradation of lignocellulose, a major component in the predicted feedstock for STAR, is under evaluation. This evaluation will enable optimization of the system as well as providing necessary information for potential downstream technologies. Influent and effluent lignin content along with CO_2 generation and O_2 consumption can be used to estimate degradation kinetics in the STAR system. These estimates can be utilized along with feedstock characteristics to predict degradation and optimize the desired parameters for STAR.

Lignin and cellulose/hemicellulose degradation are key to reducing waste volume and achieving high degradation rates both in space mission scenarios as well as on earth. Microbial cellulose utilization is responsible for one of the largest material flows in the biosphere and is of interest in relation to analysis of carbon flux at both local and global scales (Lynd et al. 2002b). Lignocellulose constituents are major components in ALS solid wastes. Feces contain approximately 30% lignocellulose, while plant matter and food residuals can contain up to 70-80% (Komilis and Ham 2003). Huge quantities of plant growth are projected for habitat scenarios – 6.7 kg/cm-d of inedible biomass is estimated for full crop diets (Hanford 2004). Optimization of solid waste systems to achieve high degradation rates of these substances and recover water, carbon, and nutrients will be crucial.

ASTM 1721 Determination of Acid-Insoluble Residue in Biomass is the method used to assess lignin content. Samples from both the STAR pilot-scale reactor as well as the filtrate bench-scale studies (See #3 above) are being assessed for lignin degradation. Preliminary studies on lignin degradation in the pilot-scale STAR system have shown degradation rates of approximately 80%. Published values for biological degradation of lignin range from 0-70%.

Future Research Directions

The bench-scale evaluation of lignin degradation and enzyme activity will continue, with completion by December, 2005. Recommendations based on the evaluation of filtrate as a wetting agent will be made to enhance degradation and minimize ESM. Publication of results will be completed in 2006.

Trainees

Post-Doctorates-	None
PhD Candidates-	Dawn Whitaker, Purdue University (0.5 Research Assistant)
MS Candidates-	Angela Nolan, Purdue University (0.5 Research Assistant, Jan–Aug 2005)
Undergraduates –	Ryan Ellis, Tom Konopka, Suhaili Muhammad, Brian Walker

Research Collaboration

- STAR researchers participate in SIMA telecons, and the Solid Waste group telecons. Participation is ongoing in the Solid Waste Working Group.

- Dawn Whitaker continues to work with Julie Levri at NASA-ARC on the OPIS project, part-time during the school year and full-time during the summer.

- Dawn Whitaker served as a reviewer for both the Biological Waste Processing and Microbial Processes Session and Advanced Life Support Missions, Requirements, Metrics, and Decision Tools Session, 2005 International Conference on Environmental Systems, Rome, Italy

- Dawn Whitaker served as a conference session moderator for the Biological Waste Processing and Microbial Processes Session, 2005 International Conference on Environmental Systems, Rome, Italy

Publications and Presentations

Whitaker, D.R., and Alleman, J.E. (2006). "Thermophilic Aerobic Solid Waste Processing for Long-term Crewed Missions." To be presented at the *Earth and Space 2006*, *10th ASCE Conference on Engineering, Construction, and Operations in Challenging Environments*, Houston, TX

Whitaker, D.R. and Alleman, J.E. (2006). "Lignin Degradation in Solid Waste Treatment Systems." To be presented at *Habitation Conference*, American Institute of Aeronautics and Astronautics, Orlando, FL.

Whitaker, D.R., Invited Speaker, W1170: Chemistry, Bioavailability, and Toxicity of Constituents in Residuals and Residual-Treated Soils Group Meeting, "Solid Waste and Biosolids in Space." January 2005, Las Vegas, NV.

Whitaker, D.R., Staton, K.L., Alleman, J.E., Lane, J.W. (2005). "Loading Balance and Influent pH in a Solids Thermophilic Aerobic Reactor." Conference Proceeding: *International Conference on Environmental System*s, Society of Automotive Engineers, Aerospace Division, Rome, Italy, Paper # 2005-01-2982.

Kuo, Y., Whitaker, D., Chiu, G., Alleman, J. (2005). "System Level Design and Initial Equivalent System Mass Analysis of a Solid-Phase Thermophilic Aerobic Rector for Advanced Life Support Systems." Conference Proceeding: *International Conference on Environmental System*s, Society of Automotive Engineers, Aerospace Division, Rome, Italy, Paper # 2005-01-2983.

Pending Research Milestones and Benchmarks

- Solids Loading Study: The loading study for the pilot-scale STAR reactor will be complete in early October, 2005.
- Bench-scale lignin degradation study: Lignin assays will be complete in December, 2005.
- Bench-scale enzyme assay: Enzyme assays begin in late September 2005, with completion estimated in November 2005.
- Filtrate Recycle Evaluation: The evaluation of filtrate recycling in the STAR will be completed in January 2006.

References

Hanford, A. (2004). "Advanced Life Support Baseline Values and Assumptions Document, Revision A." CTSD-ADV-484 A, Crew and Thermal Systems Division, NASA–Lyndon B. Johnson Space Center, Houston, Texas..

Komilis, D. P., and Ham, R. K. (2003). "The effect of lignin and sugars to the aerobic decomposition of solid wastes." *Waste Management*, 23(5), 419-423.

Lynd, L. R., Weimer, P. J., van Zyl, W. H., and Pretorius, I. S. (2002). "Microbial cellulose utilization: Fundamentals and biotechnology." *Microbiology and Molecular Biology Reviews*, 66(3), 506-577.

The PAABLO Project: Plant-based Anaerobic-Aerobic Bioreactor Linked Operation

Dr. Ronald Turco, Agronomy Department, College of Agriculture
Dr. Larry Nies, School of Civil Engineering, College of Engineering

Statement of Project Goals and Expected Results

This project will focus on adaptation and use of aerobic-anaerobic technologies for the treatment of three sources of waste (plant residues, greywater and human waste) on the Mars colony. We are proposing the development of dual systems, one to handle the combination of plant waste and greywater and one to handle the human waste. While both will result in the formation of CH_4, the plant waste-grey water system (e.g., PAABLO) will also be used to develop an organic plant growth media similar to compost. Human waste treatment system (WAABLO) use the same approach but the resulting biosolids are not used in food production. In both cases, the anaerobically processed waste streams will provide a renewable energy source and will result in a reduction in the mass plant waste present. In the long-term the anaerobic digestor will be a source of methane (leading to small scale electricity generation), produce water, treat waste materials while conserving O_2 and act as a heat sink as optimal digestor operation is achieved with an elevated temperature. Solid waste from the PAABLO system will be an ideal bedding material for the production of mushrooms and any other plant types that are difficult to grow in liquid systems. In order to maintain a high quality food, at no time do we propose using biosolids from WAABLO in food production. These anaerobic digestor systems will provide a robust treatment technology that will allow a rapid and consistent conversation of waste to energy and in the case of PAABLO needed secondary materials.

Biomass Waste Conversion with Energy Reclamation

A significant fraction of waste material (plant residue, greywater and human waste) generated during space missions contain recoverable energy when processed with an anaerobic biotreatment system. The specific objective of the project is to optimize an approach and demonstrate a Plant-based Anaerobic-aerobic Bioreactor Linked Operation (PAABLO) that efficiently converts plant residuals and greywater into fuel grade methane (CH_4), electricity, and biosolids that will be suitable for plant and fungal culture. This effort will optimize the potential for CH_4 production by aerobically treating anaerobically recalcitrant waste materials - biosolids (using specially adapted bacteria and fungi) before they are used in as a plant growth material. This step will breakdown the resistant materials, freeing soluble carbon and stabilizing the materials. In a second part of the project we will modified the PAABLO system into a system concerned with converting human waste in to CH_4 and biosolids. The Waste-AABLO (WAABLO) approach is for the conversion of human waste into CH_4 and biosolid residuals that are condensed but not used in the production of food.

Our PAABLO system will demonstrate an "energy conversion package" and ability to recycle biological waste materials. The anaerobic-aerobic bioreactor uses anaerobic technologies for the generation of CH_4. The anaerobic digesters will produce biogas which is rich in CH_4. The biogas stream will be coupled to a rectifier system that removes impurities and converts the CH_4 into hydrogen (H_2); the H_2 is used to produce electrical power in a fuel-cell.

Our WAABLO system will also demonstrate an "energy conversion package" and ability to condense and recover water from otherwise problematic biological waste materials. The anaerobic-aerobic bioreactor also uses anaerobic technologies for the generation of CH_4. The biogas stream will be coupled to a rectifier system that removes impurities and converts the CH_4 into hydrogen (H_2); the H_2.

The solids will be reduced to the fullest extent possible, compressed and stored. (However, we will test the feasibility of using these waste materials for bedding in the production of fish-food.)

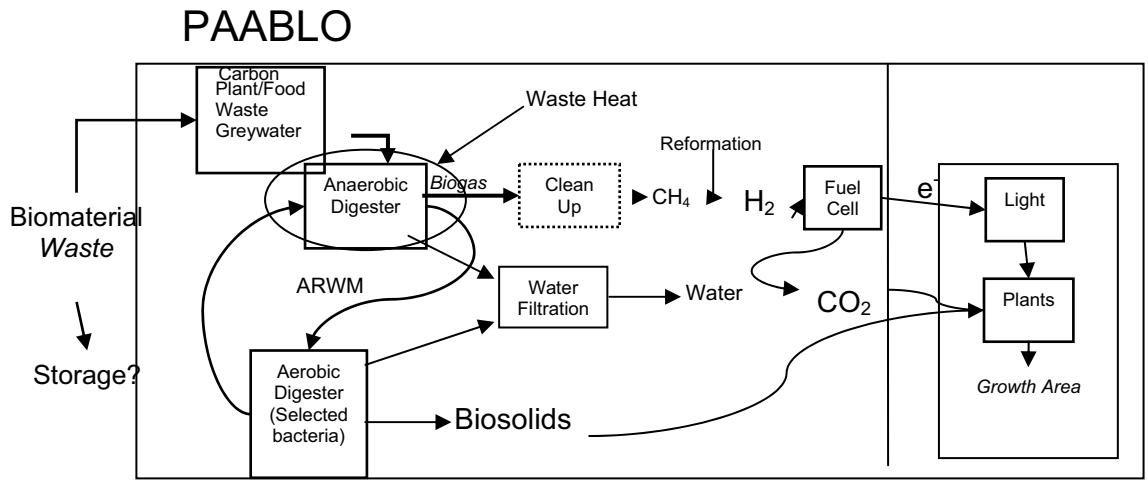


Figure 1. Conceptual design of the Plant-based Anaerobic-Aerobic Bioreactor Linked Operation PAABLO system; ARWM: anaerobically recalcitrant waste materials.

General Project Tasks

Task 1. In this task we will develop and optimize anaerobic-aerobic bioreactor system to allow the production of methane from plant-based waste materials and greywater. This will form PAABLO.

Task 2. Methods for the optimization of the PAABLO 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 PAABLO.

Task 3. 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. This will form WAABLO.

Task 4. Methods for the optimization of the WAABLO biosolids. 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.

Principal Investigator: Charles C. Glass, Ph.D.

BACKGROUND

As a part of the original proposal and first year of operation, one of the goals of this project was to evaluate the nitrification then denitrification of the condensate water scrubbed from the gas of the STAR system. The original hypothesis focused on the regeneration of zeolite through nitrification of the ammonia adsorbed to the surface. After issues with nitrification in columns during year one occurred, predominantly acclimation and the alkalinity requirements causing a higher ESM than adsorption, in the second year an evaluation of zeolites to remove ammonium from solution was performed and successfully completed. A clinoptilolite and a chabazite were both found to adsorb high concentrations of ammonium both from synthetic wastewater and from condensate water from STAR.

During the EAC meeting in the fall of 2004 a few questions from the audience seemed to suggest some disappointment that completion of the nitrogen cycle was no longer being investigated by the PI. After review of the literature the possibility of complete nitrification and denitrification is possible, with the assistance of a zeolite support matrix. In year three the development of a system and an initial experiment performing complete nitrification and denitrification were completed.

PROJECT GOALS AND OBJECTIVES

This year the PI completed all zeolite experiments and submitted the work for publication in the journal Habitation. Sequencing Batch reactors were acquired and utilized to investigate the oxidation of ammonium through nitrification followed by denitrification for complete nitrogen reduction treating the proposed flow rate of the condensate production from the STAR reactor (0.6 L/day) at a concentration up to 1000 mg/L NH_4^+-N. The goals of the project were to:

- Establish a culture on a synthetic wastewater, acclimating the biomass to an influent concentration of 1000 mg/L
- Feed the culture the condensate from the STAR reactor when the shipments are received from Purdue
- Add chabazite and clinoptilolite to the acclimated biomass to determine if this enhances the capability of the mixed culture to oxidize ammonium to nitrate
- Determine the ability to perform nitrification then denitrification in the same system, completing the nitrogen cycle

Originally this project was going to investigate the ability of a nitrification/denitrification reactor to regenerate a zeolite and return a liquid product that can be used as a nutrient supplement for the plant systems in the ALS. Nitrification and denitrification have been shown to convert nitrogen at the concentrations greater than 1000 mg/L as N (Mahne et al., 1996, Glass et al, 1997, Glass and Silverstein, 1998, Glass and Silverstein, 1999).

The investigator has begun examining the capabilities of nitrifiers, to grow on the condensate, and solve any issues of toxicity that may occur. In addition, research has shown increased nitrification rates with zeolite used as a support matrix in a sequencing batch reactor leading the PI to believe that complete nitrification/denitrification is possible at these concentrations. Unfortunately progress with actual STAR effluent was discontinued due to changes in the center structure. This, however, will not change the need

for some form of nitrogen removal after solid waste treatment as all processes that degrade proteinaceous material produce ammonia in gas or liquid form.

RESEARCH PROGRESS

Completion of Zeolite Adsorption Studies. Ammonium removal by clinoptilolite has been found to be a cost effective method in wastewater treatment over the last three decades. Clinoptilolite, a naturally occurring zeolite, has been found to be selective for ammonium ions in the presence of other cation concentrations commonly found in wastewaters. Five zeolites including two types of clinoptilolite and two types of chabazites were purchased from GSA Resources, Tucson Arizona. The last of the zeolites was a clinoptilolite from Bear River Zeolite Company (BRZ) Thompson Falls, Montana. The purpose of the project was to find a suitable means of decreasing the concentration of ammonia-rich brine fluid, which includes some organics and hydrogen sulfide from condensate from the STAR Gas Scrubber.

In Figure 1 the results of the equilibration of ammonium ion on to three zeolites in the presence of sodium and potassium ion, when treated with simulated wastewater (1000 mg NH_3 - N/L) in a batch reactor setup. The chabazite ZS500RW/H was found to be the most stable and had a maximum adsorption capacity at equilibrium (32.73 mg NH_3-N/ $g_{zeolite}$) as compared to the commonly used clinoptilolite. The chabazite at an initial concentration of 1000 mg NH_3-N/L attained 90% removal in less than one hour with only one gram of the respective zeolite. However, a maximum adsorption capacity was higher for clinoptilolite ZS403TM (36.20 mg NH_3-N/ $g_{zeolite}$) in comparision to that of ZS500RW/H (34.40 mg NH_3-N/ $g_{zeolite}$). But the most important property of retention capacity at equilibrium differs largely in the case of ZS403TM (25.82 mg NH_3-N/$g_{zeolite}$) than in the chabazite. The adsorption capacity of clinoptilolite ZK406H was also found to be consistent with the general capacity of clinoptilolites in the literature of 18 mg NH_4^+/g of zeolite.

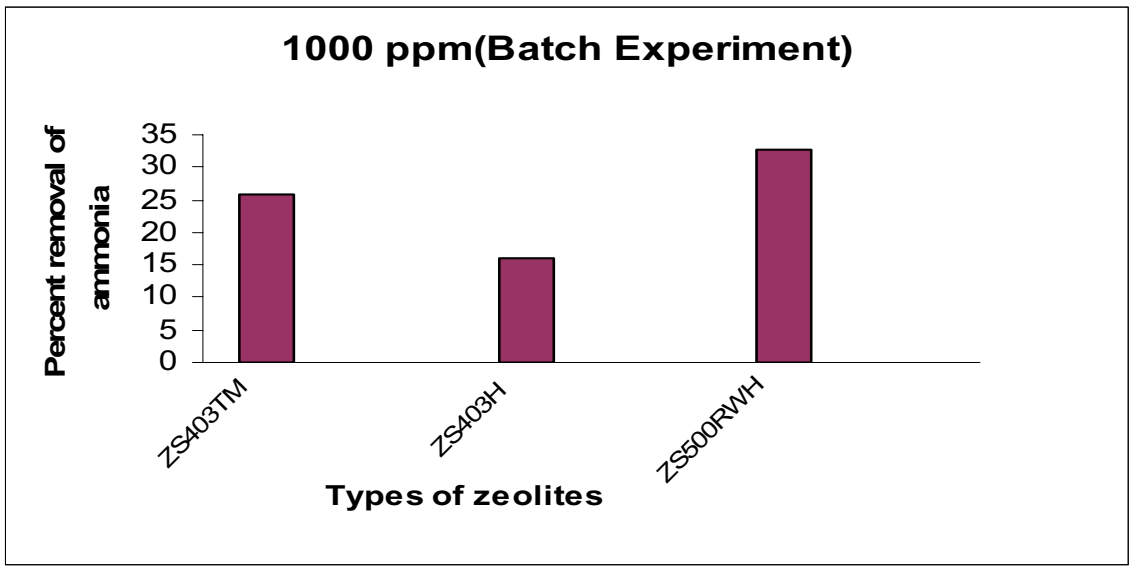


Figure 1: Adsorption capacity of various zeolite at a concentration of 1000 mg NH_3-N/L in a batch reactor.

A column study was also performed with synthetic wastewater to determine the breakthrough curves for the three zeolites. Figure 2 shows the results of the column study. The chabazite ZS500RW/H was found to outperform the other forms of zeolite which was consistent with the results of the batch reactors. This may be due to the presence of easily exchangeable sodium ions. Further, Ming et. al, 1995 and Lahav et. al, 2000 observed that the affinity of clinoptilolite and chabazite was greater for K^+ than NH_4^+. This

affinity for potassium cations over ammonium can explain the poor adsorption of ammonia for the other zeolites.

The optimum zeolite screened from the above experiments was tested over a range of concentrations of ammonia (Figure 3) to determine its maximum capacity for lower concentrations and behavior over various concentration ranges. At the 400 mg NH_3-N/L concentration the retention ability of ZS500RW/H for ammonia was comparable with a maximum adsorption of 22.57 mg NH_3-N/$g_{zeolite}$ to an equilibrium adsorption capacity of 21.81 mg NH_3-N/$g_{zeolite}$. It was observed that an increase in initial concentration 500 and 1000 mg NH_3-N/L resulted in an effectual increase in the adsorption capacity, 31.93 mg NH_3-N/$g_{zeolite}$ and 40.87 mg NH_3-N/$g_{zeolite}$ for ZS500RW/H, respectively. However these values were misrepresentative since the equilibrium adsorption capacities were 66% and 63% respectively, of the original aqueous ammonia retention.

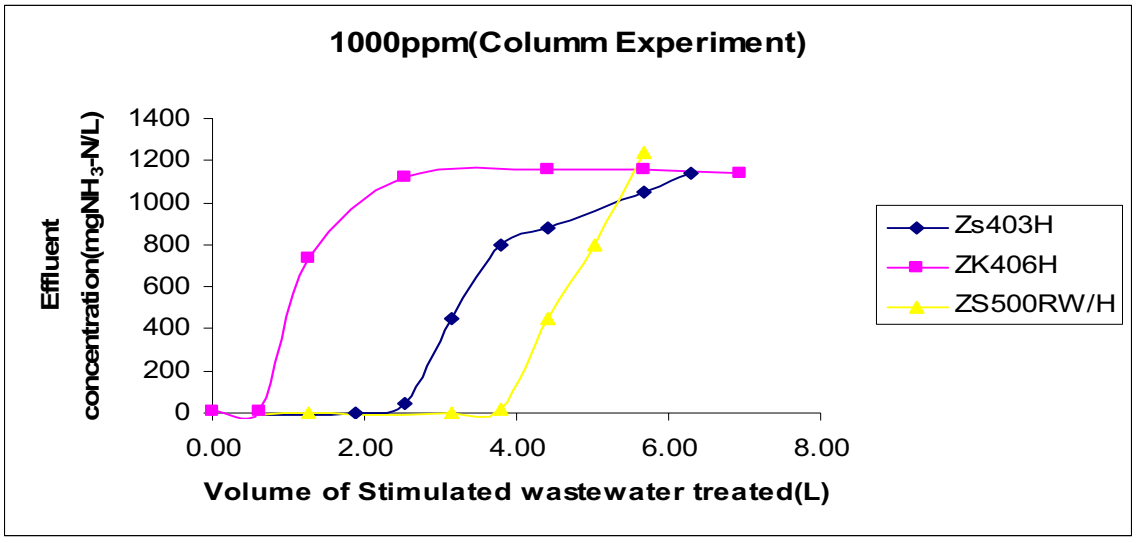


Figure 2: Adsorption capacity of various natural zeolites studied at a concentration of 1000 mg/L in a column experiment.

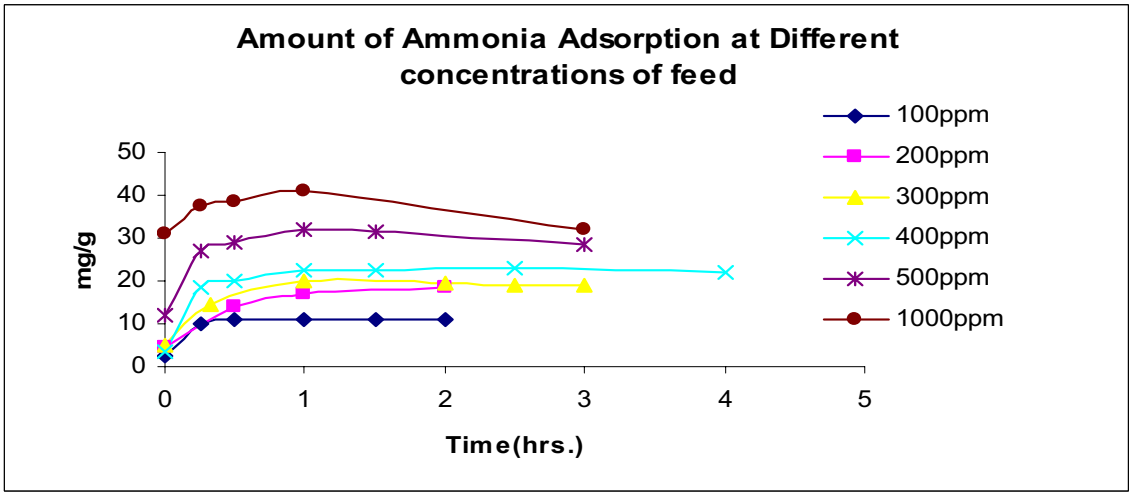


Figure 3: Extent of adsorption of ammonia over chabazite(ZS500RW/H) at different concentrations of ammonia solution.

To increase the adsorption capacity by adjusting the various parameters responsible for adsorption, different pretreatment techniques were carried out for the best zeolite and at 400 mg/L concentration of the ammonia solution, as shown in Figure 4. It was found that the heat treatment for one hour at 600 ºC showed the best results. Based on the observations of Klieve et. al (9), the heat treatment for one hour was carried out. This heat treatment resulted in weight loss due to evaporation of water and some organics which opened channels for better ammonia adsorption. During the initial five hours, the untreated zeolite ZS500RW/H had the best adsorption capacity but maintained a very low retention value, while the one hour heat pretreated sample had comparative adsorption and retention capacities. Researchers have observed that Na-exchanged natural chabazite showed solid-state transformation to a sodalite structure at 600 ºC ,whereas K-exchanged natural chabazite did not transform. However, the XRD spectra taken after the heat treatment of the samples under study did not show any such structural changes as compared to untreated samples (Figure 5).

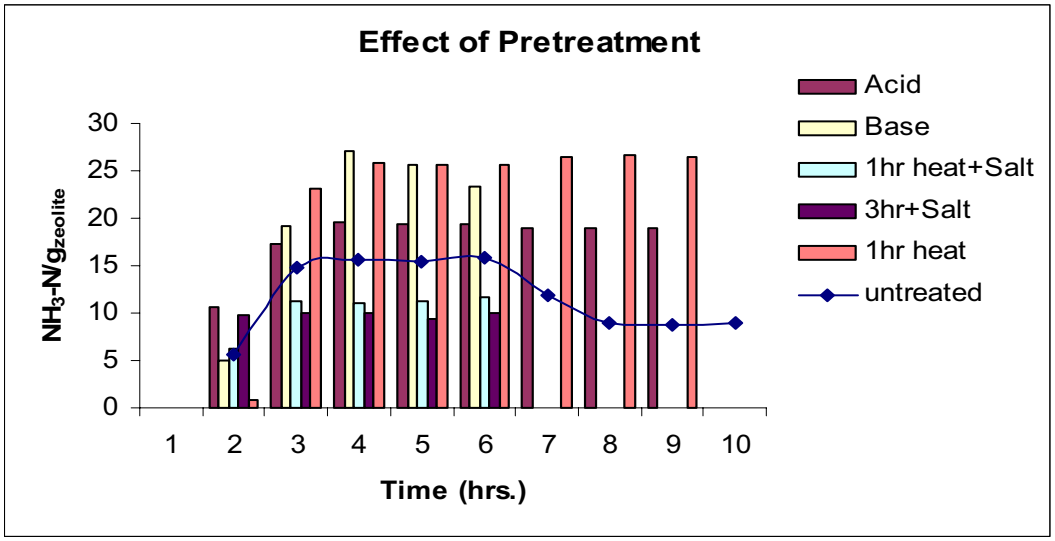


Figure 4: Comparision of the effect of various pretreatments carried out over chabazite (ZS500RW/H) with a concentration of 400 mg/L of ammonia solution.

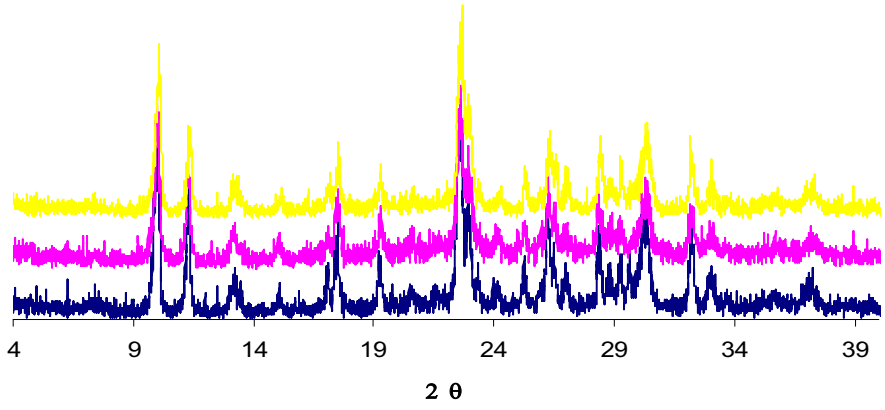


Figure 5: X-Ray Diffraction patterns for (a) natural zeolite-Chabazite (ZS500RW/H) (b)After calcinations at 600^0C for 1hour (c) After absorption of ammonia on sample (b).

A pretreatment process involving an acid treatment may result in dissolving alkali impurities which can block pores and channels within the zeolitic framework, hence promoting adsorption deep within the pores. However, zeolites with lower Si/Al ratio have been found to be unstable at lower pH, probably due to the loss of aluminum from the framework and thus, resulting in lower adsorption capacity. This can be seen from Figure 4, where at an initial concentration of 400 mg NH_3-N/L, the untreated zeolite performs better than the acid pretreated.

Another pretreatment aimed at increasing the ammonia adsorption capacity of the zeolite was treatment with sodium hydroxide. Heating in the presence of salt for varying time periods did not improve the adsorption capacity. In Figure 4, ZS500RW/H base treated had a maximum adsorption capacity (27 mg NH_3-N/$g_{zeolite}$) that surpassed that of the untreated zeolite (15 mg NH_3-N/$g_{zeolite}$) and one hour heat treated also. But the retention capacity of one hour heat treated chabazite was the best among all the other pretreated samples.

When the initial NH_3-N concentrations applied to the batch reactors was very low, ammonium uptake by the clinoptilolite zeolite was comparable to that of the chabazite zeolite. However for very high loadings of NH_3-N concentrations, chabazite with sodium ion as exchangeable ion was found to be the best, in terms of the adsorption capacity. The adsorption capacity of chabazite was enhanced by pre-heating for one hour.

Initial Nitrification/Denitrification Studies in Sequencing Batch Reactors. The objective of this phase of the study was to evaluate the ability of nitrifying and denitrifying bacteria to process ammonia to nitrogen gas. In order to investigate the bioavailability of precipitated phosphorus, SBR bioavailability experiments were conducted. During this project, we were confronted by various operational issues. This report presents those issues and also proposes some solutions.

Sequencing Batch Reactor System.

Two bench scale sequencing batch reactors (SBR) running in parallel were used to perform nitrification/denitrification process by providing aerobic followed by anoxic conditions. To ensure cycling between aerobic and anoxic conditions the two reactors, an airtight fitting was delivered air into the sealed reactors. Two motor driven mixers provided mixing during the nitrification/denitrification process. Two pH and DO probes were used for on-line pH and DO measurements using a Hydra Data logger Unit. Three double-headed pumps were used to fill and decant the SBRs and to add sodium bicarbonate as a base to the reactors in order to adjust the pH to 7. A Chrontrol timer was used to control the initial 12-hour SBR cycle which comprised of a total fill time of 15 minutes, a reaction period of 7 hours for nitrification and 4 hours for denitrification, a settling period of 30 minutes and decant time of 15 minutes.

Each reactor was seeded with 20 L of activated sludge from Blue Plains WWTP tertiary treatment process. The collected activated sludge placed in the reactors was acclimated under experimental conditions to room temperature at 22-25 ºC.

Table 1 Basic Conditions of the System

Parameters	Values
Flowrate (L/day)	0.6
NH_4-N (mg/L)	40, 100, 300, 500, 800, 1000, 1200
Temperature	22 ± 2 deg C
pH	7.0 ± 2
D.O. (Nit)	5 mg/L

D.O. (Denit)	>1 mg/L
C:N (Nit)	1:4
C:N (Denit)	2.5:1
HRT	6.7 day
SRT	
Biomass (VSS)	1000 (25:1, B:N)
Alkalinity	Added in Excess through pH control
Addition of Zeolites	Later if time permits
Phosphorus	Calculate requirement
Micronutrients	Tapwater

Synthetic wastewater consisting of condensate water scrubbed from the gas of the STAR system was going to be used with an ammonia nitrogen concentration and a phosphate phosphorous concentration. Initially synthetic wastewater was utilized in place of feed from the STAR system feed and to acclimate nitrifiers and denitrifiers in the two reactors. In Figure 6 nitrification and denitrification take place, with the large amount of methanol, measured as soluble COD, that is necessary to complete denitrification. The precipitous drop in COD at the end of nitrification is believed to be the result of aerobic heterotrophic use of the organic carbon with the dissolved oxygen present at the end of nitrification. Figure 8 confirms this drop in dissolved oxygen with the shift from aerobic to anoxic conditions during the seventh hour of the reaction cycle.

In Figure 7 only the forms of nitrogen are presented for the system. Figure 7 shows that ammonia nitrification is complete within three hours for these conditions and that there is no build up of nitrite during the reaction. During denitrification nitrite does accumulate during the reaction and is completely denitrified by the end of the reaction period. During this particular profile oxygen entered the system to interfere with the completion of the reaction, and cause the nitrification of nitrite back to nitrate. This preliminary experiment has confirmed that nitrification followed by dentrification is possible in Sequencing Batch reactors. A delicate balance between aerobic and anoxic conditions must be maintained in this type of single consortium system. We are currently increasing our initial concentration following the protocol established in the experimental design.

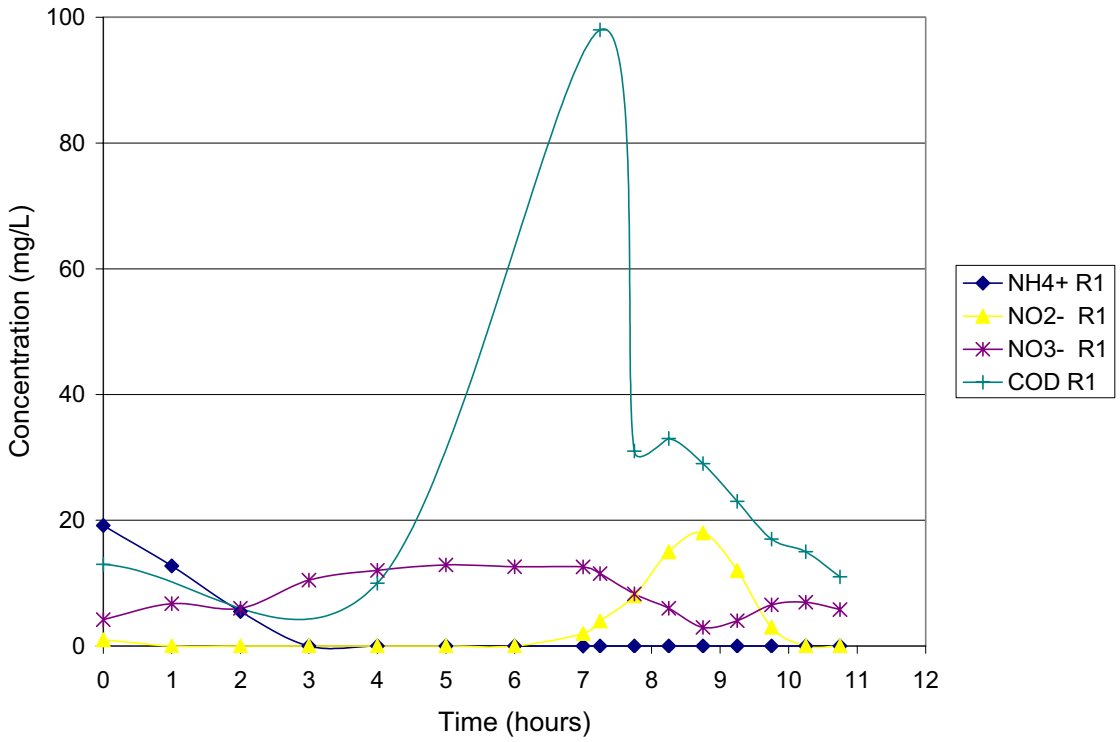


Figure 6 Nitrification and denitrification with an initial ammonia concentration of 20 mg/L-N, methanol addition as the organic carbon source measured as COD.

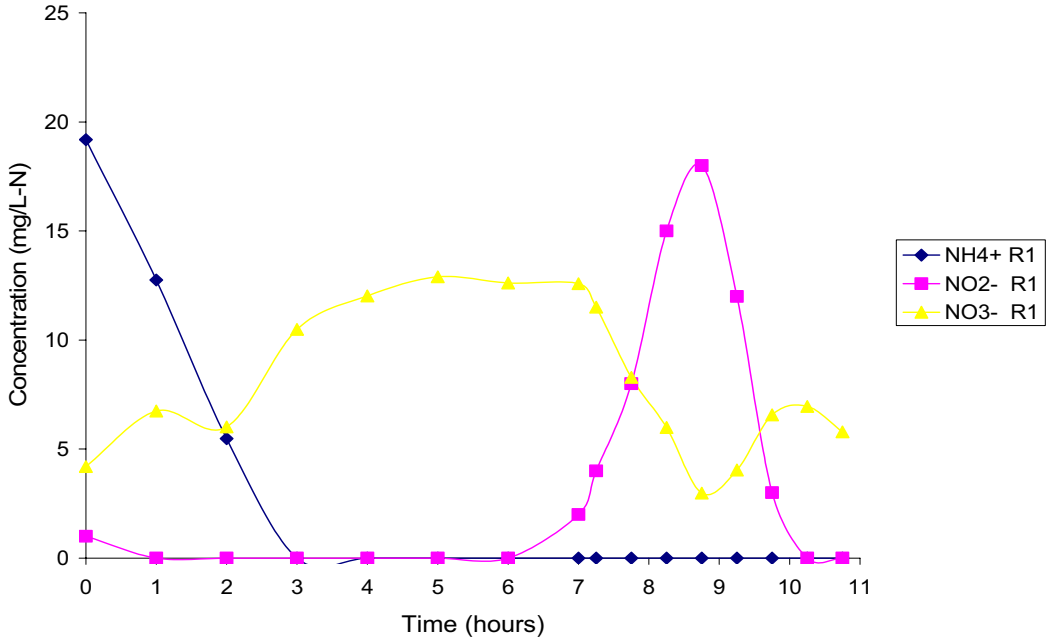


Figure 7 Nitrification and denitrification with an initial ammonia concentration of 20 mg/L-N in a without the methanol shown.

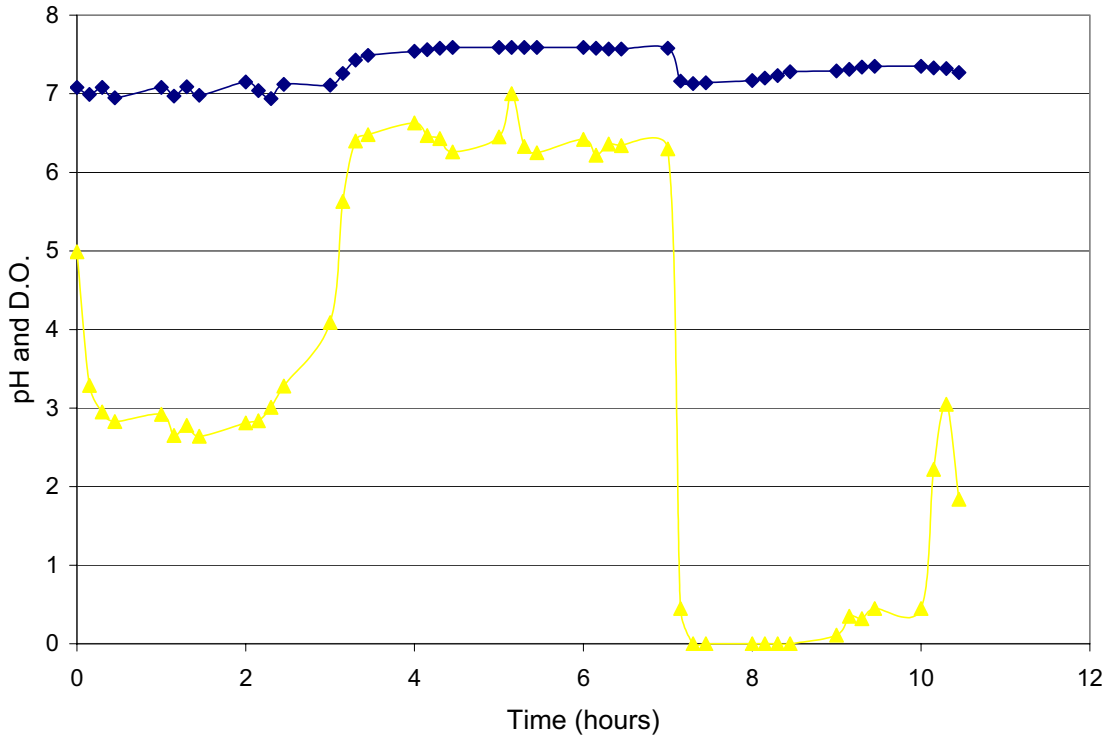


Figure 8 pH and dissolved oxygen during nitrification and denitrification.

FUTURE RESEARCH DIRECTIONS

- Establish a culture on a synthetic wastewater, acclimating the biomass to an influent concentration of 1000 mg/L
- Determine the ability to perform nitrification then denitrification in the same system, completing the nitrogen cycle
- Feed the culture the effluent rich in nitrogen from solid waste treatment
- Add chabazite and clinoptilolite to the acclimated biomass to determine enhancement of the capability of the mixed culture to nitrify and denitrify
- Optimize performance of nitrification/denitrification with zeolite while minimizing important ESM parameters

TRAINEES

M.S. Students: Ressa Chee Wah and Emma Bruce
Undergraduate Students: Hugh-Berk Sinclair and Kurt Smith

PUBLICATIONS

Glass, C.C. and Chee Wah, R. “Enhancement of Ammonium Adsorption Capacity with Pretreated Zeolites” Submitted to Habitation in July 2005.

Glass, C.C. and Chee Wah, R. (2004). "Nitrogen Recovery during Solid Waste Treatment in Advanced Life Support." *International Conference on Environmental Systems*, Paper #04-01-2514.

PRESENTATIONS

Glass, C. C. and R. Chee Wah, (2004). “Nitrogen Recovery during Solid Waste Treatment in Advanced Life Support.” Presented at the Habitation Conference, Orlando, FL.

Glass, C. C. and R. Chee Wah, (2004) "Nitrogen Recovery during Solid Waste Treatment in Advanced Life Support" Presented at the *International Conference on Environmental Systems*, Colorado Springs, CO.

Sinclair, H. (2004), “Ammonium adsorption by ion exchange using pretreated zeolites,” Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Khunjar, W. (2003), “Biological Nutrient Removal in a Multi-Stage Attached Growth System,” Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Chee Wah, R. (2003), “Ammonium removal by ion exchange using different types of zeolites,” Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Fish in Space: High-Quality Food Production Coupled with Minimization of Equivalent System Mass

Principal Investigator: Paul B. Brown, PhD

BACKGROUND

Nutrient intake by crews living on other planets will be supplied by perpetual production on site, transporting stored food, or a combination of the two approaches. Production of food on site yields fresh food products, but results in waste products that must be degraded. Any benefit of on-site food production must outweigh the costs associated with transporting food from Earth to remote colonies. To achieve this goal, food production and waste minimization must be linked within the overall flow of chemical compounds to achieve efficiencies not yet realized in earth-bound food production systems. Those linkages exist between fish and plant production.

Fish live in water and water is an essential nutrient for all plants. Further, fish excrete ammonia as their primary nitrogenous waste product and many species of plant can utilize ammonia as a source of nitrogen, also an essential nutrient for plants. Approximately 15 minerals are essential nutrients for fish, but efficiency of uptake is less than 100%, resulting in excretion of minerals from the fish. Water, nitrogen and minerals are the primary nutrients required for plant growth. Integrated fish and hydroponically grown crops (aquaponics) has been a focal area for researchers on Earth for over 30 years, but the quantitative flows of nutrients through this integrated system are poorly understood. The other unknown is gas balance between fish and plants. Fish uptake oxygen and excrete carbon dioxide, while plants uptake carbon dioxide and produce oxygen. The entire aquaponic system produces edible biomass, but there are also waste products generated from both fish and plants. The goal of our project is evaluation of fish, specifically the Nile tilapia, as a source of food and as a component in reducing equivalent system mass.

Our project began by acquiring a recognized stock of pure Nile tilapia and establishing broodstock, hatching and fry rearing production systems at the Aquaculture Research Laboratory, Purdue University. We then examined their willingness to accept several of the plant waste products under development by collaborators evaluating potential plant foods. All products were accepted by juvenile tilapia and we declared tilapia the formal species to conduct further testing. We are continuing our evaluations of waste products by examining the nutrient concentrations of products as they become available from collaborators. Our research, and research findings from collaborators, identified fiber as problematic in an Advanced Life Support System (ALS). Research this year became focused on fiber use and degradation by fish, as well as a continuation of previous research.

PROJECT GOALS AND OBJECTIVES

- Quantify nutrient concentrations of tilapia
- Quantify degradation of fiber in tilapia by fully characterizing gut microflora and enzymes secreted by fish .
- Initiate evaluations of alternative production systems relative to traditional approaches.
- Continue characterizing nutrient concentrations in waste products.
- Quantify nutrient concentrations in plant waste products that had been used for fungal (mushroom) growth and the acceptance of those food products by tilapia.
- Quantify mineral flows from fish to plants.

RESEARCH PROGRESS

Approximately 2 weeks prior to starting the third year of the NSCORT project (November 13, 2004), the Aquaculture Research Laboratory was completely destroyed by fire. Temporary facilities have been occupied, fish have been reacquired and experimental systems have been reestablished, but we were obviously delayed in starting research this year.

Nutrient concentrations in tilapia. We completed our initial evaluations of nutrient concentrations in tilapia. We conducted a near exhaustive nutrient profile for both whole fish and fillets. Fatty acid concentrations are presented in Table 1, amino acid concentrations are presented in Table 2 and a comparison of crew nutrient requirements to the nutrient concentrations found in tilapia is presented in Table 3. Amino and fatty acid requirements for crews are not presented in Table 3 as those values have apparently not been developed. Based on these data, tilapia should be considered a high-quality food for crews.

Fiber Degradation. We initiated a study in which we are quantifying gut microflora and gastrointestinal tract enzymes present in tilapia fed fiber concentrations ranging from 0 to 20% of the diet. Two new collaborations were established with this project. Dr. Ching-Ching Wu, Purdue, Animal Disease Diagnostic Laboratory is a bacteriologist with a fully equipped laboratory for quantifying both aerobic and anaerobic bacterial populations, and Dr. Jiri Adamec is a lead scientist in the newly established Bindley Biosciences Complex. The Bindley Complex has individual Genomics and Proteomics Centers. Our work will be conducted in the Proteomics Center. We will collect samples and characterize bacterial flora as a function of fiber ingestion in tilapia. Additionally, we will isolate enzymes in the gastrointestinal tract as a function of fiber ingestion. Samples will be collected from three segments of the intestine and proteins separated with one- and two-dimensional gel electrophoresis. Treatment differences will be determined with a gel imaging system and those spots will be harvested for protein characterization and quantification using LC-MS. This will be an important step in understanding fiber degradation in tilapia.

Alternative Production Systems. The integrated aquaponics systems, as developed and used on Earth, are large and cumbersome. We are exploring the potential of altering the flow of nutrients within a fish production system from the integrated aquaponics system to a stand-alone fish production system utilizing heterotrophic bacteria in combination with nitrifying bacteria. The modified system appears more efficient and our initial trial is underway. We are comparing an integrated system with a modified heterotrophic fish production system. Growth of fish as a function of nutrient inputs and resulting water quality (dissolved oxygen, pH, ammonia-N, nitrite-N, nitrate-N, chemical oxygen demand, and alkalinity) are the primary responses being quantified in this initial evaluation.

Characterization of nutrient concentrations. We recently acquired a gas chromatograph from our insurance settlement that we will use to quantify fatty and amino acids in waste products as they become available from collaborators evaluating crop species. Based on our initial evaluations of waste products, we identified those nutrient categories as limiting for tilapia growth. Fatty acids will be separated and quantified using standard methods published by the American Oil Chemists' Society employing methyl esterification of individual fatty acids. Unknowns will be compared to standards acquired from commercial sources. We will use a new method for separating and quantifying amino acids with GC and flame ionization detection. The EZ-FAST method was recently released by Phenomenex and we will be working with them to validate their new method with our specific tissues. The basics are similar to all other amino acid analysis methods in that proteins are hydrolyzed, derivatized then separated. The obvious difference is that amino acids are separated using gas as the mobile phase instead of organic solvents. Given our earlier results indicating tilapia will consume a wide range of waste products, we feel nutrient characterization is sufficient for the next year. A final feeding study will probably be warranted

once collaborators finalize decision regarding candidate species of plant. Our initial fatty and amino acid analyses are being conducted with soybean residue that has been used as a substrate for fungal growth. Working with Drs. Mitchell and Beyl, we acquired soybean residue, sent those to Dr. Beyl where she inoculated the residue with fungi. We acquired treated and untreated samples and are conducting the analyses now.

Fungi treated crops as food for tilapia. Due to limited biomass, we were only able to quantify the nutrient concentrations of soybean residue treated with fungi. We acquired inoculum from Dr. Beyl and treated a separate sample of cow pea waste, collected that product and are feeding treated and untreated cowpea waste to juvenile tilapia. Full nutrient characterization and quantification is underway. Biological responses from fish will also be quantified. This project will help direct the flow of fibrous plant residues within the ALS to maximize degradation and efficient production of food.

Quantify mineral flows. The mass balance of minerals from feed or waste products, through fish then into solution for plant use is one of the keys areas in aquaponics. We established seven aquaponics systems with fish and lettuce, then modified diets to test the limiting nutrients for plants from the fish feeds. Boron, iron and manganese appear to be the limiting nutrients for plants. We established a positive control, a negative control, and test diets with each mineral alone or in combination. This study was lost in the fire that destroyed the Aquaculture Research Laboratory and, using that well water source, all minerals were deficient for plants. In our temporary laboratory, manganese concentrations are sufficient for plant growth, so we are limited in the tests we can run. We are feeding fish in that study and will replicate the study once we move into our reconstructed building (anticipated completion date January 2006). Our goal is to quantify mass balance in a static system so our evaluation of systems can be more complete than previous efforts.

Table 1. Amino Acid Concentrations of Whole Body, Fillet and Carcass Residue of Nile Tilapia Fed a Fish Meal-Free Diet (% protein, dry matter basis).

Amino acid	Whole body	Fillet	Carcass
Methionine	1.16	3.36	0.65
Cystine	0.41	1.22	0.22
Lysine	2.19	4.58	1.23
Phenylalanine	1.03	2.03	0.58
Leucine	2.21	4.26	1.25
Isoleucine	1.23	2.39	0.69
Threonine	1.51	3.07	0.84
Valine	1.27	2.45	0.72
Histidine	0.76	1.41	0.43
Arginine	2.01	4.60	1.13
Alanine	2.11	5.00	1.18
Tryptophan	0.20	0.58	0.11
Tyrosine	0.96	1.72	0.54
Aspartic Acid	2.73	5.39	1.54
Serine	1.55	3.10	0.87
Glutamic Acid	4.41	9.29	2.48
Proline	1.86	4.33	1.04
Hydroxyproline	0.63	2.45	0.35
Glycine	2.43	6.38	1.36

Table 2. Fatty Acid Concentrations of Whole Body, Fillet, and Carcass Residue of Nile Tilapia Fed a Fish Meal-Free Diet (% lipid, dry matter basis).

Fatty acid	Whole body	Fillet	Carcass
Lauric (12:0)	0.01	0.00	0.01
Myristic (14:0)	0.91	0.17	1.31
Myristoleic (14:1)	0.06	0.00	0.07
Pentadecanoic (15:0)	0.08	0.02	0.09
Palmitic (16:0)	4.62	1.05	5.73
Palmitoleic (16:1)	1.38	0.03	1.73
Hexadecadienoic (16:2)	0.11	0.02	0.13
Hexadecatrienoic (16:3)	0.11	0.02	0.13
Hexadecatetraenoic (16:5)	0.04	0.01	0.05
Table 2, continued			
Heptadecanoic (17:0)	0.10	0.02	0.12
Stearic (18:0)	1.50	0.35	1.86
Oleic (18:1)	6.28	1.24	7.80
Linoleic (18:2n-6)	4.12	0.88	5.11
Linolenic (18:3n-3)	0.55	0.12	0.68
Octadecatetraenoic (18:4)	0.09	0.02	0.11
Arachadic (20:0)	0.06	0.05	0.07
Eicosenoic (20:1)	0.31	0.06	0.38
Eicosadienoic (20:2)	0.13	0.03	0.16
Eicosatrienoic (20:3)	0.15	0.04	0.18
Arachadonic (20:4n-6)	0.24	0.08	0.29
Eicosapentaenoic (20:5n-3)	0.28	0.05	0.34
Uncosapentaenoic (21:5)	0.04	0.01	0.05
Behenic (22:0)	0.02	0.00	0.02
Eruric (22:1)	0.02	0.01	0.02
Docosatetraenoic (22:4)	0.06	0.02	0.07
Docosapentaenoic (22:5)	0.79	0.17	0.98
Docosahexaenoic (22:6n-3)	1.22	0.36	1.50
Nervonic (24:1)	0.03	0.00	0.04
∑ n-6	4.36	0.96	5.40
∑ n-3	2.05	0.53	2.52
∑ n-3:n-6	0.47:1	0.55:1	0.46:1

Table 3. Nutritional Concentrations of a One Hundred Gram Tilapia Whole Body and Fillet Compared to Known Daily Nutritional Requirements for International Space Station Missions up to 360 D.

Nutrient	Requirement[a]	Whole Body[b]	Fillet[b]
Energy (KJ)	WHO equation[c]	2595.00	2135.00
Protein (%)	12.00-15.00	57.97	83.37
Carbohydrate (%)	50.00	6.96[d]	6.75[d]
Fat (%)	30.00-35.00[e]	23.31	4.78
Vitamin A (mg)	1.00	> 44.00[f]	> 44.00[f]
Vitamin D (µg)	10.00	160.80[f]	574.20[f]
Vitamin E (mg)	20.00	11.77[f]	19.88[f]
Vitamin K (µg)	80.00	N/D[g]	N/D[g]
Ascorbic Acid (mg)	100.00	255.52	49.90
Cyanocobalamin (µg)	2.00	8.90	9.85
Pyridoxine (mg)	2	0.03	0.06
Thiamin (mg)	1.50	0.03	0.96
Riboflavin (mg)	2.00	N/D[g]	N/D[g]
Folate (µg)	400.00	21.73	18.45
Niacin (mg)	20.00	17.05	9.43
Biotin (µg)	100.00	10.25	14.30
Pantothenic Acid (mg)	5.00	0.90	0.93
Inositol (mg)	N/R[h]	40.04	4.48
Total Choline (mg)	N/R[h]	356.40	237.60
Calcium (mg)	1000-1200	476.15	29.75
Phosphorous (mg)	1000-1200	25.87	2.72
Magnesium (mg)	350	12.75	15.42
Sodium (mg)	1500-3500	39.47	0.74
Potassium (mg)	3500	5.68	14.04
Iron (mg)	10	0.03	0.34
Copper (mg)	1.5-3.0	0.05	0.00
Manganese (mg)	2-5	0.02	0.13
Flouride (mg)	4	N/D[g]	N/D[g]
Zinc (mg)	15	1.35	0.05
Boron (mg)	N/R[h]	0.04	0.06
Cobalt (mg)	N/R[h]	0.06	0.00
Molybdenum (mg)	N/R[h]	0.57	0.10
Selenium (µg)	70	705.00	13.67
Iodine (µg)	150	N/D[g]	N/D[g]
Chromium (µg)	100-200	7.10	2.94

[a] Modified from Lane and Feedback (2002)
[b] Reported on a dry matter basis
[c] Requirements calculated with WHO equation, accounting for weight, age, sex, and activity level
[d] Reported as nitrogen free-extract
[e] Reported as percent dietary intake
[f] Reported as IU/Kg
[g] Not Determined
[h] Not Reported

FUTURE RESEARCH DIRECTIONS

- Continue quantifying critical nutrient concentrations for fish from plant waste products as they become available from collaborators.
- Determine the combinations of waste residues that meet the nutritional requirements for tilapia.
- Evaluate the ability of tilapia to reproduce when fed waste products.
- Quantify gas flows between plants, fish, water and air.
- Continue evaluation of alternative fish production systems that reduce equivalent system mass.

TRAINEES

PhD Student:	John. M. Gonzales
Undergraduate Students:	Megan Rosinski

RESEARCH COLLABORATION

- Dr. Cary Mitchell provided crop wastes for evaluation as feed.
- Dr. Mitchell provided crop waste and Dr. Caula Beyl provided fungal inocula for treating cow pea waste.
- Dr. Beyl treated soybean residue, then sent those samples back to us for nutritional characterization.
- Dr. Ching-ching Wu will be collaborating on bacterial characterization of gastrointestinal tracts of fish.
- Dr. Jiri Adamek has been instrumental in establishing a proteomics collaboration for identifying and quantifying proteins in the gastrointestinal tract of fish.

PUBLICATIONS

Gonzales, J.M., A.H. Hutson, M.E. Rosinski, P.B. Brown, Y.V. Wu and T.F. Powless. Evaluation of fish meal-free diets for first feeding Nile tilapia, Oreochromis niloticus. Journal of Applied Aquaculture. In Review.

Gonzales, J.M. and P.B. Brown. Potential impacts of Nile tilapia Oreochromis niloticus on nutrition and equivalent system mass of an Advanced Life Support System: initial considerations. Journal of Advances in Space Research. In Press.

PRESENTATIONS

Gonzales , J.M., Y.V. Wu, T. Powless and P.B. Brown. 2004. Evaluation of fish meal-free practical diets for first feeding Nile tilapia Oreochromis niloticus. Aquaculture 2004, Honolulu, HI.

Alleman, James E., P.B. Brown, C.C. Glass, B.C. Joern and J.C. Volenec. 2004. Coordinated Biochemical Stabilization and Resource Recovery with Human-, Plant-, and Food-Derived ALS Solid Wastes. Habitation 2004, Orlando, FL.

Gonzales, J.M., Jr. and P.B. Brown. 2005. Quantitative and qualitative characterizations of the nutritional impact Nile tilapia Oreochromis niloticus may offer in an advanced life support system. Aquaculture America 2005, New Orleans, LA.

BIO REGENERATIVE ENVIRONMENTAL AIR TREATMENT FOR HEALTH (BREATHE): INTEGRATED STAR OFF-GAS, CABIN AIR, AND GRAYWATER PROCESSING

Principal Investigator: M. Katherine Banks, PhD, PE

BACKGROUND

An integral part of a NASA life support system is the ability to recycle air and water. The Bio-Regenerative Environmental Air Treatment for Health (BREATHe) process is an important component of the NSCORT integrated system that will recycle air and water during a long duration (more than 365 days) mission to Mars. The BREATHe system will consist of two biotrickling filter reactors that will simultaneously treat contaminated air and water. BREATHe I will primarily treat graywater and gas effluent from a biological solids treatment process expected to generate NH_3, H_2S, and CO_2. The BREATHe system will enable efficient treatment of water and air while minimizing mass, volume, power, and crew time maintenance requirements.

The design and optimization of the BREATHe reactors will involve an in-depth developmental phase where bench scale reactors will be used to simulate full-scale systems. During the developmental phase of the project, design parameters such as size, recirculation rates, and flow rates will be optimized. It will be essential to use simulated waste streams for this study because it is not possible to obtain realistic waste streams on a regular basis Anticipated chemical concentrations will be predicted for graywater, cabin air, atmospheric condensate, and effluent gas from the solid waste treatment system. In addition, because each reactor will treat both gas and liquid phases, it also will be important to assess liquid/gas equilibrium for each chemical constituent in a complex waste matrix.

PROJECT GOALS AND OBJECTIVES

- Construct and operate a representative bench-scale biotrickling filter treatment system.
- Optimize key design parameters identified through a first generation model, specifically packing material surface area and liquid velocity.
- Assess biodegradability of surfactants of concern, sodium laureth sulfate (SLES) and disodium cocoamphodiacetate (DSCADA).
- Screen for potential surfactant degradation byproducts in bioprocessor effluent.
- Determine Monod kinetic parameters for surfactants of interest.
- Quantify Henry's constants for target gas contaminants (NH_3 & H_2S) when present as gas mixtures and in the presence of surfactant.

RESEARCH PROGRESS

Operation of Bench Scale Biotrickling Filters. Results from the first generation model presented in last year's report indicated that contaminant removal rates in the liquid phase are highly dependent on packing material surface area and liquid velocity with removal rates increasing with increases in the former and decreasing with increases in the latter. Based on these results, it was determined that packing materials are an extremely important design consideration since not only does their surface area have an effect on microbial activity, but the geometry and size can have a significant impact on liquid distribution and velocity though a biotrickling filter. The effects of several packing materials on liquid velocity, or residence time, in the trickling filters were quantified through a series of tracer tests (data not shown). Tri-pack packing material was used for bench-scale experiments conducted in 2004 where steady state TOC removal was on average 62%. It was hypothesized that use of packing material with a higher surface area would increase process performance due to a combination of increased area for microbial growth and decreased liquid velocity. Qualities of the packing materials used in this study are

summarized in Table 1. Results for TOC removal in replicate reactors are shown in Figure 1. In all cases, the reactor startup phase took approximately 40 days during which point TOC removal was highly variable. A one sided t-test showed that removal rates were not significantly improved using Biobale or Bee-cell packing material at the 95% significance level. At this point it was hypothesized that TOC removal may be limited by liquid distribution in the reactor resulting in incomplete utilization of packing material surface area. The recommended hydraulic loading rate to optimize liquid distribution in trickling filters is 1.94 m/hr, which would require a recirculation rate of 75X for the constructed bench-scale reactors. A recirculation rate of 20X was employed for data presented thus far. On day 107 of operation, the recirculation rate was increased from 20X to 75X (Figure 2). Again, TOC removal was not improved. During these experiments, surfactants were analyzed by LC-MS methods (Figures 3 & 4) to further characterize the composition of effluent TOC. Interestingly, surfactant removal was on average 99%. It has come to our attention that biodegradation of SLES and/or DSCADA may result in a recalcitrant byproduct contributing to effluent TOC.

Table 1. Packing Material Characteristics

Packing Material	Surface Area (m^2/m^3)	Diameter (cm)
Tri-packs	281	2.5
Biobale	825	0.4
Bee-cell	653	1.3

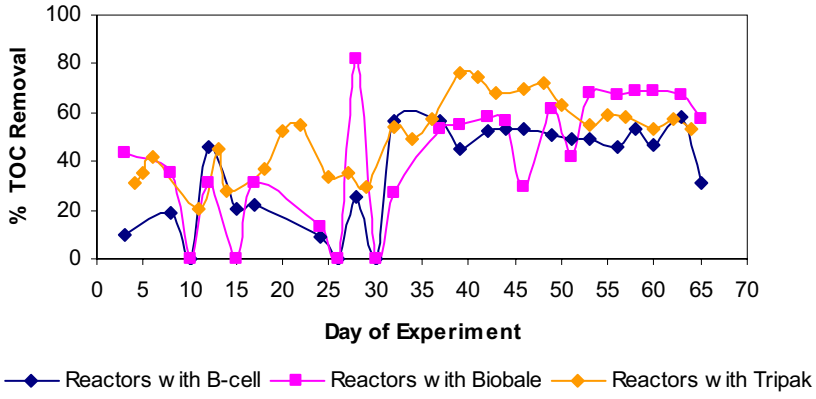


Figure 1. Average TOC removal for replicate reactors packed with different packing material (error bars not shown for clarity).

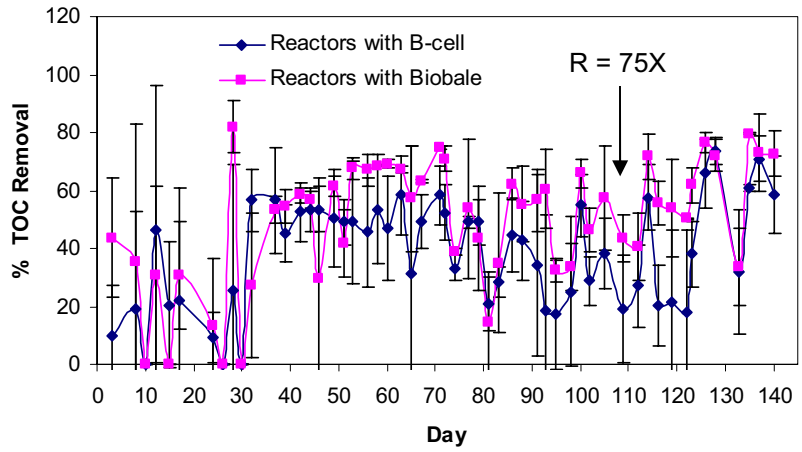


Figure 2. TOC removal before and after an increase in recirculation rate on day 107 from 20X to 75X.

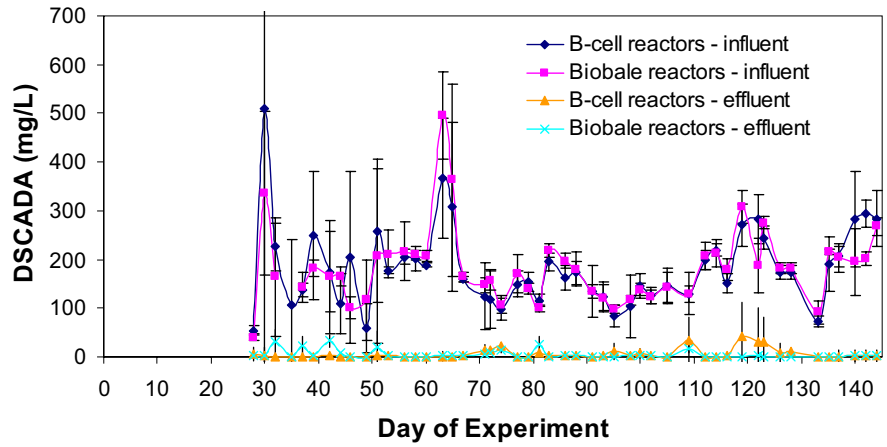


Figure3. Influent and effluent levels of DSCADA for 3 replicated reactors packed with B-cell and 3 replicate reactors packed with Biobale.

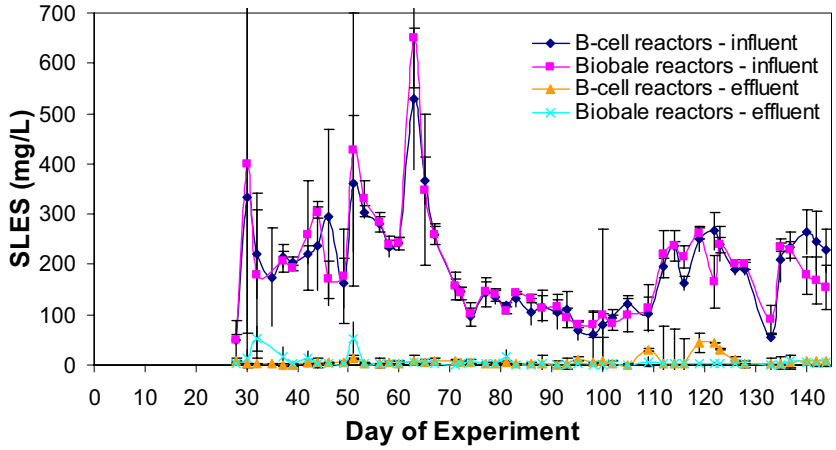


Figure 4. Influent and effluent levels of SLES for 3 replicated reactors packed with B-cell and 3 replicate reactors packed with Biobale.

Biodegradability of SLES and DSCADA. A set of shaker flask experiments was conducted to assess the biodegradability of SLES and DSCADA. Prior to the start of experiments, a mixed culture of bacteria was acclimated to SLES and DSCADA degradation. Three replicate flasks were prepared that contained 248 ppm SLES and 5% by volume SLES acclimated bacteria and another three flasks were prepared that contained 218 ppm DSCADA and 5% by volume DSCADA acclimated bacteria. One experiment set was conducted using BREATHe graywater simulant as a nutrient medium and another was tested using a minimal salts medium (MSM). SLES was readily degraded in both nutrient media with TOC approaching zero as the tests ended (Figure 5). Conversely, TOC levels did not reduce below 40 mg/L in flasks containing DSCADA (Figure 6). Samples were also tested for COD, with similar trends observed (data not shown). COD approached zero in flasks containing SLES and did not go below 100 mg/L in flasks containing DSCADA. DSCADA degradation also was examined in a rich nutrient medium, tripticase soy broth, so that a more diverse microbial population could develop that may support complete degradation of DSCADA (Figure 7). In this situation, TOC did not go below 200 mg/L, indicating that even easily degradable carbon present in tripiticase soy broth was not degraded. It is likely that a DSCADA degradation byproduct is toxic to bacteria inhibiting their ability to grow and metabolize organic carbon. Degradation pathways for amphoteric surfactants, the group to which DSCADA belongs, have not been well characterized so it is difficult to predict the byproduct that creates toxicity issues. Toxicity issues have previously been noted for amphoteric surfactants as the quaternary nitrogen group existing in alkylamphoacetate surfactants has been found to cause antimicrobial activity (Domsch, 1995). However, this was typically overcome by bacterial acclimation to the surfactant of interest, which did not work in the case presented. Toxicity issues related to DSCADA degradation may be the cause of limited TOC removal in the BREATHe I system.

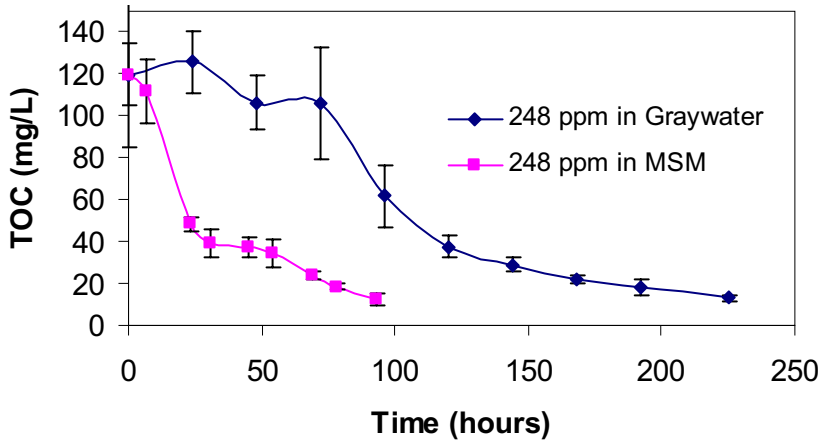


Figure 5. TOC removal in shaker flasks containing SLES.

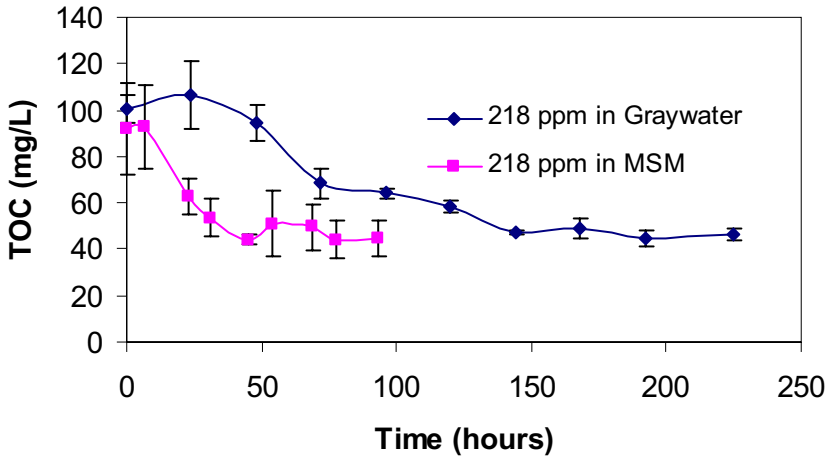


Figure 6. TOC removal in shaker flasks containing DSCADA**.**

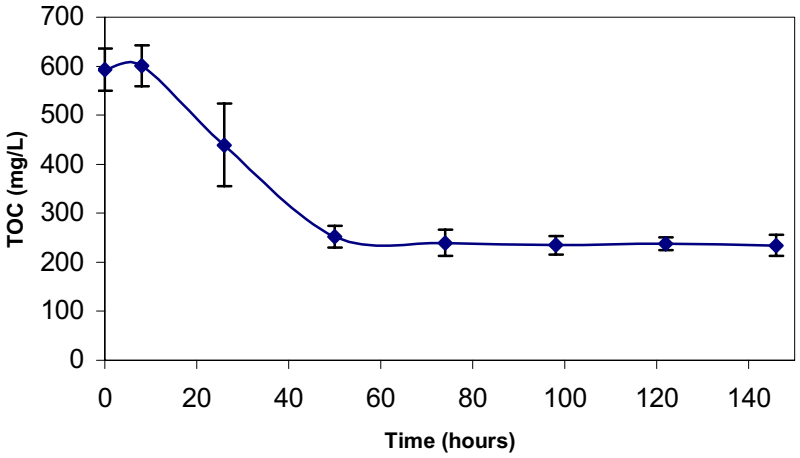


Figure 7. TOC removal in shaker flasks containing DSCADA in a tripticase soy broth medium.

Screening forPotential Surfactant Degradation Byproducts. The buildup of any contaminant within an advanced life support system could pose serious health risks to crew members. Therefore, it is important to identify surfactant degradation pathways and screen for potential byproducts in bioprocessor effluent. BDoxy biosensors were used to confirm degradation byproducts for Polyalcohol Ethoxylate (PAE), which is commonly used in laundry and dish wash detergents. BDoxy biosensors are 96 well plates containing an oxygen sensitive fluororphore. Substrate and bacteria can be added to each well and resulting fluorescence is indicative of microbial activity. Several substrates that have been identified in the literature as potential degradation byproducts of PAE were added to wells containing bacteria acclimated to PAE degradation. Substrates studied included ethylene glycol (EG), diethylene glycol, triethylene glycol, hexaethylene glycol, octaethylene glycol, glycolic acid, formic acid, glyoxilic acid, and oxalic acid. A positive response significantly higher than a control population would indicate acclimation of bacteria to that substrate and thus confirm presence as a degradation byproduct of the parent compound. Fluorescence of EG compounds is shown in Figure 8 where the y-axis is normalized relative fluorescence units (NRFU). All of the compounds had a significantly higher response than the control population

except for octaethylene glycol. The only organic acid that showed a response was glycolic acid (data not shown). Polyethylene glycols are clearly important degradation byproducts for PAE. It is quite possible that EG could be present in effluent of a bioprocessor treating PAE, particularly because it's degradation is the last step in the multi step process. EG is highly toxic for human consumption with effects such as vomiting, drowsiness, coma, respiratory failure, gastrointestinal upset, cardiopulmonary effects, renal damage, and potential fatality. EG is also a predicted degradation byproduct of SLES degradation. BREATHe effluent will be screened for EG in the future using a UV method that has previously been developed. If present in bioprocessor effluent, the fate of EG through post treatment processes such as membranes should be assessed.

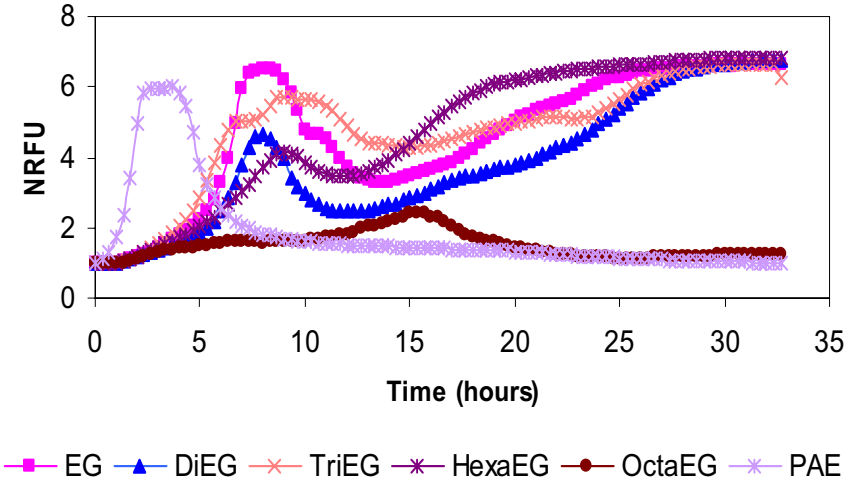


Figure 8. BDoxy results for EG compounds in a PAE acclimated inoculum.

Biokinetics of Sufactant Degradation. Monod kinetics have been determined for surfactants of interest SLES, DSCADA, and PAE using respirometry methods developed by Grady et al., 1989 (Table 2). Parameter estimates represent averages of six replicate tests.

Table 2. Monod Knetic Prameter Etimates for Srfactants of Iterest

	Growth Yield, Y (mg biomass COD formed per mg COD removed)	Decay , b (h^{-1})	Max. Specific Growth Rate, μ_{max} (h^{-1})	Half Saturation Constant, K_s mg/L COD
DSCADA	0.528	0.0059	0.0092	5.083
SLES	0.512	0.0137	0.0232	15.092
PAE	0.842	0.0011	0.0042	23.271

Mass Transfer of Gas Constituents. Henry's constants were determined for each of the combination of gases in the presence and absence of surfactants. Initially, 600 mL of air and 400 mL of solvent were allowed to equilibrate and gas measurements were made. Known volumes of calibration gas were injected into the system and the system was again allowed to equilibrate. Note that only small volumes of gas were injected into the system as Henry's Law is only valid for dilute solutions. Distribution curves allowed the Henry's constants to be determined for combinations of H_2S, CO_2, and NH_3 gases as listed in the first row of Table 3. Surfactants did not have a significant effect on solubility for any of the gas combinations tested. The largest effect on abiotic system mass transfer seemed to be the presence of CO_2,

as the solubility of both NH_3 and H_2S seemed to slightly increase. Due to the large range of Henry's constant in the literature, none of the gas mixtures and/or surfactants can be attributed to changes in solubility.

Table 3. Henry's Constants for NH_3 and H_2S in Gas Mixtures in the Presence and Absence of Surfactant

Gas Mixture $[NH_3:H_2S:CO_2]$	Milli-Q H (NH_3) [-]	Surfactant H (NH_3) [-]	Milli-Q H (H_2S) [-]	Surfactant H (H_2S) [-]
1:0:0	176.2 ± 25.4	169.7 ± 31.9	-	-
1:0:2.1	214.2 ± 40.0	198.2 ± 19.4	-	-
1:1.7:5.6	161.7 ± 35.2	185.6 ± 6.5	0.25 ± 0.06	0.31 ± 0.11
1:2.1:0	175.2 ± 26.7	201.4 ± 20.1	0.19 ± 0.16	0.24 ± 0.09
0:1:71.3	-	-	1.30 ± 0.41	1.24 ± 0.32

FUTURE RESEARCH DIRECTIONS

- Confirm the ability to simultaneously treat graywater and a waste gas contaminated with hydrogen sulfide, ammonia, and carbon dioxide.
- Determine a maximum loading capacity for both NH_3 and H_2S gases at which an increase in contaminants becomes detrimental to reactor performance.
- Provide information to the systems group about how BREATHe I performs under a variety of conditions.
    - Generate data for models to determine how BREATHe I should be operated and pretreatment requirements for waste streams
- Validate the physicochemical component of the developed mathematical model using NH_3 gas and clean water.
- Characterize the biodegradation pathway for DSCADA.
- Use BDoxy biosensors to screen for most important degradation byproducts for SLES and DSCADA.
- Screen for EG and other trace contaminants of concern in BREATHe I effluent.
- Optimize performance of BREATHe I while minimizing important ESM parameters

TRAINEES

PhD Students: Sybil Sharvelle and Eric McLamore
Undergraduate Students: Katherine Graham, Erin Malony, Chris Ghattas, Joi Dunham, Rebecca Lattayak, Stephan Clark, Elizabeth Skvarenina

RESEARCH COLLABORATION

- Banks co-chaired the ICES session on "Biological Treatment for Water Recycling" with Jay Garland from KSC. Due to the large number of papers submitted to this session, two periods, morning and afternoon, were devoted to this topic, 2004.
- Kennedy Space Center – Jay Garland is a member of Sybil Sharvelle's PhD committee. Sybil has traveled to KSC several times for research interaction, 2004.
- Johnson Space Center – Banks has communicated with researchers at JSC and Texas Tech. to provide updates on NSCORT progress with BREATHe, 2004 and 2005.
- Banks attended the NASA Biological Water Treatment Workshop held in Houston, TX, 2004.
- Banks visited the University of Florida's NASA Commercialization Center, 2004.

- BREATHe meetings are conducted with Al Heber's and K. Banks' research groups, 2004 and 2005.
- Monthly Water Group Telecons are conducted with PIs and students from Purdue and Howard, 2004.
- Sharvelle collaborated with Kim Jones' research group at Howard University to determine a realistic simulant of BREATHe effluent that can be used to test membrane efficacy, 2005.
- Sharvelle presented at the Gordon Research Conference on Engineering for Space Sciences in Les Diablerets, Switzerland, 2005.
- Banks traveled to KSC to present research results to ALS group, 2005.
- McLamore and Banks are serving as co-chairs for an ALS session at the 2006 ASCE Earth and Space Conference, Houston, TX.

PUBLICATIONS

Sharvelle, Sybil, Eric McLamore, Yong Sang Kim, Stephen Clark, and M.K. Banks (2005). "Characterization of Effluent from Biological Trickling Filters Treating Graywater in Advanced Life Support Systems." *Habitation*, under review.

Shah, Neepa, Sybil Sharvelle, and M.K. Banks (2005). "Influence of Support Media Characteristics on Biofilm Activity in Graywater Treatment Systems for Advanced Life Support." *Habitation*, under review.

Sharvelle, Sybil, M.K. Banks, Eric Mclamore, Yong Sang Kim, and Stephen Clark (2005). "Evaluation of Trickling Filter Performance for Graywater Treatment in ALS Systems." *International Conference on Environmental Systems*, Rome, Italy.

Sharvelle, S. E., K. Banks, K. Graham and E. Maloney (2004). "Graywater Treatment Using Biofilm Reactors for Water Recycling in Advanced Life Support." *Proceedings of the ASCE Earth and Space Conference*, Houston, TX.

Sharvelle, S. E., M. K. Banks and E. Maloney (2004). "Surfactant Biodegradation for Application to Advanced Life Support Water Recycling Systems." *International Conference on Environmental Systems*, Paper #04-01-2513.

Sharvelle, Sybil, Banks, M. K., A. J. Heber (2003), "Wastestream Characterization for a Packed Bed Biofilter Intended for Simultaneous Treatment of Graywater and Air," *International Conference on Environmental Systems*, Paper #03ICES-182.

PRESENTATIONS

Banks, M. K., (2005). "Biodegradation of target surfactants in the BREATHe system" Presented at Kennedy Space Center ALS Research Group, FL.

Sharvelle, S., M.K. Banks, and Eric McLamore (2005). "Biological Treatment of Graywater and Waste Gas for Advanced Life Support." Presented at the Gordon Research Cenference, Les Diablerets, Switzerland.

Sharvelle, Sybil, M.K. Banks, Eric Mclamore, Yong Sang Kim, and Stephen Clark (2005). "Evaluation of Trickling Filter Performance for Graywater Treatment in ALS Systems." Presented at the International Conference on Environmental Systems, Rome, Italy.

Lattyak, R., S. Sharvlle, and M.K. Banks (2005). "Determination of Microbial Kinetics for Degradation of Surfactants in NASA Advanced Life Support Treatment Operations." Presented at the Student Undergraduate Research Funds Summer Symposium, Purdue University, West Lafayette, IN.

Skvarenina, E., S. Sharvlle, and M.K. Banks (2005). "Assessment of Surfactant biodegradability during Graywater Recycling for Advanced Life Support Applications." Presented at the Student Undergraduate Research Funds Summer Symposium, Purdue University, West Lafayette, IN.

Clark, S., S. Sharvlle, E. McLamore and M.K. Banks (2005). "Multicomponent Gas Mass Transfer in a Novel Bioreactor." Presented at the Student Undergraduate Research Funds Summer Symposium, Purdue University, West Lafayette, IN.

Banks, M. K., S. Sharvelle, and Y. Kim (2004), "Graywater and Gas Treatment for ALS using BREATHe," Presented at the Habitation Conference, Orlando, FL.

Banks, M. K. (2004), "Biological Treatment for Graywater and Cabin Air," Presented at the University of Florida Seminar Series, Gainesville, FL.

Heber, A.J., S.-H. Lee, H. Huang, S. Sharvelle, M. K. Banks, Y. S. Kim. (2004). "Testing biofilters for advanced life support." Poster presented at the Habitation Conference, Orlando, FL.

Layattak, R. (2004), "Identification of Surfactant Degraders in BREATHe," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Clark, S. (2004), "Operation and Maintenance of BREATHe," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Kim, Y. S. (2004), "Gas/Liquid Phase Equilbruim in BREATHe," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Banks, M. K., Sharvelle, S., and Y. S. Kim (2004), "BREATHe System: Design and Operation," Presented at the NASA Biological Workshop, Houston, TX.

Sharvelle, S. E., M. K. Banks and E. Maloney (2004). "Surfactant Biodegradation for Application to Advanced Life Support Water Recycling Systems," Presented at the International Conference on Environmental Systems, Colorado Springs, CO.

Sharvelle, S. E., K. Banks, K. Graham and E. Maloney (2004). "Graywater Treatment Using Biofilm Reactors for Water Recycling in Advanced Life Support." Presented at the ASCE Earth and Space Conference, Houston, TX.

Sharvelle, S. (2003), "BREATHe Treatment for Water and Air," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayettte, IN.

Maloney, E. (2003), "Design of BREATHe Reactors," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Graham, K. (2003), "Biodegradation of Surfactants in BREATHe," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayette, IN.

Sharvelle, S., M. K. Banks, and A. Heber (2003), "BREATHe Treatment for Air and Water," Presented at the International Conference on Environmental Systems, Vancouver, Canada.

MEMBRANE PROCESSES IN ALS

Principal Investigator: Kimberly L. Jones, Ph.D.

BACKGROUND

Membrane processes such as reverse osmosis are typically utilized in a water recovery system, including wastewater recycling for space missions, as wastewaters (grey water, urine) processed via biological processes will require final polishing to meet stringent potable/reuse drinking water standards mandated by NASA and EPA. Reverse osmosis (RO) membranes are well suited to remove contaminants from water and have been evaluated for use as a critical part of the treatment process in wastewater recycle during space missions. However, reverse osmosis membranes suffer from fouling, low flux, and high pressure requirements, especially during use for wastewater recycling. In fact, fouling has been identified as the major problem for the application of RO membranes to wastewater recycling applications for space missions. Fouling is typically defined as pore plugging and/or pore blocking resulting from the accumulation and deposition of material within the membrane pores and on the membrane surface. One way to mitigate fouling is to implement strategic pretreatment processes to reduce the contaminant load on the RO membrane system.

In the ALS NSCORT system, biological (PABLO-WABLO and BREATHe) physical (microfiltration) and chemical (UV disinfection) pretreatment will precede the final membrane polishing step (typically RO). This level of pretreatment is unprecedented in previous NASA water recovery systems and should greatly increase the lifetime of the polishing membrane, reduce fouling of that membrane, which will result in reduced ESM for the polishing membrane. It is hypothesized that, with such pretreatment steps, larger pore, higher flux nanofiltration (NF) membranes may be attractive alternatives to RO for the final polishing step. NF membranes have much reduced pressure and energy requirements when compared to RO membranes and will have much lower ESM. This project investigates the MF pretreatment membrane and NF polishing membrane and will optimize the integrated membrane system for wastewater recovery. All membrane materials used in the system will be low-fouling, high flux materials under development at Howard University.

PROJECT GOALS AND OBJECTIVES

- Modify commercially available NF membranes to improve efficiency for targeted contaminant removal
- Synthesize novel new NF membranes with increased flux and rejection properties
- Modify commercially available MF membranes to mitigate biofouling

RESEARCH PROGRESS

NF membranes are being synthesized at Howard University to decrease fouling and increase salt rejection for water reuse applications. NF membranes operate under much lower operating pressure than RO membranes, resulting in significantly reduced ESM for the system. Thus, focus is presently on developing new NF membranes to be used as alternatives to traditional high pressure RO membranes for recovery of space mission wastewater.

Thrust 1: Synthesis of novel new NF membranes with increased flux and rejection properties

Nanofiltration membranes modified to increase negative surface charge (indicated by increasingly negative zeta potential, Figure 1) exhibited higher salt rejection when compared to unmodified membranes (Figure 2). These membranes were modified by an ion implantation technique, which implanted the electronegative F^- ion into the active layer of the membrane in order to render the membrane more negative and increase rejection by electrostatic repulsion.

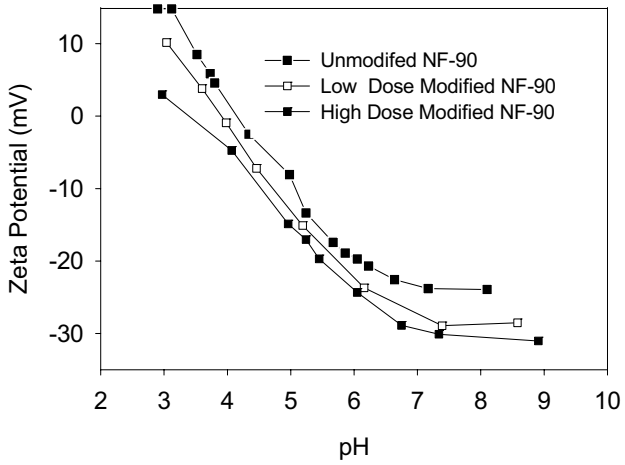


Figure 1. Zeta Potential of unmodified and modified cellulose acetate (SP28) membranes as a function of pH. Background electrolyte 0.001 M KCl.

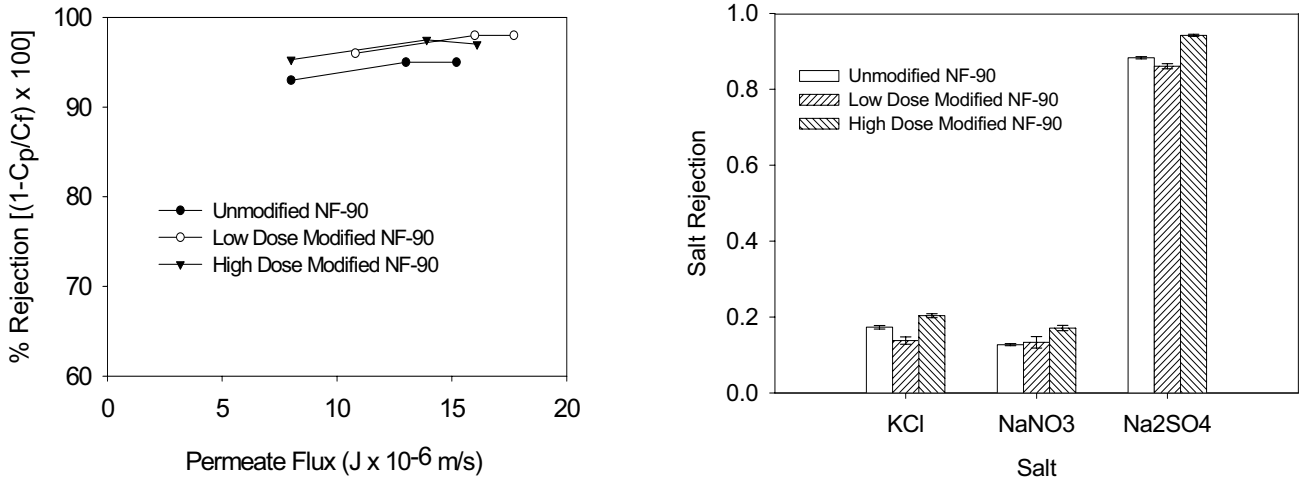


Figure 2. Rejection of organics (left) and salt rejection (right) for unmodified and modified polyamide (NF-90) membranes. Flux = 3.5 x 10^{-6} m/s. ΔP = 70 psi (483 kPa), 140 psi (966 kPa) and 190 psi (1310 kPa) respectively for unmodified, low modified and high modified. Salt concentration = 0.005 M KCl, 0.005 M $NaNO_3$ and 0.005 M Na_2SO_4 (ionic strength = 0.0125, 0.0125 and 0.005 M respectively). Feed pH = 6.4 ± 0.2

Based on these studies, membrane synthesis experiments were undertaken to develop novel new membranes with controllable pore size and surface charge, which will allow for higher flux and rejection.

Synthesis and Characterization. Cellulose acetate (CA) membranes were spun onto a polypropylene backing (Figure 3, left). The rejection properties of the CA membranes were varied by changing the initial concentration and spinning quenching conditions. These experiments were undertaken as part of a collaboration with the Nanobiotechnology Center at Cornell University. Effective pore sizes were measured by pressure driven rejection of uncharged solutes. The limiting rejection of an uncharged solute is directly proportional to the ratio of solute radius to pore radius. The surface charge was evaluated by electrokinetic analysis/streaming potential measurements. The zeta potential of the membrane, determined from electrokinetic analysis (EKA) was measured as a function of pH.

Results. The zeta potential (and thus the charge of the CA membranes) is negative and increases with increasing pH (Figure 3, right). The isoelectric point is pH 3.5, but the maximum zeta potential for basic pH increases with acetate concentration. Uncharged solute rejection shows a 1.7 nm pore radius for 25% acetate membranes, classifying them as nanofiltration with filtration mechanisms dependent on charge and steric hindrance. These properties are ideal for separation of salts such as NH_4^+, Na^+, and Cl^-. Membranes with molecular weight cutoffs (MWCOs) of 300, 350, and 600 Da have been fabricated, which should also reject residual surfactant monomers that may remain following BREATHe. Experiments are ongoing to determine the rejection of salts, organics and surfactant monomers from the synthesized membrane.

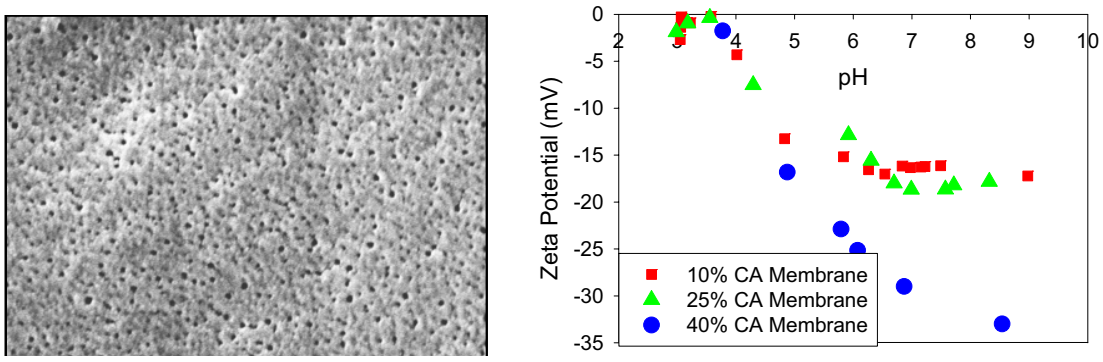


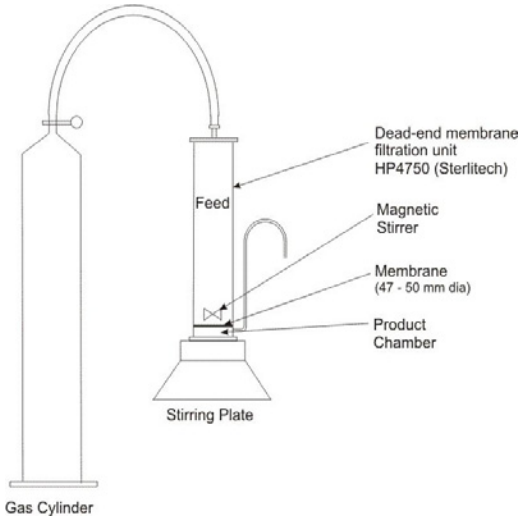
Figure 3. SEM of CA membrane (left) and zeta potential of synthesized CA membranes as a function of pH (right). The plots show CA membranes with increasing acetate concentrations. The solution pH was increased incrementally and the zeta potential was measured at each value.

Thrust 2: Reduction of Biofouling in Microfiltration Membranes using Membrane Modification

MF membranes will be used as pretreatment for the NF membranes. The large pore size in MF membranes results in lower pressure and higher flux than for the other pressure driven membranes, making MF membranes ideal for the low ESM requirements in ALS NSCORT. The largest problem with the MF membranes will likely to due to biofouling, as these membrane follow directly behind BREATHe, which may leak biosolids. The MF membranes will reject biosolids, but these materials may accumulate on the membrane surface and reduce flux. Experiments were begun to modify MF membranes by grafting acrylic acid onto the surface of these membranes, which will increase hydrophilicity of the surface and smooth the morphology, which reduces biofilm accumulation on the surface.

Flux. Polyethersulfone (PES) membranes of pore sizes 0.1 microns and 500 kDa were modified. Filtration experiments were carried out in an Amicon stirred cell at a fixed pressure of 20 psi (Figure 4). Flux experiments were conducted with clean water to establish initial permeability, then simulated wastewater was used in order to quantify the biofouling extent of the membranes.

Figure 4. Schematic of stirred cell for biofouling experiments.



Biofouling. Simulated wastewater was filtered for at least 6 hours or until the flux was at steady state. The fouled membrane was rinsed and replaced in the filtration cell and filtered with clean water. The membranes were the placed in a sodium pyrophosphate solution to remove the bacteria. The bacteria were then quantified using the pour plate method.

Modification. Acrylic acid was the monomer chosen mainly because of its antimicrobial properties, its ability to reduce irreversible fouling and its hydrophilicity. The membranes were coated with a photoinitiator by soaking them in 0.25M benzophenon in ethanol. The membranes

were dried and acrylic acid with the inhibitor removed was placed on the active side of the membrane. The membranes were then UV irradiated and after polymerization the membranes were extracted with methanol/water solution and washed with sodium hydroxide Ultrapure water to remove any non-grafted polymer, monomer or residual initiator.

Results: Flux declines of the modified and unmodified MF membranes are shown in Figure 5 using clean water. The modified membrane showed less severe flux decline than the unmodified membrane, most likely due to the increased hydrophilicity of the modified membrane.

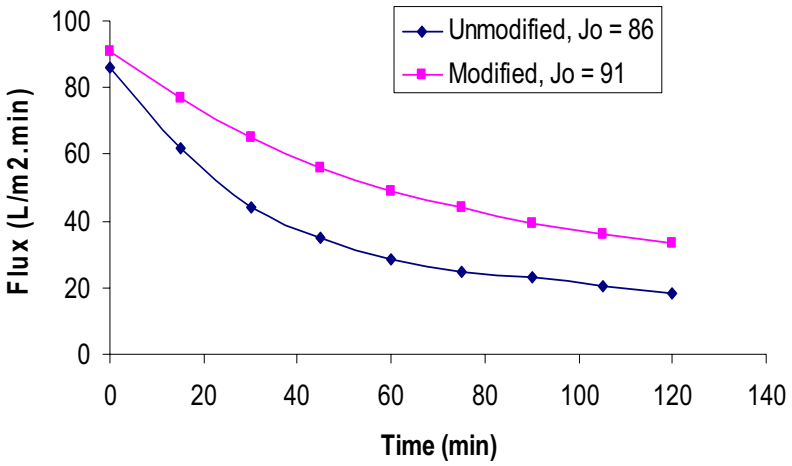


Figure 5. Flux decline of 0.1 μm PES membranes. Membranes were modified using acrylic acid. Pressure 20 psi, clean water flux.

Normalized flux values are shown in Figure 6 using synthetic wastewater. Wastewater is a surrogate for BREATHe effluent, with contaminant values reported from Purdue University. The modified MF membrane exhibited enhanced flux after modification, while the modified UF membrane showed reduced flux following modification. Mechanisms governing this behavior are being investigated.

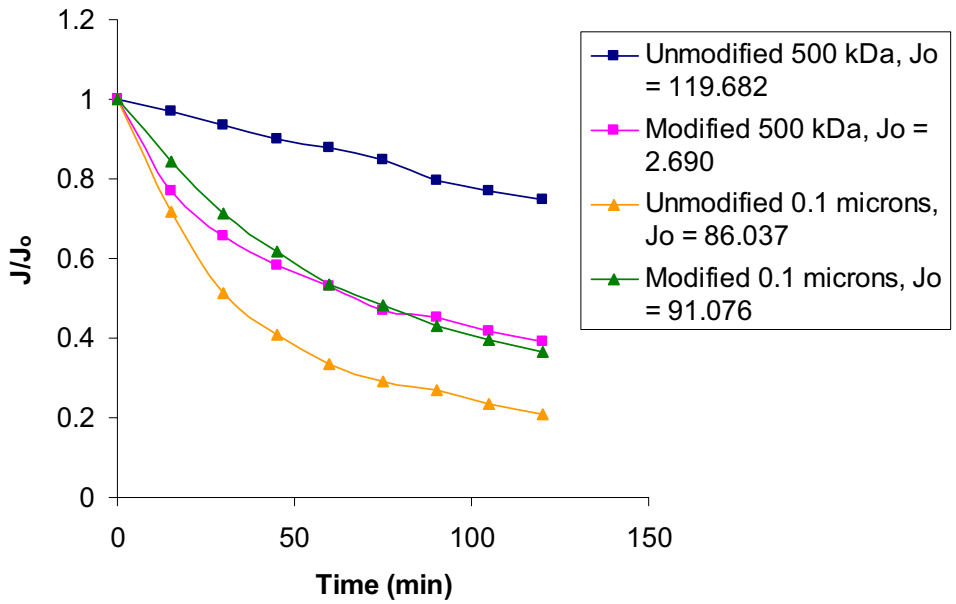


Figure 6. Flux reported as normalized flux for modified and unmodified UF and MF membranes using synthetic wastewater. UF membrane has MWCO of 500 kDa, MF membrane has a pore size of 0.1 μm.

FUTURE RESEARCH DIRECTIONS

- Evaluate flux characteristics of the synthetic CA membranes.
- Synthesize robust polyamide NF membranes with higher charge and larger pores to enhance flux and rejection.
- Determine fouling and rejection characteristics of synthesized NF membranes using synthetic wastewater (UV disinfected MF effluent).
- Quantify the effect of surfactants on fouling of NF membranes (commercial and newly developed materials)
- Determine the mechanisms for flux enhancement (or reduction) following modification of MF membranes using synthetic wastewater, then BREATHe effluent.
- Evaluate the effect of MF modification on biofouling using surrogate (E. coli), then BREATHe effluent.
- Integrate UV disinfection following MF membrane to quantify the effect on potential biofouling of RO/NF membranes.
- Evaluate the effect of the modification on backwashing and cleaning of MF membrane. Cleaning strategies will focus on options utilizing the least chemicals (eg. backwashing, NaOH wash).
- Develop a flux model to predict long term operation of both membrane systems, including backwashing and cleaning schedule.

TRAINEES

Graduate Students: Samantha LaHee, Oluwaseyi Kayode, Joshua Abitoye

Undergraduate Students: Zenobia Lewis, Michael Chestnut

RESEARCH COLLABORATIONS

- Student from Membrane Group traveled to Purdue (Banks Laboratory) in order to develop methods for an experimental project to determine the effect of biofouling on reverse osmosis efficiency.
- Monthly Water Group Telecons are conducted with PIs and students from Purdue and Howard.
- Traveled to Houston, presented results of synthesis to Membrane group at Rice University, including Karen Pickering, from NASA water recovery group.
- Discussed proposal on biofouling with Jay Garland (KSC), and PIs from Nanobiotechnology Center (NBTC) at Cornell University.
- Worked within NBTC to develop new generation membranes for use in NASA space mission applications.

PUBLICATIONS

Mukherjee, P, Jones, K., and Abitoye, J. "Surface modification of nanofiltration membranes by ion implantation", *Journal of Membrane Science*, (2005), 254/1-2 pp 303-310.

Abitoye, J, Mukherjee, P. and Jones, K. "Ion Implantation: Effect on Flux and Rejection Properties of NF Membranes", *Environ. Sci. Technol*. (2005) *39*(17) pp 6487 – 6493.

Jones, K., Leevy, J., and LaHee, S., High-Flux, Low-Fouling Membrane System for Wastewater Recycle in Space Missions, *SAE* 2004-01-2464

PRESENTATIONS

- La Hée, S. Jones, K., " Biofouling in Microfiltration Membranes" Presented at the National Society of Black Engineers Conference, Boston, MA, 2005.
- La Hée, S. Jones, K. " The Reduction of Biofouling in Microfiltration Membranes using Membrane Modification" Presented at Howard University Graduate Symposium, Washington, DC and GEM National Consortium Conference, Boston, MA, 2005.
- Jones, K., Tesema, M., "Adsorption and Fouling Mechanisms in UF and MF Membranes", *228th ACS National Meeting*, Philadelphia, PA, August 22-26, 2004
- Mukherjee, Parna; Jones, Kimberly; Abitoye, Joshua, O.; "Surface modification of nanofiltration membranes by ion implantation", *North American Membrane Society (NAMS) Conference,* Honolulu, Hawaii 2004.
- Jones, K., "Membrane Modification to increase Performance", presented at Rice University, 2004.
- Jones, K., LaHee, S., "Membrane modification to reduce Biofouling", presented at the University of Illinois, April 2005.

LIQUID FREEZE-THAW (LIFT): URINE PROCESSING FOR ADVANCED WATER RECOVERY

Principal Investigator: James E. Alleman, PhD

BACKGROUND

Resource recovery, including that of urine water extraction, is one of the most crucial aspects of long-term life support in interplanetary space travel. This effort consequently examined an innovative approach to processing raw, undiluted urine based on low-temperature freezing. A strategy uniquely different from NASA's current emphasis on either 'integrated' (co-treatment of mixed urine, grey, and condensate waters) or 'high-temperature' distillation processing strategies, whereby this liquid freeze-thaw (LiFT) procedure avoids both chemical and microbial cross-contamination concerns while at the same time securing highly desirable reductions in likely energy requirement levels. In essence, this research effort investigated an innovative, first-generation eutectic freeze concentration strategy for extraction of potable water from urine. The existing project's experimental unit, which couples the sequential steps normally associated with conventional eutectic freeze crystallization (e.g., seed crystal growth followed by ice ripening, concentration, washing, extraction, and final ice-water recovery), has proven capable of generating high-quality product water, (i.e., greater than 99% reduction of contaminant concentrations; inorganics, organics, biological and antibiotics).
However, the attainable levels of percentile water recovery have to-date been constrained to ~30-35% levels due to sub-optimal conditions with the existing batch-type push-and-pull plunger steps used for the critical ice concentration and washing steps. Despite this fact, a theoretical water recovery value between approximately 85 and 95% for a continuously operating system has been determined based on the experimentally determined eutectic point of a binary urea / water solution, since the theoretical eutectic of urea at first glance appears to be the major limiting contaminant in eutectic freeze concentration of a urine waste stream. Overall, this research effort focused on several aspects of freeze concentration, including an overview of the basic technology and its various pragmatic applications, a comparison of percent contaminant removal between a ternary urine solution and an ersatz urine solution, and the possibility of urea extraction for downstream crop fertilization use.

PROJECT GOALS AND OBJECTIVES

This research effort is addressing four aspects of freeze concentration for potable water extraction from urine. Each of these aspects has been identified as a goal for the purpose of this research.

- Theoretical modeling of freeze concentration process and contaminant migration
- Detailed evaluation of inorganic and organic migration to product water
- Detailed evaluation of possible low level contaminants including biological and antibiotic / pharmaceutical compounds entering product stream
- Detailed evaluation of potential urea recovery from brine solution

RESEARCH PROGRESS

Theoretical Modeling
For successful design and operation of freeze concentration processes, the determination of the temperature bounds on water recovery is required in addition to the identification of the limiting components. The complexity arises due to the number of salts, salt hydrates and other complexes, acids, and bases in the solution and their interactions with each other. Each of these components will form a

eutectic with water. Theoretical modeling, or solution phase diagram development, consists of the equilibrium observations between multi-component mixtures or solutions as a function of composition and temperature. Another point to consider are that complex mixtures consisting of several components, such as the ersatz urine solution, can be reasonably condensed to binary and/or ternary models despite the fact that solute addition progressively depresses the freezing point of solution. Multi-component (greater than two components) solutions have proven to be difficult without the aid of computer modeling, which in itself is an expensive proposition and could have a relatively low level of precision since computer models are based on chemical literature data and thermodynamic properties of solution that have never been experimentally proven. In essence, these computer models are utilized as a general process operation predictor to narrow down the range of operating temperatures required for the freeze concentration process. Thus, binary, and in some cases ternary, models can be developed based on experimental and literature data that should provide reasonable estimates of operating parameters that are not significantly different from those generated by computer models.

The theoretical modeling approach was based on the binary system of urea / water since it has been determined that urea is most probably the major limiting component for urine freeze concentration. Therefore, a theoretical freezing point depression curve was determined based on the solute/solvent composition as well as the molal freezing point depression constant of the solvent, water. The resulting curve can be fitted to a second-order polynomial equation and then compared to experimental data as shown in Figure 1. The results of the eutectic point determination experiment indicate a very good correlation between predicted and actual results. An initial examination of the results reveals a step decrease in temperature between the theoretical freezing point depression curve and the experimental results for the urea extraction due to supersaturation conditions of urea in the solution.

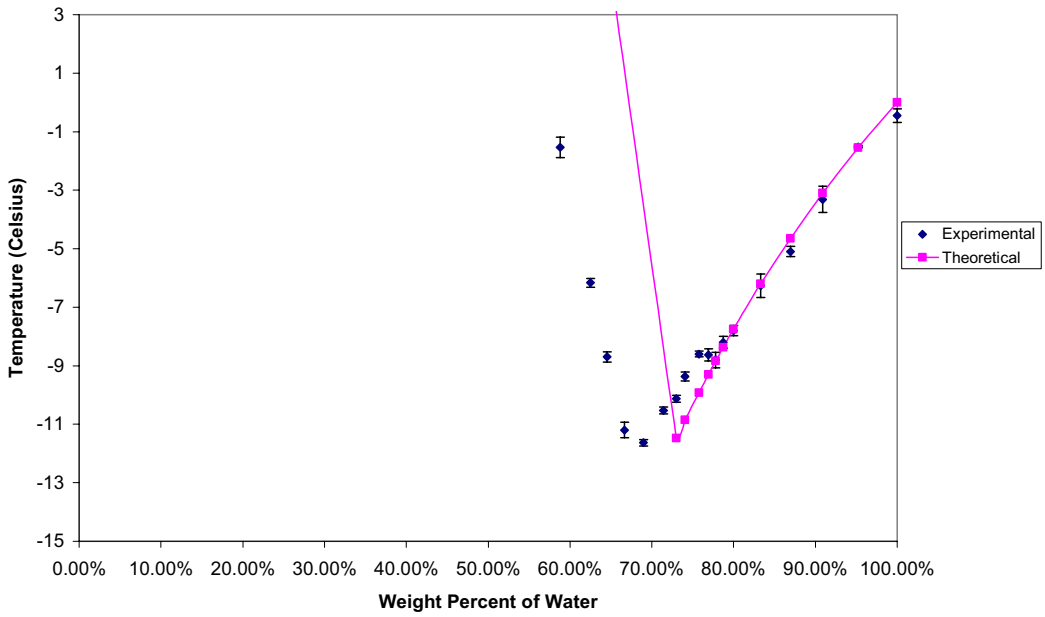


Figure 1: Experimental Eutectic Point Determination of a Urea / Water Solution

Inorganic / Organic Migration

This multi-faceted objective was to determine contaminant migration into the product water for both a ternary urine solution (urea, sodium chloride, and water) and the transit ersatz urine wastewater solution.

From the results displayed in Table 1 for the ternary solution it is obvious that the freeze concentration process is potentially a feasible option for potable water production from a urine waste stream; however, to facilitate a better conception of the capability of the freeze concentration process, the multi-component ersatz solution was tested. This portion of the study was based on the results of the ternary solution with the purpose of determining appropriate variables in the operation.

Antibiotic and Biological Removal

Since the beginning of this research emerging concerns have developed in regards to other contaminants that may enter the water cycle of an advanced life support system. These concerns correlate with the recent interest in the environmental field that has focused attention on low level contamination issues of personal care products and pharmaceuticals in water bodies used as a source of potable water. Space based resource recovery can mimic Earth bound environmental contamination problems especially in the case of antibiotic contamination. In the event of crew member illness, a large portion of non-metabolized antibiotic will be released to the water cycle through the urine; therefore, developing a technology that is capable of removing this contaminant from the waste stream is required to prevent potential negative downstream reuse impacts such as unintended human consumption and the development of bacterial resistance.

The main objective of this study was to determine the capability of the freeze concentration process in the removal of Tylosin. Initial estimates predicted high removal rates due to the fact that Tylosin, along with many other antibiotics, is a relatively large molecule. Table 3 displays the results of the aforementioned study. As predicted the freeze concentration process was successful in removing a vast majority of the Tylosin, below the minimum detectable limit, in the model urine solution thus making it a good candidate for low level contaminant removal in a closed loop system.

Table 1: Freeze Concentration Results of Ternary Urea / NaCl / Water Solution

Temperature (ºC)	Average Initial Conductivity (mS/cm)	Average Product Conductivity (mS/cm)	Average Percent NaCl Removal	Average Initial Total Nitrogen (mg/L)	Average Product Total Nitrogen (mg/L)	Average Percent Urea Removal
-6.0	3.89	0.02	99.44%	5204.09	4.99	99.90%
-7.0	3.98	0.03	99.30%	5203.75	10.39	99.80%
-8.0	3.72	0.03	99.16%	5212.42	9.60	99.82%
-9.0	4.11	0.03	99.25%	5209.67	8.33	99.84%
-10.0	4.10	0.09	97.93%	5209.08	13.83	99.73%

A more detailed analysis was performed on both the ersatz urine solution and the product water including ph, conductivity, total organic carbon, total nitrogen, PO_4^{2-}, SO_4^{2-}, K^+, Na^+, Ca^{2+}, and Mg^{2+}. Results of the experiment are shown in Table 2. The results of the ersatz urine solution mimic those of the ternary solution to the same degree of contaminant percent removal. Consequently, it is apparent that the freeze concentration process is efficient at removing nearly all contaminants for the key reason of ice crystal lattice exclusion of contaminants during formation.

Table 2: Freeze Concentration Results of the Ersatz Urine Solution

Temp. (ºC)	pH		Cond. (mS/cm)			TN (mg/L)			TOC (mg/L)		
	Initial	Product	Initial	Product	% Rem.	Initial	Product	% Rem.	Initial	Product	% Rem.
-6.0ºC	6.32	6.5475	7.88	0.03	99.61	2480	14.4	99.42	1100	1.63	99.85
-7.0ºC	6.35	6.6375	7.96	0.03	99.60	2620	16.7	99.36	1860	1.93	99.90
-8.0ºC	6.35	6.4625	7.98	0.03	99.59	2400	10.8	99.55	1460	5.35	99.63

Temp. (ºC)	PO_4^{2-}			SO_4^{2-}		
	Initial	Product	% Rem.	Initial	Product	% Rem.
-6.0	107.6	1.48	98.63	1080	5.35	99.50
-7.0	83.2	0.63	99.24	1114	1.84	99.83
-8.0	88.9	0.80	99.10	1057	2.19	99.79

Temp. (ºC)	K^+			Na^+			Ca^{2+}			Mg^{2+}		
	Initial	Product	% Rem.	Initial	Product	% Rem.	Initial	Product	% Rem.	Initial	Product	% Rem.
-6.0ºC	2316	0.65	99.97	3753	9.2575	99.75	81	0.37	99.54	413	0.07	99.98
-7.0ºC	2060	0.30	99.99	3487	9.54875	99.73	68	0.205	99.70	381	0.05	99.99
-8.0ºC	2380	0.57	99.98	3747	14.7	99.61	69	0.57	99.17	435	0.04	99.99

Antibiotic and Biological Removal

Since the beginning of this research emerging concerns have developed in regards to other contaminants that may enter the water cycle of an advanced life support system. These concerns correlate with the recent interest in the environmental field that has focused attention on low level contamination issues of personal care products and pharmaceuticals in water bodies used as a source of potable water. Space based resource recovery can mimic Earth bound environmental contamination problems especially in the case of antibiotic contamination. In the event of crew member illness, a large portion of non-metabolized

antibiotic will be released to the water cycle through the urine; therefore, developing a technology that is capable of removing this contaminant from the waste stream is required to prevent potential negative downstream reuse impacts such as unintended human consumption and the development of bacterial resistance.

The main objective of this study was to determine the capability of the freeze concentration process in the removal of Tylosin. Initial estimates predicted high removal rates due to the fact that Tylosin, along with many other antibiotics, is a relatively large molecule. Table 3 displays the results of the aforementioned study. As predicted the freeze concentration process was successful in removing a vast majority of the Tylosin, below the minimum detectable limit, in the model urine solution thus making it a good candidate for low level contaminant removal in a closed loop system.

Table 3: Freeze Concentration Results for Tylosin Removal from a Ternary Urine Solution

Ripening Temperature (ºC)	**Initial Concentration (mg/L)**	**Final Concentration (mg/L)**	**Percent Reduction**
-6ºC	27	<0.2	>99%
-8ºC	27	<0.2	>99%
-10ºC	27	<0.2	>99%

In case of the possibility of crew member urinary tract infections, the freeze concentration process may prevent biological migration to the product water; therefore, a biological migration study was performed. Despite the fact that urine is normally a sterile waste stream it may contain human pathogens, most predominantly *E. coli*, such as the case in a urinary tract infection. Therefore, the possibility of microbial migration from the urine solution to the product water was studied on the batch freeze concentration process by doping the ersatz urine solution utilizing the ATCC 25922 strain of *E. coli.*

Figure 2 represents the control group of the biological experiments. Each control solution, distilled water, the ersatz urine solution, and tryptic soy broth was doped and underwent three separate scenarios to compare the vitality of the *E. coli* in each case. Three cases were considered. The first was to determine the number of colony forming units per 100 mL in an initial control solution. It can be assumed that the initial values between the different control groups cannot be compared since the doped count of *E. coli* may vary between freeze-dried ampules, and dilutions thereof. The latter cases set the baseline for *E. coli* vitality for ambient conditions and for a basic freeze-thaw cycle condition.

Once the control was defined the ersatz solution was doped and underwent the freeze concentration process to determine the migration of *E. coli* into the product water stream. Table 4 displays the results of this experimental group. From these results it is obvious that the freeze concentration process is effective in removing greater than 99% of the *E. coli* in the influent stream. Further tests would need to be performed in order to verify this result on a continuous freeze concentration process.

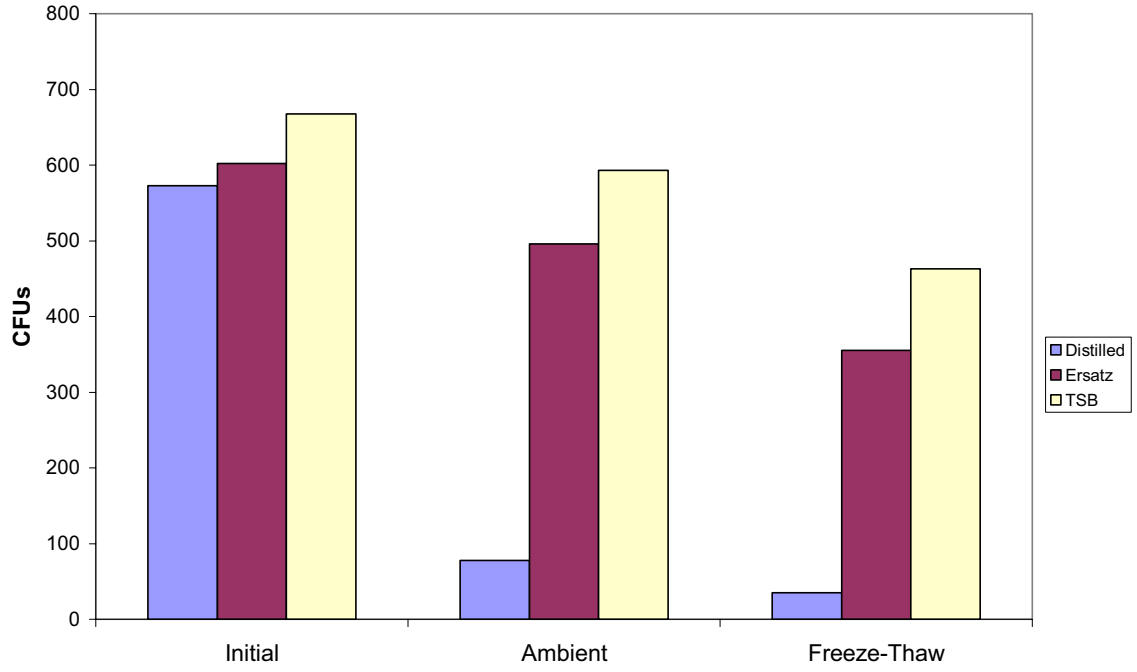


Figure 2: Control Groups of E. coli ATCC# 25922

Table 4: Results of the Biological Removing Capacity of the Freeze Concentration Process

(CFUs)	**Initial**	**Concentrated Brine**	**Product**
Sample 1	424	243	1
Sample 2	424	381	1
Sample 3	651	573	5
Sample 4	651	502	0
Sample 5	643	316	0
Sample 6	643	420	1

Urea Recovery

This exciting research effort will focus on the potential recovery of urea along with trace organics to be utilized as a nutrient source for plants in a long-term advanced life support initiative. The foundation of this recovery lies in the fact that the eutectic point of urea lies above the eutectic point of NaCl therefore its separation from salt brine may be possible. In order to achieve such separation a dual freeze concentration system aligned in series may be the optimal method as depicted in Figure 3. The first concentration would extract water while the second concentration would extract a urea / water solution. Future experiments based on the utectic theoretical model will pursue the possibility of this recovery.

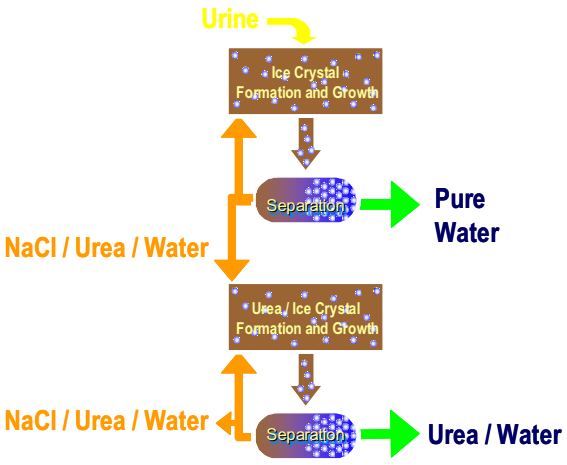


Figure 3: Two Stage Urea Extraction Process

FUTURE RESEARCH DIRECTIONS

In this last year of LiFT's funding, future research is limited to the following areas:

- Confirm the ability to remove biological contaminants from a doped urine ersatz solution.
- Determine the feasibility of urea extraction for downstream plant fertilization use via a dual freeze concentration process operating in series.
- Assess the energetic capabilities of the freeze concentration process and compare it to other physical chemical processes for water recovery including distillation and filtration.

RESEARCHERS

PhD Student: Jeff Schmidt

RESEARCH COLLABORATION

- Johnson Space Center – Alleman has communicated with researchers at JSC to provide updates on NSCORT progress with LiFT
- Alleman has tracked down and communicated with a prior freeze concentration NASA researcher, Kevin Alexandre, to compare and discuss applications of the technology and its feasibility.

PUBLICATIONS

Schmidt, J.M. and Alleman, J.E. (2005). "Urine Processing for Water Recovery via Freeze Concentration," *International Conference on Environmental Systems*, Paper 2005-01-3032.

PRESENTATIONS

Schmidt, J.M. and Alleman, J.E. (2005). "Urine Processing for Water Recovery via Freeze Concentration," Presented at the *International Conference on Environmental Systems*, Rome, Italy.

Schmidt, J.M. and Alleman, J.E. (2004), "Liquid Freeze-Thaw Treatment (LiFT) for ESM Enhanced Urine Water Recovery," Presented during the Technical Interchange Meeting (TIM), September.

Alleman, J.E. and Schmidt, J.M. (2004), "Liquid Freeze-Thaw Treatment (LiFT) for ESM Enhanced Urine Water Recovery," Presented at the ALS-NSCORT External Advisory Committee Meeting, Washington, D.C.

Schmidt, J.M. and Alleman, J.E. (2004), "LiFT:Post-LiFT Urine Treatment Technologies," Presented at the NSCORT Summer Symposium, Purdue University, West Lafayettte, IN.

Schmidt, J.M. and Alleman, J.E. (2003), "Urine Processing via Sublimation Technology," Poster Presented at the ALS-NSCORT External Advisory Committee Meeting, West Lafayette, IN.

POTABLE WATER DISINFECTION SUBJECT TO EXTEDED SPACE TRAVEL CONSTRAINTS – COMPLEMENTARY WATER DISINFECTION

Principal Investigator: Ernest R. Blatchley III, PhD, PE

BACKGROUND

Long-term space missions pose a number of challenges, including provision of safe potable water for the crew. Bringing water from earth sources for long-term space missions beyond near-Earth orbit is not practical for several reasons, including costs on the order of $40,000/gallon. Therefore, the National Aeronautics and Space Administration (NASA) has been focused on developing closed-loop water treatment processes, which do not require water re-supply. The use and reuse of water in a closed-loop system poses a number of challenges for water disinfection, including poor "source" water quality, diverse microorganism populations, and evolution of microogransisms, which may result in the emergence of more resistant strains.

To overcome the hurdles of a close-loop treatment system, a complementary disinfection process has been developed to target a wide range of microorganisms. The disinfection process uses ultraviolet (UV) radiation as the primary disinfectant and a chemical disinfectant (iodine) as the residual disinfectant. UV radiation was selected as the primary disinfectant because it is effective at inactivating a broad spectrum of microorganisms and has minimal potential for the formation of disinfection byproducts. Iodine, which is effective at inactivating many microorganisms and is less likely to react and form disinfection byproducts than other halogens, was selected as the residual disinfectant because it has the potential for dual use as an on-line UV monitor and a disinfectant. Figure 1 shows the process diagram for the UV/iodine disinfection system.

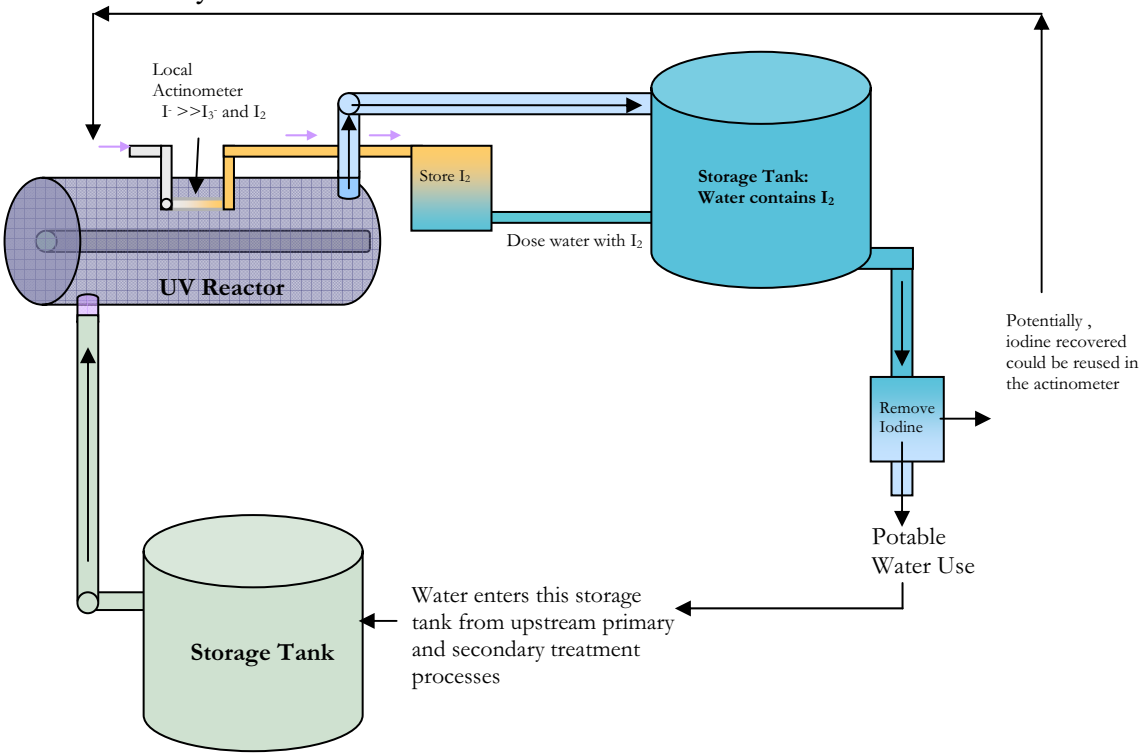


Figure 1. Schematic representation of complementary disinfection system for potable water.

PROJECT GOALS AND OBJECTIVES

- Determine whether complementary disinfection using UV radiation and iodine will provide more effective microbial inactivation than either method alone.
- Develop a chemical actinometer that can monitor the UV disinfection process, in addition to providing sufficient quantities of iodine to achieve residual disinfection. The dual purpose of the chemical actinometer may reduce chemical restock requirements.
- Investigate whether the microbial check valve (MCV), which was developed in the 1970s by NASA, can be used to store and deliver the photochemically-produced iodine.
- Evaluate the effectiveness of using Vitamin C to remove iodine from potable water, while providing a beneficial residual for the astronauts.

RESEARCH PROGRESS

Complementary Disinfection:
Experiments were performed to investigate the independent and combined effects of UV and iodine using *Bacillus subtilis* spores as the challenge microorganism. Spore forming bacteria have been recognized as one of the "hardiest" forms of life on Earth. *B. subtilis* have been widely studied as a surrogate for other spore-formers in laboratory settings because they are non-pathogenic and their resistance to many forms of disinfection has been documented in published literature. Furthermore, they have been identified by the United States Environmental Protection Agency (USEPA) as a challenge microorganism suitable for the design of UV disinfection systems.

Figure 2 shows the results for *B. subtilis* spore inactivation by UV irradiation at 222 nm, 254 nm and 282 nm. For each of the wavelengths investigated, the reduction in the concentration of viable spores occurred in three distinct stages: a lag period (or shoulder) where disinfection is slow, until a "threshold dose" is reached, at which a straight-line (first-order) decrease in the viable population is observed. The first-order portion of the curve is followed by a transition period where the disinfection effectiveness is slowed until finally, a tailing period is observed.

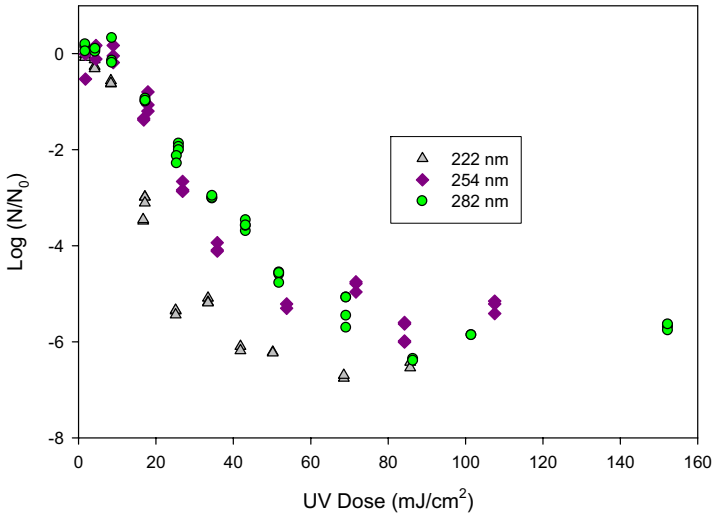


Figure 2. UV and *Bacillus subtilis* spores Dose-Response Behavior.

To investigate *B. subtilis* spore susceptibility to iodine, batch experiments were conducted. The results from these experiments are shown in Figure 3. To examine the validity of the CT concept, two initial iodine concentrations were used. Data from both experiments are shown on Figure 2. The results suggest that the inactivation behavior of the spores can be estimated by a first order process (i.e. no shoulder or tailing regions were observed). The fit of a first order model is shown on Figure 3. Based on this model, a dose of approximately 1400 mg-min/L is required to achieve 2-$\log_{10}$ units of inactivation.

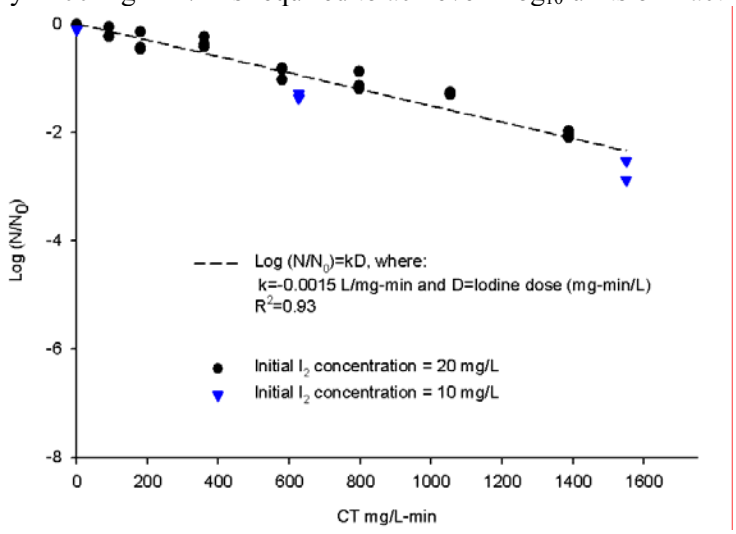


Figure 3. Model Fit for Inactivation of *B. subtilis* Spores by Iodination (pH=5). N_0 is the concentration of viable spores prior to iodine exposure.

Sequential disinfection experiments were performed by irradiating a suspension of *B. subtilis* spores using a collimated UV source, and then subjecting it to iodination in a batch system. For each wavelength investigated (254 nm and 282 nm) the spores were exposed to three different UV doses. The first dose corresponded to the shoulder region of the UV dose-response curve (see Figure 2), the second dose corresponded to the region of first-order decay, and the third dose corresponded to the region just before tailing. Following each of these doses, the spores were exposed to a known concentration of iodine and the dose-response behavior was observed. Figure 4 shows the results of sequential disinfection for 254 nm. Figure 5 shows the results of sequential disinfection for 282 nm.

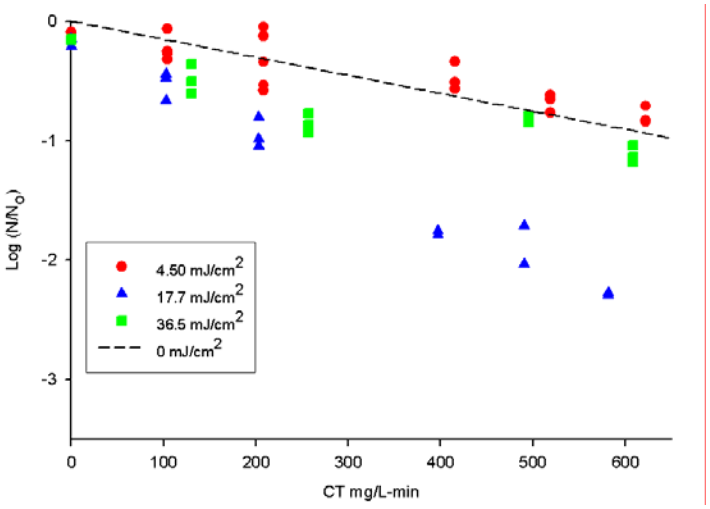


Figure 4. Inactivation of *B. subtilis* spores by UV radiation (254 nm) followed by iodination (pH=5). N_0 is the concentration of viable spores following UV radiation, but prior to iodination.

In Figure 4 the effects of sequential disinfection are most apparent when the spores were pre-irradiated (254 nm) with a dose of 17.7 mJ/cm^2. This dose corresponds to the portion of the UV Dose-response curve in between the shoulder and tailing regions (see Figure 1). The doses of 4.5 mJ/cm^2 and 36.5 mJ/cm^2 represent the shoulder and tailing portions of the UV dose-response curve (Figure 2), respectively. Neither of these doses resulted in increased inactivation by iodination. At a dose of 4.5 mJ/cm^2, it is hypothesized the spores may not have acquired enough damage to increase their susceptibility to iodination. At a dose 36.5 mJ/cm^2, the majority of the spores had already been inactivated (over four-logs) (Figure 2). It is hypothesized that some spores within the population display inherent resistance to UV irradiation; it is possible that these attributes of the spore also result in increased resistance to chemical disinfectants, such as iodine.

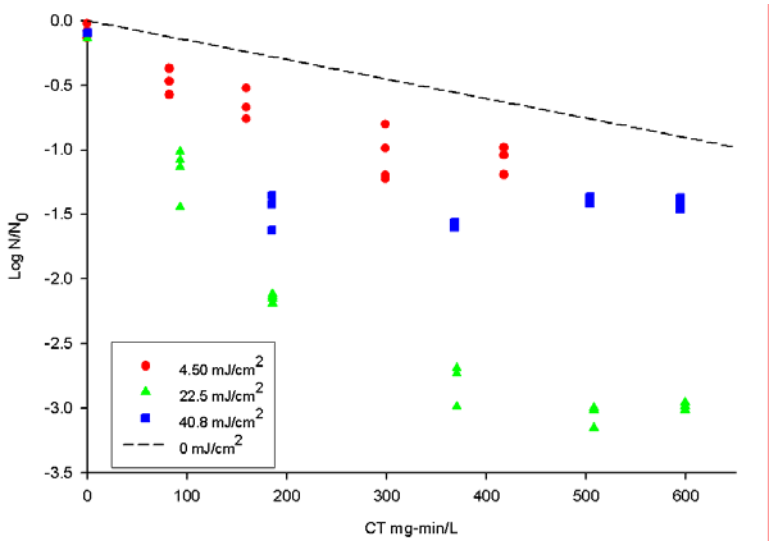


Figure 5. Inactivation of *B. subtilis* spores by UV radiation (282 nm) followed by iodination (pH=5). N_0 is the concentration of viable spores following UV radiation, but prior to iodination.

Figure 5 shows the effects of sequential disinfection when the spores were pre-irradiated using a UV source that emitted at 282 nm. The results indicate that only small increases in iodine susceptibility were observed for the UV doses that correspond to the shoulder and near-tailing regions (4.5 mJ/cm^2 and 40.8 mJ/cm^2, respectively). However, when the spores were pre-irradiated with a UV dose that corresponds to the first-order portion of the UV dose-response curve (22.5 mJ/cm^2), iodination resulted in over 2-logs more inactivation than was observed when the spores were not pre-irradiated. It should also be noted that for pre-irradiation doses of 22.5 and 40.8 mJ/cm^2, tailing behavior was observed.

Statistical analysis of the data presented in Figures 4 and 5 indicates that differences between inactivation kinetics for sequential disinfection compared to iodination alone were statistically significant ($p=0.05$) for all of the 282 nm pre-irradiation experiments. For the 254 nm experiments, differences in inactivation kinetics were only statistically significant ($p=0.05$) for the 17.7 mJ/cm^2 pre-irradiation experiment.

A numerical model was developed to describe the effects of UV and iodine on *B. subtilis* spores. The model was based on the hypothesis that phenotypic variation in a microbial population is responsible for the existence of a small fraction of the microbial population that has physiological characteristics that enhance its chances of surviving various forms of externally-applied stress, despite the susceptibility of the population majority.

Chemical Actinometry:

Iodide (I^-) and iodate (IO_3^-) are used as the chemical actinometer to monitor the efficacy of the UV system. The chemical actinometer system to be employed in this application consists of a small capillary tube installed within the UV reactor through which an I^-/IO_3^- solution is pumped. When exposed to UV radiation, this solution will form triiodide (I_3^-);

Laboratory experiments have been performed with collimated-beam devices using conventional low-pressure Hg lamps, as well as excimer sources to determine the quantum yield for photochemical production of triiodide from the iodide/iodate actinometer at UV wavelengths of 222 nm, 254 nm, and 282 nm. Additional experiments have been performed using a small quartz capillary tube installed parallel to the lamp wall. The iodide-iodate actinometer solution was pumped through the capillary tube at a constant flow rate. The data from these experiments confirm the application of the iodide-iodate actinometer as a means to measure the local irradiance emitted from an excimer source. Figure 6 shows the experimental setup for the capillary experiments.

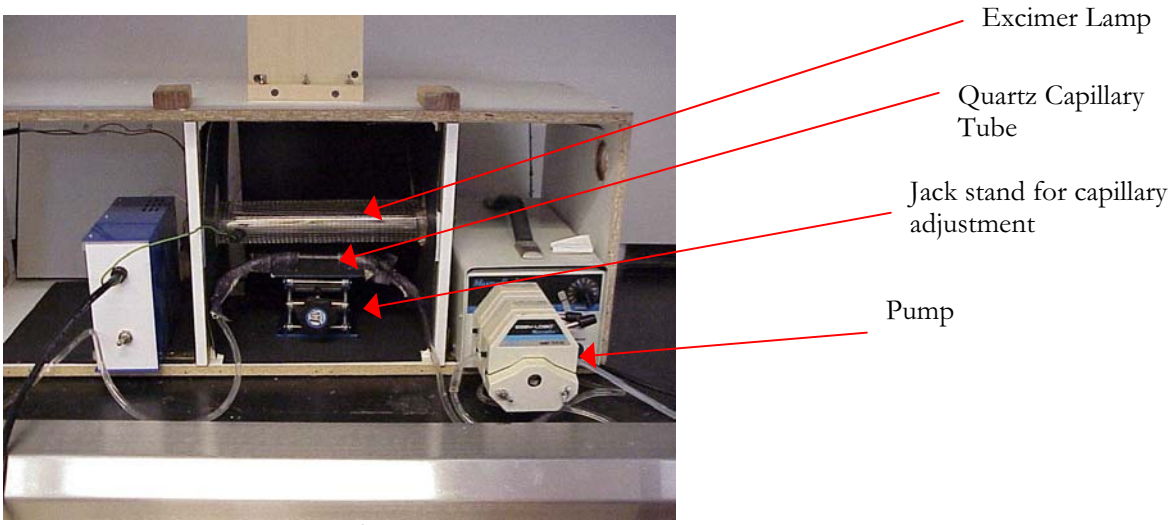


Figure 6. Experimental Setup for Capillary Experiments.

The capillary tube was located at various distances from the lamp surface while the iodide-iodate actinometer was pumped through the capillary at a constant flowrate. The data from these capillary experiments are shown in Figure 7.

By positioning the capillary at a location within the proposed reactor where minimum dose delivery is expected, proper operation of the UV disinfection system can be monitored and verified. For instance, at a given distance from the lamp, the actinometer solution should produce a given amount of triiodide based on the calculated exposure time. Correlating this data to a standard curve, will allow the UV system to be monitored.

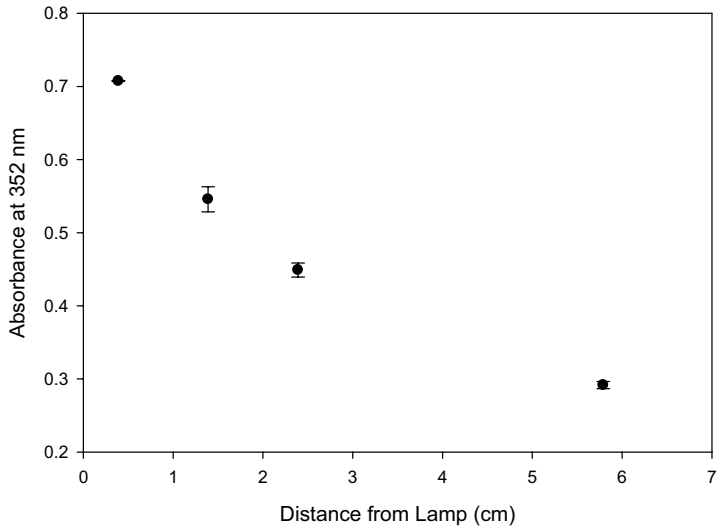


Figure 7. Actinometer Response for a Single Lamp Capillary Flow Reactor (Flowrate= 30.5 mL/min, Error shown as standard deviation, n=3)

Residual Disinfectant Production:

To limit the use of additional chemicals, the residual disinfectant selected for the closed-loop disinfection system was I_2. I_2 can be produced using the photo-reacted actinometer solution (containing a mixture of I^-, IO_3^-, I_3^-, and trace quantities of several other iodine forms) and ascorbic acid (Vitamin C). Vitamin C was selected as a reagent for iodine production because it provides a beneficial anti-oxidant residual for the astronauts. Equation 1 shows the overall chemical reaction that takes place to form I_2. As shown in this equation, iodate is the form of iodine that will react with ascorbic acid to form I_2. Iodate (IO_3^-) is present in the photo-reacted actinometer solution at a relatively high concentration (0.1 M).

Equation 1: $2IO_3^- + 5AA \rightarrow 5DHAA + 6H_2O + I_2$

Where:
AA = Ascorbic Acid ($C_6H_8O_6$)
DHAA = Dehydroascorbic Acid ($C_6H_6O_6$)

Following the production of iodine from the reaction of ascorbic acid and iodate, I_2 will be added to water that has already been treated by ultraviolet irradiation (Figure 1). The water containing I_2 will be stored until ready for consumption. At the point of use, prior to consumption, the I_2 and other iodine species will be removed because of potential adverse health effects associated with iodine consumption.

Iodine removal will be achieved using ascorbic acid and ion exchange. Ascorbic acid will be added in slight excess to ensure the formation of iodide (I^-). Equations 2 through 4 show the reaction of various iodine species with ascorbic acid to form iodide.

Equation 2: $I_2 + AA + H_2O \rightarrow DHAA + 2I^- + 2H^+$
Equation 3: $I_3^- + AA + H_2O \rightarrow DHAA + 3I^- + 2H^+$
Equation 4: $2IO_3^- + 6AA \rightarrow 6DHAA + 5H_2O + 2I^-$

Once all forms of iodine have been converted to iodide, the water will pass through an ion exchange column, where the iodide will be removed. Experiments conducted using Rohm and Haas Amberlite IRA-400Cl ion exchange resin confirm that iodide can be removed from solution via ion exchange and that the process can be described using a Langmuir Isotherm.

TRAINEES

PhD Students: Kelly L. Pennell, PE

RESEARCH COLLABORATION

- Umpqua Research Company: Communicated with URC about the use of MCV in long-term space missions. URC supplied Purdue with MCV resin for laboratory testing.
- Johnson Space Center: Discussed the use of Vitamn C in the disinfection process with Michelle Perchonok about the use of Vitamin C in the water disinfection process.
- NSCORT Summer Undergraduate Fellowship Program—Hosted Andy Hai Ting of Howard University (2004).

- Educational Outreach—Visited two fifth grade classes at Challenger Elementary in Howell, Michigan. Discussed NSCORT research and conducted science activities (2004).
- Johnson Space Center—Visited the Water Quality Laboratory and Microbiology Laboratory at JSC (2003).

PUBLICATIONS

Pennell, Kelly and Ernest R. Blatchley III. "Complementary Disinfection (UV Irradiation and Iodination) for Long-term Space Missions: Preliminary System Design." Conference Proceeding: International Conference for Environmental Systems (ICES). Paper # 2004-04-2516. 2004.

Pennell, Kelly, Zorana Naunovic, and Ernest R. Blatchley III. "Effect of Sequential Disinfection on *Bacillus subtilis* Spores using Ultraviolet Irradiation and Iodination." Conference Proceeding: *Water Quality and Technology Conference (WQTC).* November 2005.

Pennell, Kelly and Ernest R. Blatchley III. "Dual Use of the Iodide/Iodate Actinometer to Monitor Ultraviolet Irradiation and to Provide a Residual Disinfectant." Conference Proceeding: *Water Quality and Technology Conference (WQTC).* November 2005.

PRESENTATIONS

Pennell, Kelly and Ernest R. Blatchley III. "Photochemical Kinetics of the Iodide/Iodate Actinometer and Iodine Dose-Response for *B. Subtilis* Spores." Oral Presentation to the NASA Specialized Center of Research and Training (NSCORT) Advisory Board. Howard University, Washington DC. 2004.

Pennell, Kelly and Ernest R. Blatchley III. "Photochemical Kinetics of the Iodide/Iodate Actinometer and Iodine Dose-Response for *B. Subtilis* Spores." Oral Presentation to the NASA Specialized Center of Research and Training (NSCORT) Advisory Board. Howard University, Washington DC. 2004.

Pennell, Kelly and Ernest R. Blatchley III. "Water Disinfection using UV Irradiation and Iodination for Long-Term Space Missions." Oral presentation at the 5th Annual Environmental Research Symposium-West Lafayette, Indiana. 2004.

Pennell, Kelly and Ernest R. Blatchley III. "Complementary Disinfection using UV Irradiation and Iodine." Oral Presentation at the NASA Specialized Center of Research and Training (NSCORT) Summer Fellowship Symposium-West Lafayette, Indiana. 2004.

Pennell, Kelly, and Ernest R. Blatchley III. "Optimization of Physical and Chemical Disinfection Processes Subject to Extended Space Travel Constraints." Oral Presentation to the NASA Water Quality and Microbiology Laboratories at Johnson Space Center-Houston, Texas. 2003.

Pennell, Kelly, Zorana Naunovic, and Ernest R. Blatchley III. "Water Disinfection System: Complementary Use of Ultraviolet (UV) Irradiation and Iodine." Oral Presentation to the NASA Specialized Center of Research and Training (NSCORT) Advisory Board-West Lafayette, Indiana. 2003.

Pennell, Kelly, Zorana Naunovic, Dennis A Lyn, and Ernest R Blatchley III. "Water Disinfection for the Mission to Mars." Poster presentation at the 4th Annual Environmental Research Symposium-Lafayette, Indiana. 2003.

Pennell, Kelly and Ernest R. Blatchley III. "Water Disinfection for Long Term Space Missions." Oral Presentation at the NASA Specialized Center of Research and Training (NSCORT) Summer Fellowship Symposium-West Lafayette, Indiana. 2003.

POTABLE WATER DISINFECTION SUBJECT TO EXTEDED SPACE TRAVEL CONSTRAINTS – UV IRRADIATION

Principal Investigator: Ernest R. Blatchley III, PE, Ph.D.

BACKGROUND

Disinfection is an integral component of a closed-loop water reuse system intended for extended space missions. The disinfection process is one of the last water treatment operations and it must ensure inactivation of waterborne microbial pathogens. The goal of this research is to design an ultraviolet (UV) disinfection reactor that will inactivate pathogenic microorganisms present in the wastewater generated during long-term space missions, such that complete reuse (*i.e.*, direct potabilization) can be accomplished. This design must ensure microbial inactivation efficacy, as well as minimize volume, mass, power and maintenance requirements. The means to achieve this design goal is a numerical modeling tool developed in this research, which is based on Computational Fluid Dynamics (CFD), UV radiation intensity field models and microbial inactivation kinetics. The inputs to this numerical model are the desired reactor size and geometry, the inlet velocity and boundary conditions, the UV lamp output power and radiation intensity profile, as well as the characteristics of the aqueous media. The outputs of the model are the UV dose distribution delivered to the microorganisms traversing the reactor and the degree of microbial inactivation achieved. Based on these outputs, the performance of the UV reactor can be assessed for the entire range of practical operating conditions.

PROJECT GOALS AND OBJECTIVES

- Develop a numerical method for evaluation of process performance of various hypothetical reactor geometries and operational parameters
- Validate the modeling tool by conducting microbial inactivation experiments with existing hardware and simulating the process numerically
- Identify and procure a non-mercury-containing UV source suited for long-term space missions
- Perform UV dose-response experiments with challenge microorganism and non-mercury-containing UV source
- Devise and program a model for solving the intensity field around the chosen UV source
- Use the modeling tool to investigate candidate reactors
- Choose the best reactor design based on model results and build and test a physical prototype reactor

RESEARCH PROGRESS

During the third year of the NSCORT ALS project, research has been focused on testing the effectiveness of candidate UV sources and utilizing the developed numerical modeling tool to finalize designs for the disinfection reactor that will satisfy constraints of microbial inactivation and ESM minimization.

Testing of candidate UV sources. One of the main constraints imposed on the design of UV disinfection reactors for extended space missions is the restriction of use of mercury-containing lamps, as defined by the existing safety regulation documents for the International Space Station (NASA JSC, 2004). Therefore, two alternative non-mercury-containing excimer lamps have been examined as potential UV sources in disinfection reactors. Both excimer lamp systems satisfy the required criteria that they emit radiation of germicidal wavelengths and are efficient enough to be used for water treatment systems. These are the KrCl* (the asterisk notation denotes an excited molecular complex, which has no stable ground state under normal conditions) and the XeBr* excimer lamps, which emit nearly monochromatic radiation at 222 nm and 282 nm, respectively. Experiments were conducted employing the *Bacillus*

subtilis spores as a challenge organism to asses the effect of UV irradiation at wavelengths of 222 nm and 282 nm on spores. *Bacillus subtilis* were selected for these experiments because they are non-pathogenic to humans and they exhibit relatively high resistance to UV radiation. It can be hypothesized that a system that inactivates these spores effectively will perform well in response to organisms with lower resistance to UV irradiation; most protozoan parasites and vegetative bacteria are less-resistant to UV radiation than *B. subtilis* spores. Results are shown in Figure 2 of the Complementary Water Disinfection portion of this report. Both excimer sources were effective for inactivation of the challenge organism. However, employing radiation at 222 nm was shown to be impractical as the transmission of UV radiation through a representative water sample at 222 nm is much lower that at higher wavelengths of interest, i.e. at 282 nm. This was determined by analyzing effluent samples from the Bio-Regenerative Environmental Air Treatment for Health (BREATHe I) effluent. In the proposed NSCORT ALS water recycling and treatment loop, biological treatment in the BREATHe I reactor is followed by a micro-filtration membrane and UV disinfection. To obtain a representative influent water sample for the UV reactor, effluent samples were collected on two different days from six operating pilot scale BREATHe I reactors, and these samples were then pre-filtered with a 0.45 micrometer membranes to simulate the effect of the micro-filtration membrane. The water samples were then used for the examination of their optical characteristics at 222 nm and 282 nm. The average transmittance values at 222 nm and 282 nm were 16.7 % and 89.9 %, respectively. Therefore, it was concluded that XeBr* excimer radiation at 282 nm penetrates the aqueous media much more effectively. This characteristic, along with higher degree of electrical efficiency and longer lifetimes for the XeBr* lamp as compared to the KrCl* lamp, influenced the choice of the XeBr* source over the KrCl* source.

UV Intensity field modeling for the XeBr excimer source.* As the physics of excimer formation and UV radiation emission differ from those of UV radiation emitted by mercury-based sources, the Line Source Integration model used to describe the intensity profile emanating from a low-pressure mercury lamp source could not be used for the XeBr* source. The new model for excimer lamps, named the Surface Power Apportionment for Cylindrical Entities (SPACE) model, calculates radiation received at any point around the cylindrical lamp as a contribution from a large number of point sources radiating from the interior annual space of the excimer lamp. The SPACE model also accounts for the refraction, reflection and absorbance effects of the quartz lamp envelope and the media surrounding the lamp. A schematic of a typical excimer lamp is presented in Figure 1, and Figure 2 depicts the basic principle of calculating the radiation intensity received at point A with the SPACE model. Figure 3 shows the intensity contours for a XeBr* lamp calculated with the SPACE model.

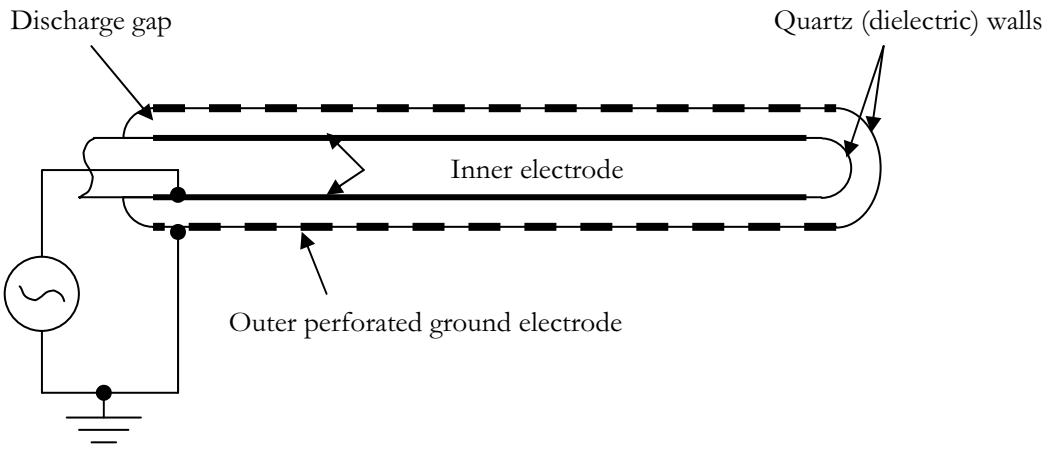


Figure 1. Cylindrical excimer source with annular discharge gap.

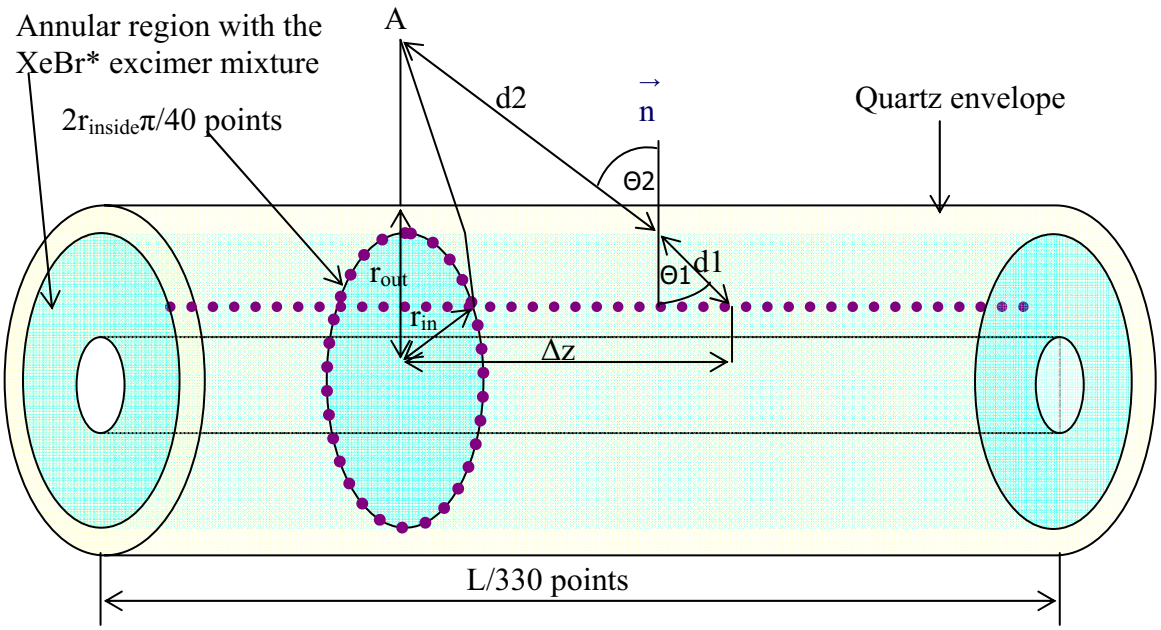


Figure 2. Principle of calculation for UV intensity values at any point around an excimer lamp with the SPACE model.

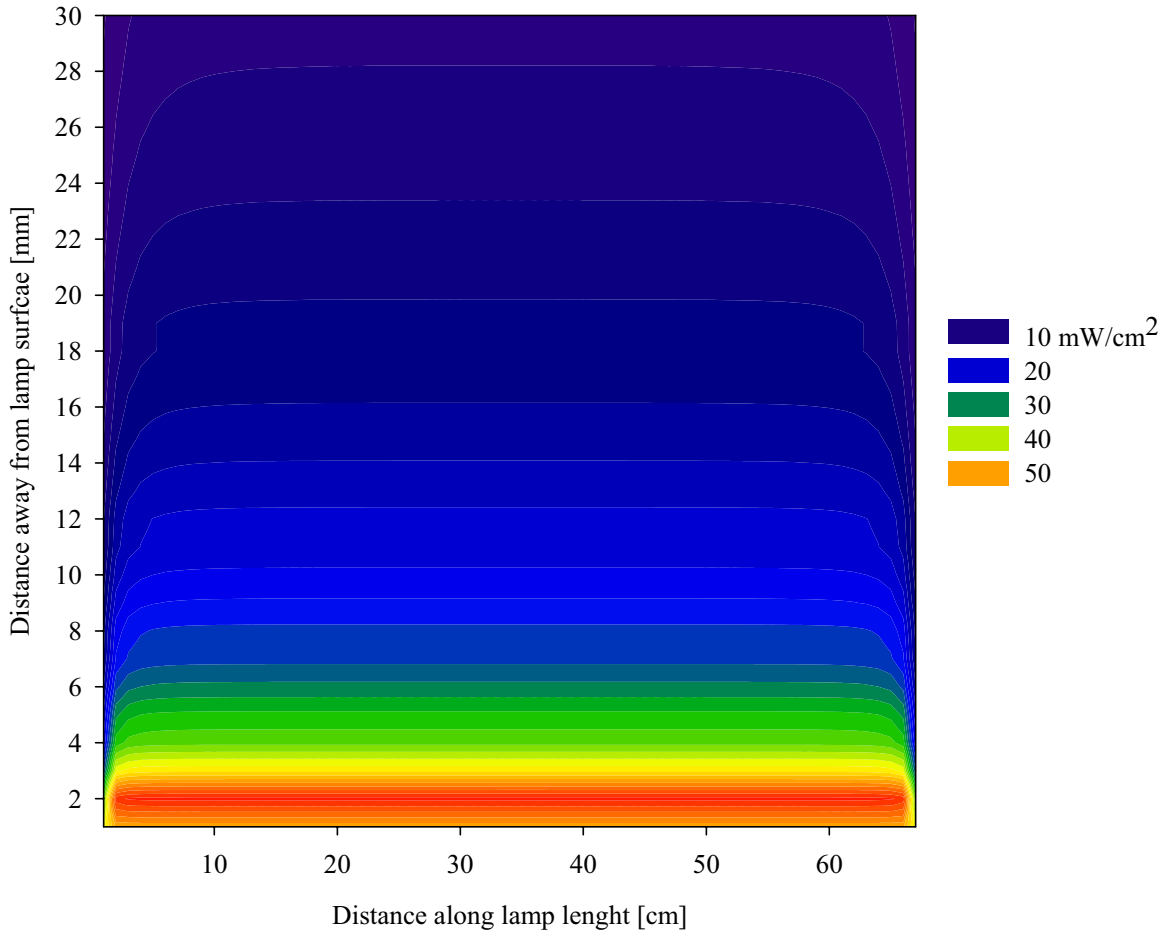


Figure 3. Intensity contours calculated with the SPACE model for a 66 centimeters long XeBr* lamp, with output power of 20 W and 90% water transmittance

Investigating the hydraulic dynamics and inactivation efficiency of candidate reactors. Computational modeling softwares GAMBIT and FLUENT were used to draw the reactor geometry and solve the fluid flow and microbial trajectories within the reactor for defined operating parameters. The microbial trajectory data was integrated with the SPACE intensity model and the measured inactivation kinetics of *Bacillus subtilis* spores. This enabled the calculation of overall process efficiency defined as the degree of microbial inactivation achieved by the reactor. Different reactor geometries were compared based on this parameter and their mass, volume and power usage, as these parameters influence the ESM value of the system. The investigated reactor variations included a 90 degree inlet elbow and a straight inlet, three different reactor diameters and internal baffle structures, two different reactor lengths and a range of output power values for a XeBr* excimer lamp with a 2.54 cm diameter. Figure 4 illustrates two of the investigated reactor geometries with different inlet configurations and baffle design. All reactors exhibited a higher degree of microbial inactivation efficiency with internal baffles as compared to a reactor without any baffles, and the straight inlet configuration provided the most optimal hydraulic conditions. The interior baffle structure around the lamp shown on Figure 4b polarizes the flow and effectively creates a spiral flow pattern through the reactor, influencing the delivery of a more uniform dose distribution UV dose to the microbes traversing the reactor. This is the chosen design and the microbial trajectories through such a reactor are shown in Figure 5. The XeBr* excimer lamp output power specifications have also been determined and a prototype lamp will be custom made for the NSCORT ALS UV disinfection reactor by the manufacturer.

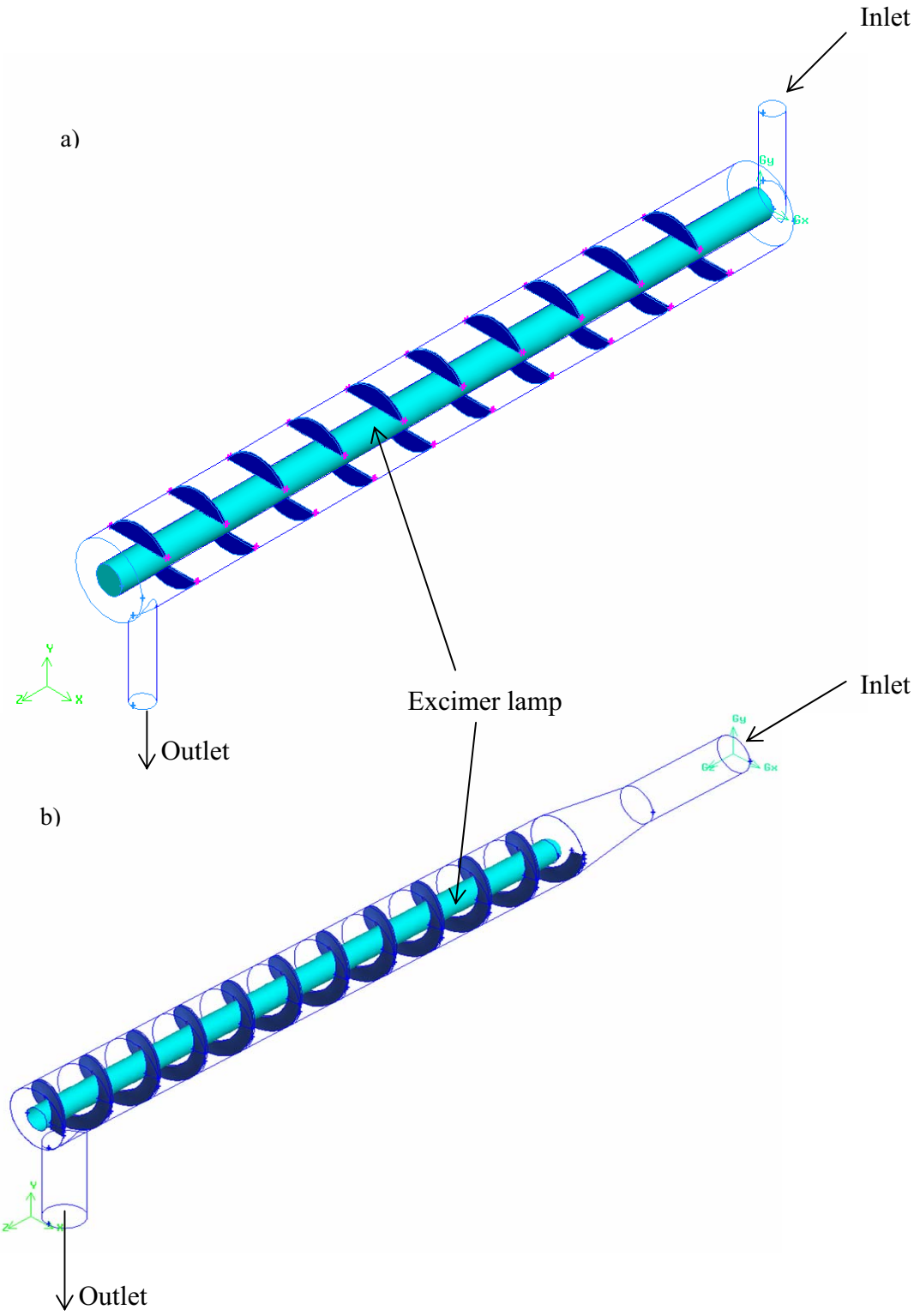


FIGURE 4. TWO REACTORS WITH DIFFERENT INLET CONFIGURATIONS AND INTERNAL BAFFLE DESIGN. THE EXCIMER LAMP IS CENTRALLY POSITIONED IN THE REACTOR.

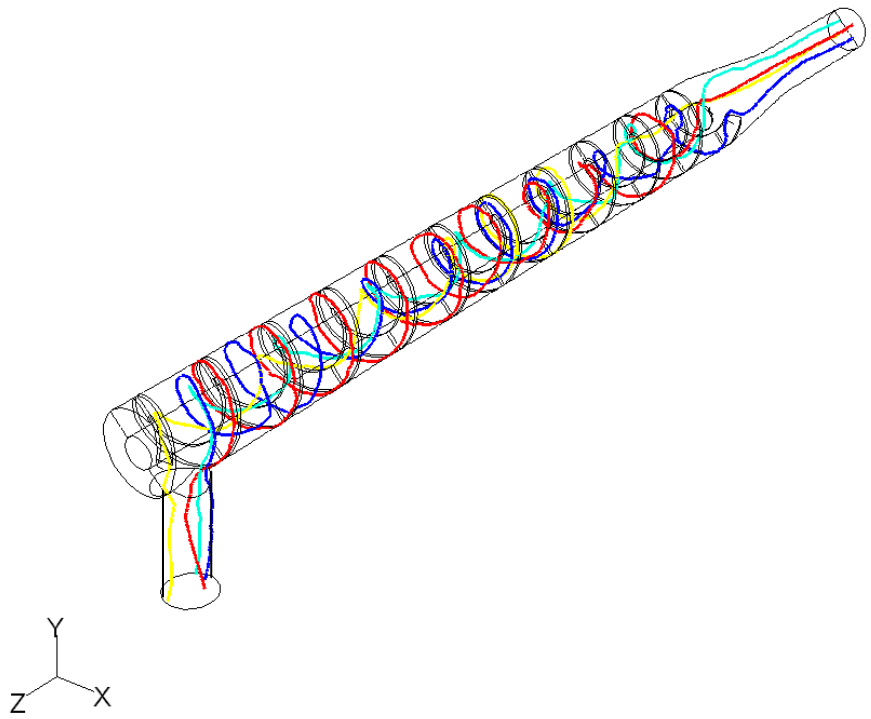


FIGURE 5. FOUR MICROBIAL TRAJECTORIES THROUGH A REACTOR WITH AN INTERNAL SPIRAL BAFFLE AT AN INLET FLOW RATE OF 0.5 L/S.

FUTURE RESEARCH DIRECTIONS

- Meet with manufacturer of excimer lamp to present to them the desired lamp specifications and procure a custom built XeBr* lamp
- Construct reactor housing for the lamp with the internal spiral baffle
- Perform microbial inactivation experiments on the excimer disinfection reactor to verify numerical model predictions

TRAINEES

PhD Students: Zorana Naunovic

RESEARCH COLLABORATION

- Ondeo Degremont Inc, Richmond, Virginia provided the model UV reactor for experimental research
- USHIO Inc, Cypress, California provided two excimer lamps as non-mercury UV sources

PUBLICATIONS

Zorana Naunovic, Dennis Lyn, Ernest Blatchley III (2005) "Modeling and Design of an Ultraviolet Water Disinfection System." *Proceedings of the International Conference on Environmental Systems(ICES)*, Paper 2005-01-3061Rome, Italy, July 2005.

Zorana Naunovic, Dennis Lyn, Ernest Blatchley III (2004) "Process performance of ultraviolet disinfection systems for long-term space missions." *Proceedings of the International Conference on Environmental Systems (ICES)*, Paper 2004-01-2538, Colorado Springs, Colorado, 2004.

PRESENTATIONS

Zorana Naunovic, Dennis Lyn, Ernest Blatchley III, "Process Performance of Ultraviolet Disinfection Systems for Long-term Space Missions," Presentated at the NASA Specialized Center of Research and Training (NSCORT) Summer Fellowship Symposium, Purdue University, West Lafayette, Indiana, August 2004.

Zorana Naunovic, Dennis Lyn, Ernest Blatchley III, "Ultraviolet Disinfection Systems for Long-Term Space Missions," Presented at the 5th Annual Environmental Science and Engineering Institute (ESEI) Symposium, Purdue University, West Lafayette, Indiana, April 2004.

Zorana Naunovic, "Ultraviolet Water Disinfection for Long-Term Space Missions," Space Advanced Life Support Class Lecture, School of Civil Engineering. Purdue University, West Lafayette, Indiana, April 2004.

Zorana Naunovic, "Potable Water Disinfection for Long-Term Space Missions", Presented at the Hydraulics/Hydrology Civil Engineering Seminar, Purdue University, West Lafayette, Indiana, October 2003.

Zorana Naunovic, Pennell Kelly, Dennis A Lyn, Ernest R Blatchley III, "Water Disinfection for the Mission to Mars". Poster presented at the *4th Annual Environmental Science and Engineering Institute (ESEI) Symposium*, Purdue University, West Lafayette, Indiana, April 2003.

GAS-PHASE REVITALIZATION USING BIOFILTERS IN ADVANCED LIFE SUPPORT

Principal Investigator: Dr. Albert J. Heber, Ph.D., P.E.
Professor of Agricultural and Biological Engineering, Purdue University

Co-Investigator: Dr. M. Katherine Banks, Ph.D., P.E.
Professor of Civil Engineering, Purdue University

BACKGROUND

Bioregenerative life support systems will play a crucial role in future long-term space missions. Continuous removal of gas and liquid-phase pollutants using biological treatment methods are possible for long term missions, and it is relatively inexpensive compared with other physico-chemical techniques.

The Bio-Regenerative Environmental Treatment for Health (BREATHe) will decontaminate the pollutants in both air and water using biofiltration process. BREATHe I will primarily treat gray water. BREATHe II will clarify habitat air and atmospheric condensate.

To enable efficient removal of a broad spectrum of gaseous trace contaminants from the cabin air, the design and operation of ALS BREATHe II system will need to be optimized based on results from bench scale reactors. Thus it is necessary to generate ersatz cabin air and evaluate bench scale reactor performance operated with different reactor configurations, packing material, gas residence time, liquid recirculation rates, contaminant loading rates, etc. The degradability of major trace contaminants by biofiltration process needs characterization as well.

PROJECT GOALS AND OBJECTIVES

- Optimize the design and operation of ALS biofiltration process to maximize the trace contaminant removal efficiency, from perspectives of both microbiology and engineering, while minimizing the Equivalent System Mass (ESM).
- Develop macrokinetics and microkinetics models for ALS biological gas phase treatment systems.
- Delineate technological barriers for use of biofiltration in ALS systems.

RESEARCH PROGRESS

Simulation of Biofiltration and Indoor Air Quality in the ALS System. A simulation of biofiltration processes was developed to predict the removal efficiency of the gas phase contaminants in the ALS cabin during long term space missions. The influences of physical, chemical, and biological parameters (e.g., liquid recirculation rate, air flow rate, support matrix surface area, matrix depth, Henry's law constant) on the removal efficiency for various gaseous contaminants were studied. The simulation results suggest that biodegradation of the trace contaminants by biofiltration is most significantly influenced by Henry's law coefficients. High removal efficiencies can be obtained for compounds with relatively low values of Henry's Law coefficient (e.g., butanol, acetone). Pollutants with high Henry's law coefficients (e.g., methane, ethylene, carbon monoxide) are difficult to eliminate in a biofilter. This can be explained by the fact that these pollutants have an unfavorable gas-liquid partition, and the pollutant concentration in the biofilm is too low to sustain a high biodegradation rate. Microbial parameters such as half-saturation coefficients also influence removal efficiency. Removal efficiencies for different compounds as a function of bioreactor packed bed depth were also predicted. Modeling efforts are also directed towards taking into consideration the effects of reduced gravity on the biofiltration process. The water film

thickness is predicted to increase at reduced gravity, which might be disadvantageous for biofilter operation, due to mass transfer resistance of contaminants and oxygen.

The one-box model coupled with the biofiltration model was used to predict cabin air quality and biofiltration performance for the purposes of guiding the design of ALS biofilters. Using the one box model and assuming complete removal of all major trace contaminants by the biofiltration process, the contaminant concentrations present in the cabin for 600-d travel with 6 crew members were predicted at different air flow recirculation rates (Figure 1).

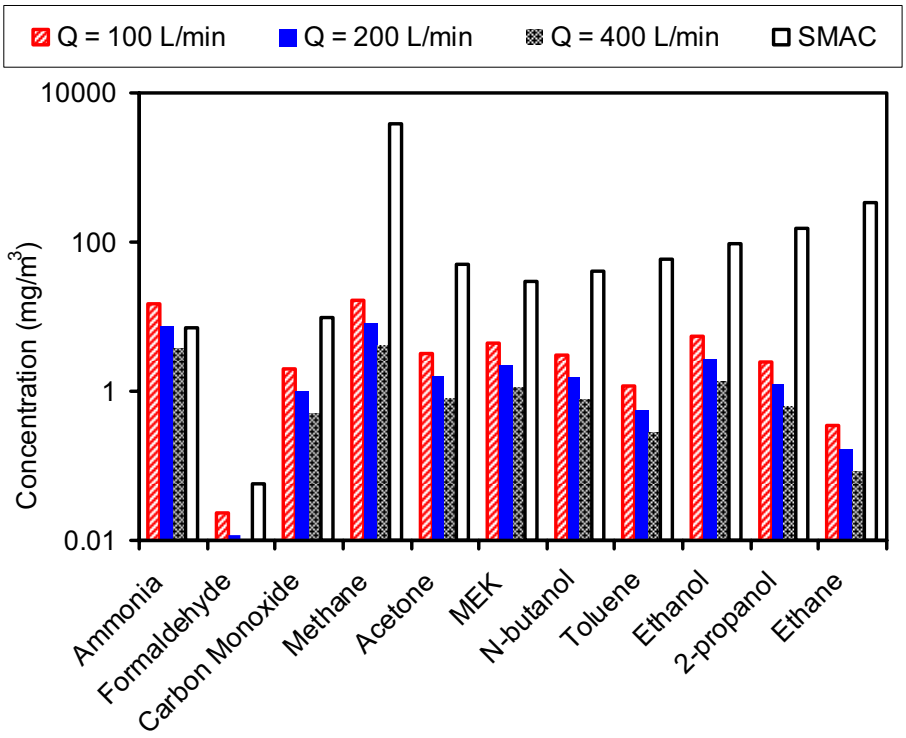


Figure 1. One-box model prediction of contaminant concentrations in cabin air during 600 d mission (6 crew members) assuming 100% of removal efficiency by biofiltration.

Completion of BREATHe II Lab Setup. Over the past 9 months, we have completed the setup of a state-of-the-art lab for testing the BREATHe II biofiltration system. The ersatz cabin air stream is generated using permeation ovens for acetone and n-butanol, and double pattern needle valves for injection of carbon monoxide, methane, ethylene, and ammonia from concentrated gas cylinders. The ersatz air stream was prehumidified to a desired humidity level prior to gas injection by double pattern needle valve control of steam generated by a steam generator. We use computer controlled solenoid valves on the concentrated gas cylinders and a pressure switch on the gas mixing manifold to uphold lab safety and prevent shock loading of the bioreactors in the event of power failure or air compressor failure. Using precision orifices, a stainless steel air supply manifold uniformly distributes the ersatz cabin air to each bioreactor. Liquid recirculation systems for the biotrickling filters and the liquid spray systems for the biofilters were designed and constructed to distribute liquid over the reactor packing material. The Fourier Transform Infrared (FTIR) spectrometer gas analyzer conducts multi-component analysis of methane, CO_2, *n*-butanol, ammonia, acetone, ethylene, and carbon monoxide of bioreactor inlets and outlets.

The laboratory (Figure 2) is capable of testing up to 24 bioreactors with identical inlet concentrations and flow rates. Testing multiple bioreactors simultaneously facilitates powerful factorial experimental design by operating them at different reactor configurations, packing medium types, gas residence times, and liquid types. The computer-controlled sampling and measuring systems allow automatic sequential gas sampling and measurement of contaminant concentrations, airflow rates, air temperature and relative humidity, etc., from each of the 24 bioreactors. The data acquisition and control system, achieved using LabVIEW™ software, allowed automatic, continuous, and real-time gas monitoring and data collection.

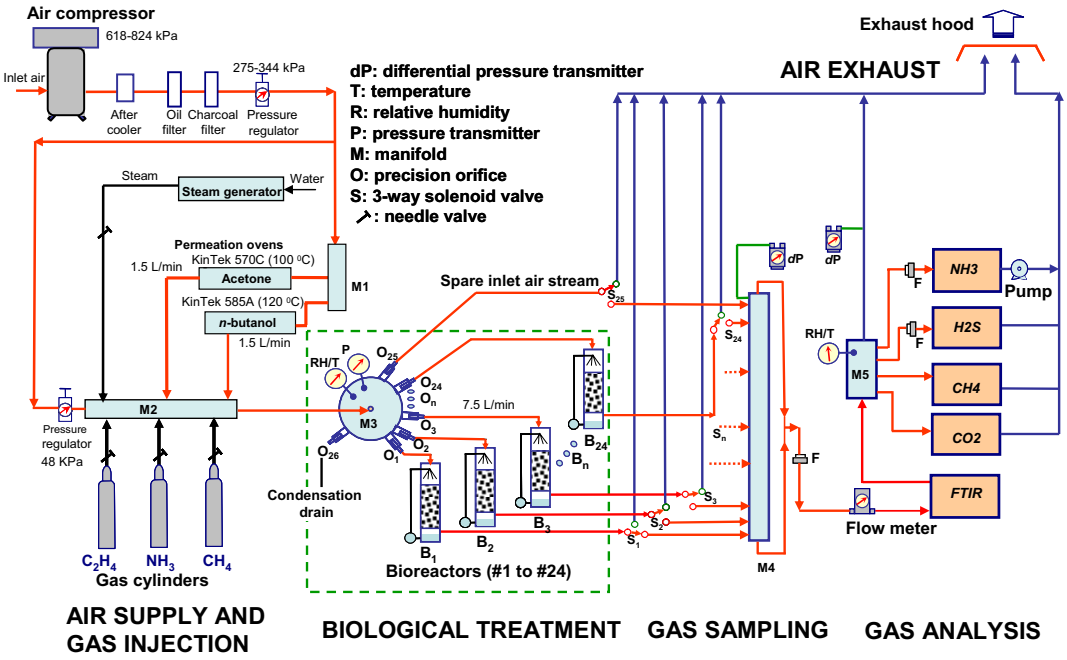


Figure 2. Schematic of the BREATHe II Lab Setup.

Biofiltration Experiment. The first experiment is underway to investigate the feasibility of biofilters and biotrickling filters to remove multiple contaminants most commonly found in cabin air during long duration space missions. The model waste gas stream contains a five-component mixture of acetone, *n*-butanol, methane, ethylene, and ammonia at influent concentrations ranging from 6 to 25 ppm_v. Ammonia was not injected to the air stream initially because of technical problems, but was introduced on day 30 of the test. The prehumidified waste gas stream (90% RH) was distributed evenly to six biofilters and four biotrickling filters for biological treatment, each operated with an empty bed residence time (EBRT) of 30 s. The airflow of each reactor is 7.5 L/min, and nutrients are recirculated throughout the biotrickling filters at 0.2 L/min, while 100 mL of nutrient is sprayed onto the top of the biofilters every 12 h. The packing media types among the ten reactors include perlite (2), polyurethane foam (2), and a mixture of compost, wood chips, and straw (2) in the biofilters and perlite (2) or polyurethane foam (2) in the biotrickling filters. The bioreactors were inoculated with activated sludge from a local wastewater treatment plant except the compost biofilters. Duplicate reactors were run for each of the five operating strategies.

As shown in Figures 3-5, acetone and *n*-butanol were degraded completely within 4 to 10 days after reactor startup for all the reactors except the foam biofilters. Lower acetone and *n*-butanol removal efficiencies observed in foam biofilters were consistent with the visual observation that biomass

established much more slowly on the foam biofilter beds than other reactors. Methane and ethylene removal efficiencies remained extremely low within 25 d of reactor startup for all reactors, indicating the slow acclimation and proliferation of methane- and ethylene-degrading microorganisms. The significant differences observed between the removal efficiencies of acetone, *n*-butanol, methane, and ethylene were consistent with conclusions of computer simulations, i.e., pollutants with high Henry's Law coefficients (e.g., methane, ethylene) are difficult to eliminate with biofiltration. Since the experiment is still in the startup phase, the system may need more time to establish the microbial consortia capable of degrading each pollutant, and long-term continuous operation of the bioreactors is therefore warranted to assess the feasibility of biofiltration for treating such multi-component trace contaminants. The lessons learned and the results of the current test will be used to design the next experiment.

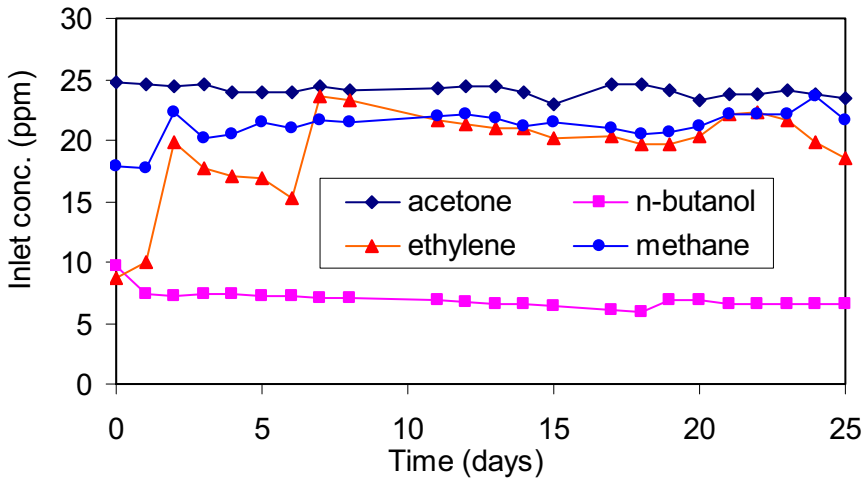


Figure 3. Bioreactor inlet contaminant concentrations.

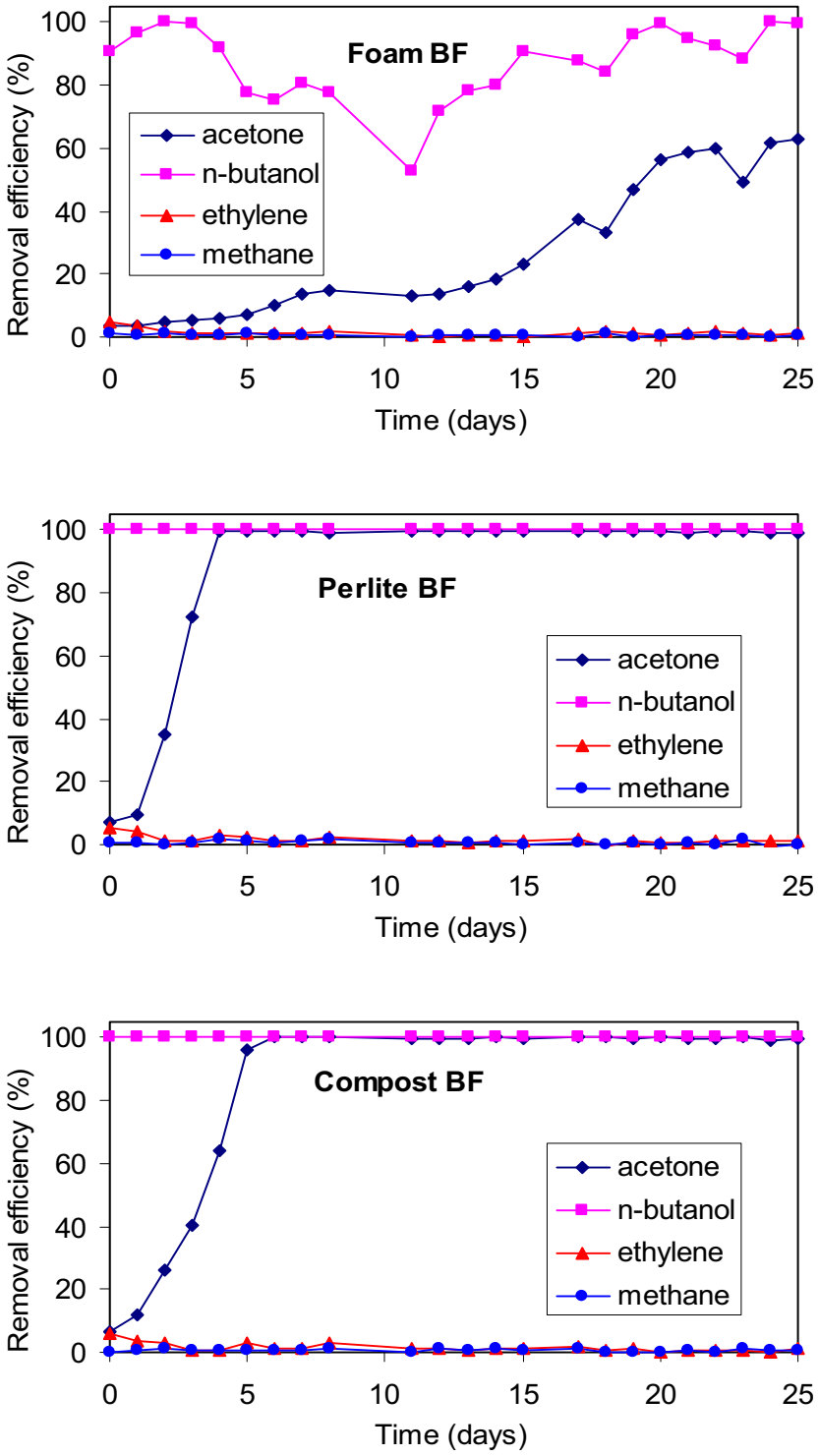


Figure 4. Contaminant removal efficiency for biotrickling filters: foam biofilter (top), perlite biofilter (middle), and compost biofilter (bottom).

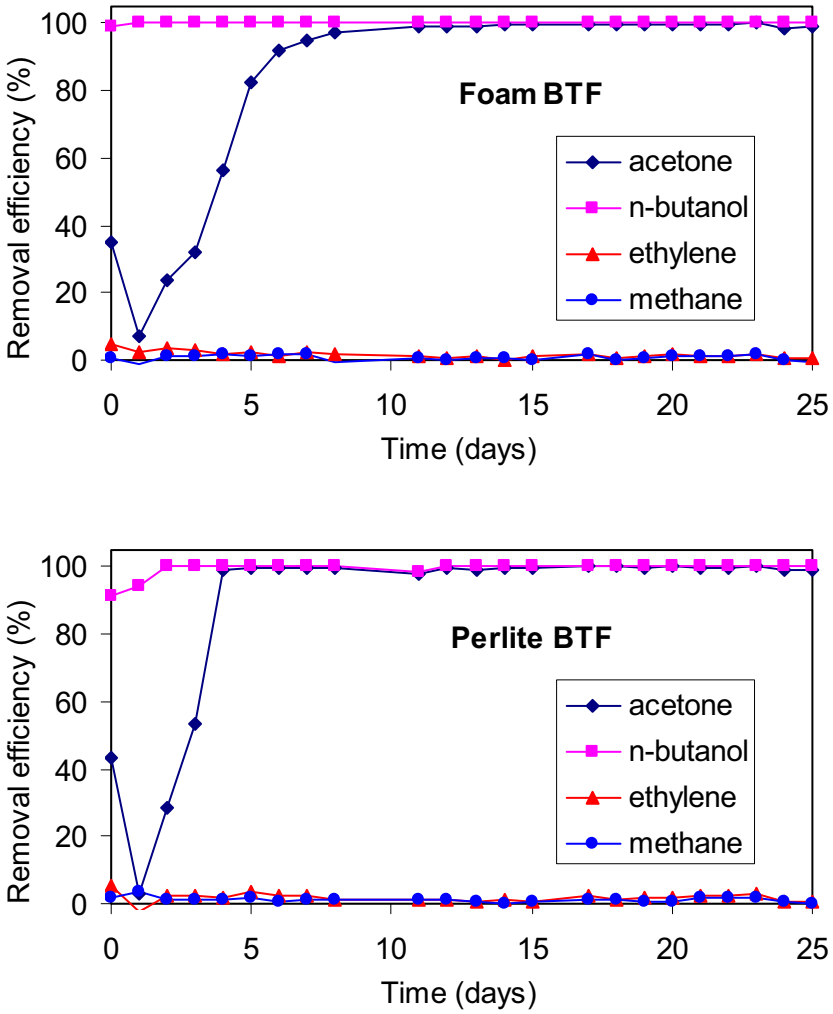


Figure 5. Contaminant removal efficiency for biotrickling filters: foam biotrickling filter (top), perlite biotrickling filter (bottom).

FUTURE RESEARCH DIRECTIONS

- Obtain physical, chemical, and microbial parameters through experiments as input parameters to the simulation model.
- Calibrate and validate the biofiltration model for predicting BREATH II performance in ALS systems.
- Conduct profile studies along the height of each reactor to evaluate any substrate degradation interactions due to kinetic inhibition and/or catabolic repression.
- Determine optimal reactor configuration, packing medium, empty bed residence time, liquid recirculation rate, and contaminant loading rate for contaminant removal. Optimize performance of BREATHe II while minimizing important ESM parameters.
- Qualitatively determine whether hazardous intermediate metabolites are emitted from the bioreactors and optimize reactor operation to minimize such release.

- Analyze the spatial and temporal variation of the microbial community structure in the bioreactors using either conventional cultural or molecular techniques.
- Test removal of other major ALS trace contaminants and single out the recalcitrant contaminants for further study. Identify major limitations to effective removal of such recalcitrant contaminants and develop methods to overcome these limitations.
- Test the feasibility of using cabin condensate as a recirculation liquid.
- Delineate technical barriers to biofiltration process for ALS cabin air contaminant removal, such as microgravity issues, biomass accumulation and clogging of the reactor bed, nutrient limitation, moisture control, etc.

TRAINEES

Congna Li, Air Quality, Postdoctoral Research Assistant, Agricultural and Biological Engineering, Purdue University

Sang-hun Lee, Air Quality, Ph.D. student, Agricultural and Biological Engineering, Purdue University

Hong Huang, Air Quality, M.S. student, Agricultural and Biological Engineering, Purdue University

RESEARCH COLLABORATION

1. Collaboration with Research Project #6 on development and discussion of biofiltration system and modeling.

2. Collaboration with Research Project #15 group on calculation of ESM values.

3. Initiated collaboration with Charles Niederhaus, NASA Glenn Research Center to evaluate the two-phase flow under microgravity or partial gravity conditions.

PUBLICATIONS AND PRESENTATIONS

Lee, S.H., Heber, A.J., and Banks, M.K. (2005). Simulation of air quality in ALS system with biofiltration. *International Conference on Environmental Systems*, Rome, Italy, Paper 05-01-3111.

Li, C., Heber, A.J., Huang, H., Ni, J.-Q., Lee, S.H., and Banks, M.K. (2005). A new lab for testing biofiltration for advanced life support. *International Conference on Environmental Systems,* Rome, Italy, Paper 05-01-3060.

Sharvelle, S.E., Banks, M. K., Heber, A.J. (2003). Wastestream characterization for a packed bed biofilter intended for simultaneous treatment of graywater and air, *International Conference on Environmental Systems*, Paper 03ICES-182.

Heber, A.J., Lee, S.-H., Huang, H., NI, J.-Q., Sharvelle, S.E., Banks, M.K., and Kim, Y.S. (2004). Testing biofilters for advanced life support. Poster presented at the *Habitation Conference*, Orlando, Florida, Jan. 5-7.

Li, C., Heber, A.J., Lee, S.-H., Ni, J.-Q. (2006). Biofiltration for gaseous trace contaminant removal from spacecraft during long-term space missions. Abstract submitted to *Habitation Conference*, Orlando, Florida, Feb. 5-8.

Biosolids Dewatering and Nutrient Recovery of Solid-Phase Thermophilic Aerobic Reactor (STAR) Effluent Using Various Plant Species

Principal Investigators

Dr. Jeffrey J. Volenec: Professor, Department of Agronomy, Purdue University
Dr. Brad C. Joern: Professor, Department of Agronomy, Purdue University

Trainees

Shane Howard (M.S. Student)
Amy Berg (M.S. Student)
Kess Berg (Ph.D. Student)
Suzanne Cunningham (Research Physiologist)
Kali Frost (Undergraduate Student)

Research Collaborations

Dr. James E. Alleman: Solid-Phase Thermophilic Aerobic Reactor (STAR) Processing of Fecal, Food, and Plant Residues.
Dr. Paul Brown: Waste Treatment Using Tilapia**.**
Dr. Caula Beyl: Waste Treatment Using Fungi

Project Goals and Objectives

Identify biomass specie(s) best suited for dewatering and capturing nutrients in STAR waste effluent.
Develop a cropping system that utilizes the dewatered biosolids form the STAR reactor to grow crops for food, mushroom growth and fish feed.
Investigate plant growth substrates for STAR effluent dewatering process.

Research Progress:

Experiment 1:

Candidate species for dewatering of biosolids included the following grasses, legumes, and food crops from the NASA ALS program:

Grasses	**Legumes**	**Food Crops**
Palaton Reed canarygrass	Endura Kura Clover	Super Dwarf Rice
Dakotah Switchgrass	Kopu II White Clover	USU Apogee Wheat
Eastern Gamagrass	Jumbo Ladino Clover	Micro-Tina Tomato
Bronson Tall Fescue	Big Trefoil	Triton Pepper
Millennium Tall Fescue		USU Perigee Wheat
Shawnee Switchgrass		

Plants of each species were established in 1 L pots containing coarse silica sand and established using a complete nutrient solution during two months of establishment in the greenhouse. Legumes were inoculated with the appropriate strain of *Rhizobium* to facilitate symbiotic fixation of atmospheric dinitrogen gas into plant-available forms. Pots were placed in a greenhouse set to control temperature (25ºC±5ºC) and photoperiod was extended to 15 h with artificial lighting. After establishment, pots were grouped into three replicates each containing four pots of each species. The following four STAR effluent treatments were randomly assigned to pots of each species within each replicate: control (no effluent); 1/2 acre-inch of effluent (86 mL per 1 L pot); 1 acre-inch of effluent (172 mL per 1 L pot); and

2 acre-inches of effluent (344 mL per 1 L pot). Cups were placed under pots to catch liquid that drained from the bottom of the pots following effluent application. Pots remained in the greenhouse and were watered with reverse-osmosis water as needed. Photos of plants were taken one week after effluent application. Plants were destructively sampled two weeks after effluent application to obtain dry weights of roots and shoots, and tissues for mineral analysis. Poor establishment prevented inclusion of three replicates of Eastern Gamagrass. Both wheat cultivars developed very rapidly, and were setting seed after two months so they were not included in this study, but were evaluated in a subsequent study.

During the week following effluent application there was no visible injury to any species caused by the ½ acre-inch effluent application. Species exhibiting the least injury included kura clover and switchgrass, while tall fescue and reed canarygrass exhibited mild and moderate injury, respectively, to at the 2 acre-inch effluent rate. Leaf tips of rice were necrotic on plants provided 1 and 2 acre-inch effluent rates, but otherwise these plants were relatively uninjured. Several species receiving 2 acre-inches of effluent were severely injured including big trefoil, Ladino clover, and pepper. Tomato appeared to be most sensitive to effluent application with severe injury exhibited by plants provided 1 and 2 acre-inch effluent treatments.

Experiment 2
Candidate species for dewatering of STAR effluent in Experiment 2 included the following grasses, legumes, and food crops:

Grasses	Legumes	Food Crops
Palaton Reed canarygrass	Endura Kura Clover	USU Apogee Wheat
Dakotah Switchgrass	Kopu II White Clover	Micro-Tina Tomato
Millennium Tall Fescue	Jumbo Ladino Clover	Triton Pepper
	Big Trefoil	USU Perigee Wheat

Three plants of each of the eleven candidate species were established in 1 L pots containing a coarse silica sand and provided nutrient solution for four weeks. Pots were placed in a greenhouse set to control temperature (25ºC±5ºC) and photoperiod of 15 h with artificial lighting. Plants growing in 1 L pots were then placed in a second 1 L pot for submersion into one of three treatments (STAR effluent, nutrient solution, and deionized water). All three treatments were delivered via a tube inserted into the bottom 1 L pot. Treatments were delivered daily to each plant specie, recording volume for water use and efficiency data. After twenty five days, plants were destructively harvested and divided into herbage, crown and root components and analyzed for B, Ca, Cu, Fe, K, Mg, Mn, N, NO_3, Na, P, S, and Zn composition.

Water transpiration increased as plant mass increased for water and Hoagland's treatments, but not with the STAR treatment. All species grown in STAR effluent exhibited reduced growth and enhanced senescence. Hoagland's nutrient solution generally increased tissue concentrations for most elements tested. Tissue sodium concentrations of plants grown with STAR effluent were significantly greater than that observed with the other treatments.

Experiment 3
The following candidate species were included for dewatering STAR effluent in Experiment 3. These plants were selected based on their potential for growth in the anoxic effluent environment.

Cattail
Rush
Kopu II White Clover
Reed Canarygrass
Super Dwarf Rice

Three plants of each of the five candidate species were established in 1 L pots containing an illite-montmorillinite-silica substrate (Turface) and provided nutrient solution for four weeks. Pots were placed in a greenhouse set to control temperature (25ºC±5ºC) and photoperiod of 15 h with artificial lighting. Plants growing in 1 L pots were then placed in a second 1 L pot for submersion into one of two treatments (STAR effluent and nutrient solution). Treatments were administered in the same fashion as in experiment 2. After one week, the STAR treatment was substituted with deionized water due to limited STAR effluent availability. Plants were destructively harvest after four weeks and divided into herbage and root components for dry weight analysis.

Cattail was the only specie that outperformed the nutrient solution treatment in terms of total dry weight production. Cattail also was the only specie observed to have roots actively growing in STAR effluent. Total dry matter production of all other species was less than that of the nutrient solution treatment.

Planned Research for 2005:
Based on poor plant performance when placed in the STAR effluent and EAC feedback, we had planned to work with Dr. Alleman's group to dewater the STAR effluent via freeze-drying or direct physical solid-liquid separation, mixing the solids with an inert and recoverable/recyclable silica glass root growth media, and use food crops to recover and recycle nutrients from the STAR residuals.

This proposed change in direction was sent to the project director in February 2005. In February 2005, the project director unilaterally eliminated this project from the program because he wanted "to de-emphasize the role of plants in waste processing in space habitats and to go in a different direction." This was the only meeting we ever had with the project director during this project. Funding was provided only to partially cover trainee costs and close out costs during 2005, so no additional experiments were conducted in 2005.

One of the greatest challenges we faced throughout this project was the lack of available STAR effluent. Dr. Alleman requested funding to build a second reactor in 2004, but our understanding was that his request was denied by the project director.

Publication:

S.M. Howard, J.J. Volenec, B.C. Joern, J.E. Alleman, D.R. Whitaker. 2004 "Biosolids Dewatering of Solid-Phase Thermophilic Aerobic Reactor (STAR) Effluent Using Various Plant Species. Agron. Abstr.

SYSTEMS GROUP EXECUTIVE SUMMARY

This year was a transition year for the systems group researchers (investigators, postdoctoral staff, graduate students, and technical staff) from learning and exploratory mode, which can be mainly characterized by proof-of-concept studies to build a viable toolbox of systems analysis methodologies suitable for ALS analysis, e.g. SimOpt (SIMulation based OPTimization), Markov Theory and Model Predictive Control, towards well structured and coordinated efforts to improve their readiness level for repeated use in answering questions that matters to NASA and ALS community at large.

As systems group was undergoing this transition, the need for expanding the scope of systems group, which was set limited only to ALS-NSCORT technologies at the original center proposal, is recognized. Consequently, a supplemental proposal in collaboration with Dr. Drysdale has been developed and submitted to NASA. This proposal was crafted to expand the systems group's analysis and modeling scope to all ALS technologies and to adopt lifecycle management view to study 15-20 year Mars Base ALS system evolution which recognizes the fact that Mars Base ALS system will not stay as it was deployed at the very first mission. Unfortunately, support and resources requested in the supplemental proposal could not be made available at that time as NASA was undergoing through major re-organization. However, another unfortunate development within the systems group, resignation of Dr. Trimble of Howard University from ALS-NSCORT, made some funds available at the ALS-NSCORT to pursue a portion of the activities proposed in the supplemental support request. Consequently, Dr. Lasinski and Dr. Russell joined to the systems group as fulltime Post-Doctoral Research Fellows to fill the void generated per successful completion of Dr. Applequist's Post-Doctoral appointment, and to support the expanded scope of the systems group and the implementation of life-cycle management view. Furthermore, a proof-of-concept study has been initiated in collaboration with Envision Center at Purdue University on visualizing the evolution of Mars Base and its ALS system.

It is our pleasure to report YanFu Kuo's graduation from the program (who has successfully fulfilled the MS degree requirements in Mechanical Engineering), and Selen Aydogan's peer reviewed journal publication.

Besides its research charter, systems group has also engaged in several outreach activities such as giving invited lectures at the McKenzie Career Center to Mr. Martin's high school class, generating projects based on the ALS problem in Chemical Engineering Design Course (ChE 450) lead by Dr. Pekny, supervising a senior undergraduate student summer research project (Rebecca Alway-Cooper of Chemical Engineering), editing a "baby BVAD" intended to be used in the project "Equivalent Systems Mass Analysis of Plant Growth" with high school students lead by outreach group of ALS-NSCORT. Systems group has also continued leading and supporting center-wide project management, roadmap development, process map revisions and ESM evaluation of ALS-NSCORT technologies initiatives and activities.

As systems group we are excited with our new vision and hope that our near-term future work will fuel high impact results for ALS community in addition to scholarly success.

Seza Orcun
Integrated Systems Group – Focus Area Lead

SYSTEMS MODELING IN ALS: A SIMULATION BASED OPTIMIZATION APPROACH TO MODEL AND DESIGN OF AN ADVANCED LIFE SUPPORT SYSTEM

Principal Investigator: Joseph F. Pekny, PhD

BACKGROUND

An Advanced Life Support System (ALSS) is subject to many operating degrees of freedom. The choice of optimal values for these, e.g. values which would minimize the amount of re-supplies or provide a safe environment for the crew members, can only be made by considering the performance objectives of the integrated system. Furthermore, an ALSS will be subject to many dynamic factors such as time-varying boundary conditions, system parameters that drift with mission time, and unplanned operating events. A novel approach, SIMulation based OPTimization (SIMOPT), is proposed to understand the dynamics of the ALSS.

The SIMOPT approach involves the integration of a deterministic optimization model which uses average system parameter values to generate planning decisions with a discrete event Monte Carlo simulation model which represents the evolution of the system over time in the presence of uncertainties in key system parameters. A typical SIMOPT timeline involves several applications of the Deterministic Optimization (DO) model at different time points to set the degrees of freedom of the system, i.e. re-supply amounts or inventory levels for CO_2, O_2, H_2O or food, optimally. In between each DO optimization within a timeline, the simulation is used to mimic the system response under uncertainty. The simulation is used to introduce uncertainty in key model parameters, such as changes in the yield of crop growth in an ALSS, or exceptional discrete events, e.g. malfunction of waste water treatment equipment in an ALSS. Thus, the response being observed during a simulation run will differ from the set values determined by the DO optimization algorithm. In fact when the simulation observed values and the optimization predicted values differ significantly (such occurrences are called trigger events), a new optimization is performed to re-set the degrees of freedom of the system. The uncertainty introduced through random changes in parameter values and exceptional events in the simulation generates a different timeline, i.e. sequence of trigger events, at each replication. The results of a significant number of timelines generated for each scenario are aggregated to study the long term expected behavior of the ALSS.

PROJECT GOALS AND OBJECTIVES

- Development of a computational framework and analysis for studying the real-life behavior of an ALSS.
- Development of a protocol for investigating the possible design configurations for an ALSS.
- Demonstrating the performance of the developed computational framework by case studies.

RESEARCH PROGRESS

Development of the computational framework and analysis tool. Figure 1 presents the SIMOPT structure developed to observe the overall behavior of the ALS systems. For a specific ALSS scenario, the simulation is replicated 50 times (test were done with different number of runs and it is observed that using more than 50 runs does not affect the initial results significantly), to generate 50 different timelines. This time series data is processed using time-series data mining techniques to determine the safety zones for re-supplies. The safety zone (safety buffer) for a given variable represents the range of acceptable values for that variable for the specific time interval. For example, the safety buffer for O_2 re-supply gives the maximum and minimum amount of O_2 that would be required by the system for any given day. Safety buffer values are fed to the ALSS optimization module as constraints. The optimal solution, which

minimizes the re-supplies that are difficult to procure or transport in a given ALSS scenario and the amount of untreatable waste, is fed as operating conditions to the ALSS simulation and it is replicated several times to see if the data in the created timelines agree with the predetermined safety zones. If the amounts of re-supplies are within the predetermined safety zones (with a specified confidence level) for the components O_2, CO_2, H_2O and food, SIMOPT is terminated generating the safety zone values. However, if trigger events occur, which means that the data collected from the timelines are not within the predicted safety zone values, then the safety zone determination algorithm is called to scan and search the timeline series data to update the safety zone values, which are then sent to the optimization as new constraints. This loop continues until no more trigger event is observed in the system.

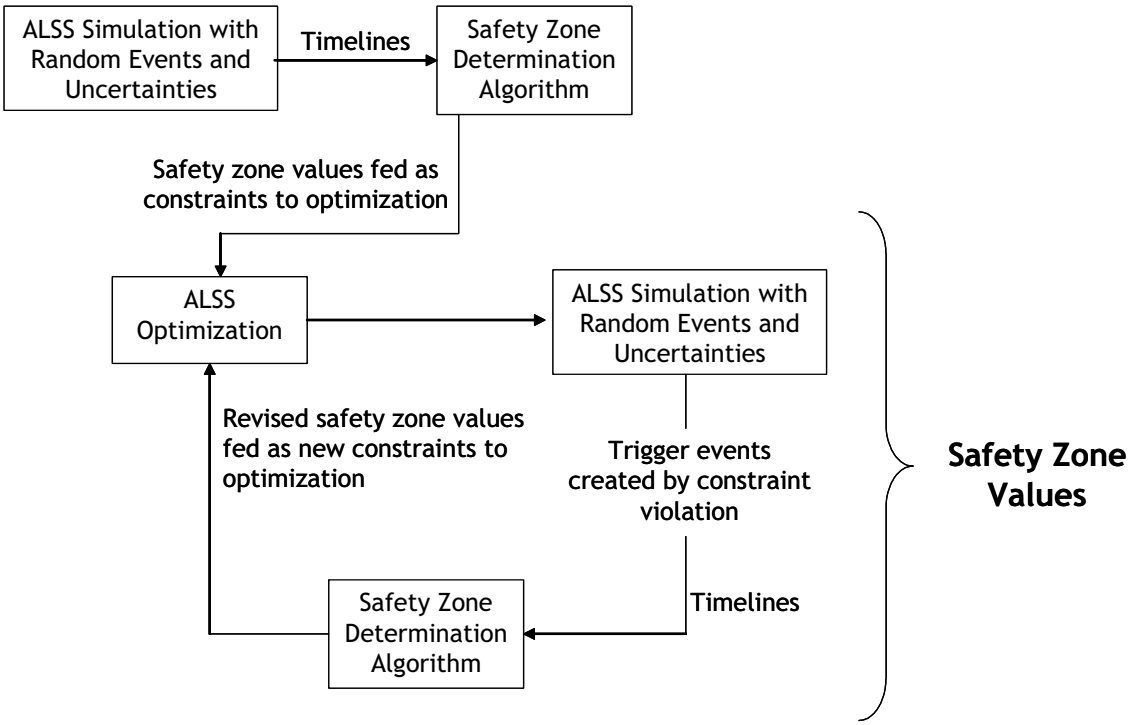


Figure 1. Developed SIMOPT structure for ALSS

Three different life support system scenarios with different technology levels are modeled and the overall behaviors of these systems with their impact on the overall waste generation are investigated using the described SIMOPT framework for a 600 day Mars base mission. These scenarios are:

(1) Scenario 1: No waste recovery system, where all supplies are shipped from earth,

(2) Scenario 2: Physicochemical (PC) waste recovery system, in which food is supplied from earth while significant portion of air and water are regenerated by PC systems, and

(3) Scenario 3: A composite bioregenerative and PC waste recovery, where food is grown on site while regenerating air and water.

It is concluded that with the current technology levels, for a 600 day mission to Mars, PC waste recovery technology (Scenario 2) seems to be the most promising one. However, bioregenerative & PC waste recovery system (Scenario 3) might become more efficient by processing human and inedible crop wastes separately, resulting in an increase in the waste water recovery efficiencies. Additionally, different options of solid waste recovery system (e.g. bioreactors) should be considered to lower the oxygen requirement of the solid waste utilization for Scenario 3. Therefore, a more hybrid system of

bioregenerative and PC technologies might be the best option. This is left as a future study. The detailed results of this study can be found in Aydogan *et al.* (in press).

Development of a Crop Planting Scheduling Module. The current version of the SIMOPT framework for ALSS does not allow for scheduling changes throughout the course of the simulation. For example, for a bioregenerative ALSS scenario, if some or all of the crop area is lost during a simulation, it is not replenished until the next planting schedule. However, a new optimization could be performed to determine a new planting schedule at that state of the system and those results can be used for the rest of the simulation. This would increase the utilization of the resources, which is crucial for the ALSS. Therefore, a planting schedule model has been developed to overcome this problem.

In this study, optimum crop planting schedule that would minimize the ESM of a bio-regenerative ALSS is determined using an advanced crop planting scheduling model in conjunction with a diet optimization model. Mixed Integer Linear Programming (MILP) models are developed to determine the best crop planting schedule and optimum diet for the crew-members. Given the activity schedule of the crew members, the diet optimization module constructs a diet cycle of 20-30 days that would meet the necessary nutritional requirements observing a predetermined diet variety. In doing so the diet optimization tries to minimize the overall system ESM. Necessary biomass amounts calculated by this model are fed into the crop planting scheduling model as the demands. Given these demands and growth parameters for these crops, the crop planting scheduling model determines the best planting schedule that will optimize the system behavior, i.e., the one that would minimize ESM. Figure 2 gives the graphical representation of the diet optimization and crop planting scheduling models.

The diet optimization and crop planting scheduling modules has been tested for a 600 day Mars mission with a crew of six. The crew is assumed to be formed from three men and three women members. The weights of the crew members are determined by randomly sampling from a triangular distribution with a maximum value of 98.5 kg (95th percentile American male), minimum value of 41.0 kg (5th percentile Japanese female) and a nominal value of 70.0 kg (Hanford, 2004, Table 3.3.1). The height is assumed to be proportional to the weight and lastly, it is assumed that the light weight crew members are females. For each crew member, a daily activity schedule is prepared for 30 day cycles. The time allocations for a nominal crew schedule table (Hanford, 2004, Table 3.3.3) are utilized for the preparation of the crew schedules. Throughout the 30 day cycle, it is assumed that each crew member takes two days off as vacations (after completion of their extravehicular activity (EVA) weekdays). In a 30 day cycle, there are four weeks, each one of which has five week-day schedules and two weekend schedules and two vacation days. Additionally, it is assumed that two crew-members, one man and one woman team, does a four hours of EVA for every weekday throughout a week.

The MILP formulation of the diet optimization, for described crew characteristics and their activity schedules, is solved using CPLEX (Ilog Inc., 2001). The objective function is 1225 kg ESM for 30 days. This makes the overall ESM of the optimum system as 24500 kg for a 600 day mission. The diet generated for 30 days is assumed to repeat until the end of the 600 day period. Using ingredients of the each recipe in the diet, the demands for each crop are calculated. The crop planting scheduling formulation, given the demand and the crop data, calculates the best crop planting schedule, i.e., the one that would minimize the overall re-supply. The total amount of re-supply that is required for 600 days, including the supplies that can not be grown on site, is 1480.8 kg. The total amount of supplies that can not be grown on site add up to 581.6 kg and the rest of the re-supply is to cover the first cycle time of each crop. The proof of concept study showed that these models can be used to predict the planting schedule for a bio-regenerative ALSS and the crop planting schedule can be linked to the crew activity schedules.

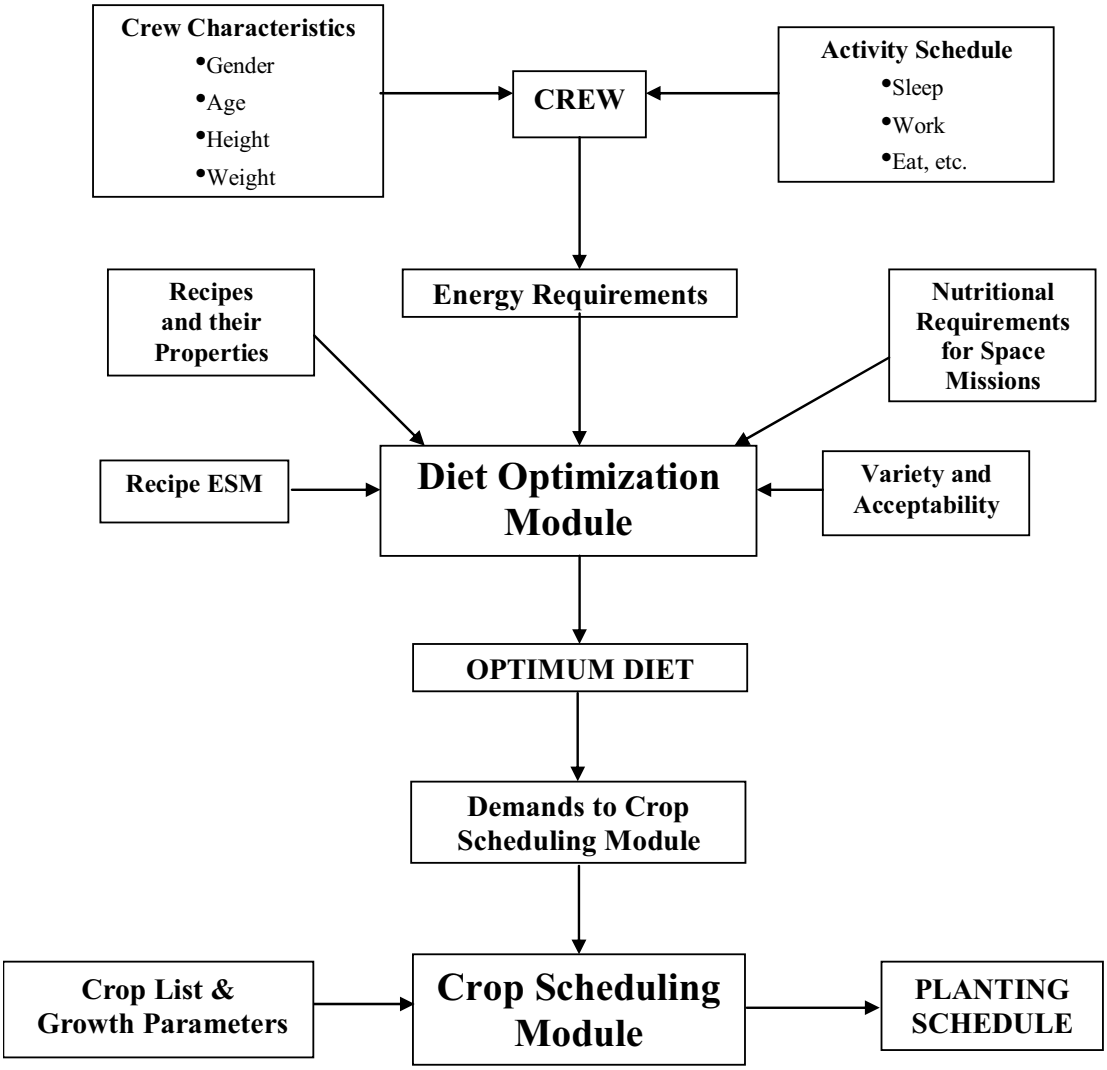


Figure 2. Graphical representation of the diet optimization and crop scheduling models

FUTURE RESEARCH DIRECTIONS

- Extend the SIMOPT approach to determine the optimum technology list and the deployment schedule for an ALSS station that would meet the crew member requirements.
    - The recipe database of the Diet Optimization model will be populated with the addition of new recipes gathered from NASA recipe database in order to overcome feasibility problems related to the limited number of recipes.
    - The Technology Selection formulation, which will determine optimum technology list and their deployment schedule given the crew requirements and the mission scenario, will be developed.
    - ALSS discrete-event simulation will be revised to incorporate the technology deployment schedule determined by the Technology Selection Module.
    - Rule Generation Algorithm can determine the safety zone values. Technology success assessment routines will be added to revise the technology list and characteristics.
- Demonstrate the performance of the framework with case studies.

- The developed framework will be used to determine the behavior of an ALSS for a 600 day Mars mission. For a mission of this length, there will not be any technology deployment schedule since it would not involve any additional trips throughout the course of the mission. The solution will determine which technologies will have a better chance of utilization for that mission and the supply levels. The results of this study will be compared to the current ALSS suggestions for the same mission in literature.
- The system will be run for a 15 year Mars base mission to generate technology deployment schedules and determine the bases evolution.

TRAINEES

PhD Students: Selen Aydogan

RESEARCH COLLABORATION

- Mike Ewert suggested that a study to determine the effect of crew size and characteristics on the system ESM can be done with the models presented in the May 19th SIMA teleconference. Selen Aydogan contacted Mike Ewert for example crew profile suggestions.
- Selen Aydogan contacted Dr. Michelle Perchonok to receive the recipe database that is developed for ALSS diet. These recipes will be used to populate our current recipe database within the diet optimization module.

PUBLICATIONS

Aydogan, Selen, Orcun, S., J. F. Pekny, "Effect of Different Waste Recovery Systems on the Overall Waste Generation Rates for an Advanced Life Support System", International Journal of Environment and Pollution, in press.

Aydogan, Selen, Orcun, S., Blau, G., Pekny, J. F. , G.V. Reklaitis, (2005) "Determining Optimum Planting Schedule Using Diet Optimization and Advanced Crop Scheduling Models", *SAE Technical Papers*, 2005-01-2815.

PRESENTATIONS

Aydogan, S., S. Orcun, G. Blau, J. F. Pekny, and G.V. Reklaitis, (2005) "Determining Optimum Planting Schedule Using Diet Optimization and Advanced Crop Scheduling Models", Presented at the *Systems Integration, Modeling, and Analysis (SIMA) Teleconference*, May 19th 2005.

Aydogan, S., S. Orcun, G. Blau, J. F. Pekny, and G.V. Reklaitis, (2005) "Determining Optimum Planting Schedule Using Diet Optimization and Advanced Crop Scheduling Models", Presented at the *International Conference on Environmental Systems*, Rome, Italy.

Aydogan, S.(2005) "A Simulation Based Optimization Approach to Modeling and Design an Advanced Life Support System", Presented at the *14th Annual Graduate Research Symposium* at School of Chemical Engineering, Purdue University, West Lafayette, IN.

Aydogan, S., S. Orcun, G. Blau, J. F. Pekny, and G.V. Reklaitis, (2005) "A Simulation Based Optimization Approach to Modeling and Design an Advanced Life Support System", Poster presentation at the *Cyber Environment Workshop*, Purdue University, West Lafayette, IN.

Dink D., S. Aydogan, V. Varma, G.E. Blau, J. F. Pekny, G. V. Reklaitis, S. Orcun, and R. L. Rardin (2005) "Healthcare and Life Support System Optimization", Poster presentation at the *14th Annual Graduate Research Symposium* at School of Chemical Engineering, Purdue University, West Lafayette, IN.

REFERENCES

Hanford, A.J. (2004) Advanced Life Support Baseline Values and Assumptions Document, NASA/CR – 2004 – 208941, Johnson Space Center, Houston.

Ilog Inc., (2001) "ILOG CPLEX 7.1 Reference Manual", Ilog Inc.

SYSTEMS MODELING OF ADVANCED LIFE SUPPORT

Principal Investigator **Dr. George T-C. Chiu, PhD., Associate Professor, Mechanical Engineering, Purdue University**
Dr. Bin Yao, PhD., Associate Professor, Mechanical Engineering, Purdue University

Cumulative Research Progress to Date:

ESM estimation for STAR
A detailed initial ESM estimation for STAR with a Mars surface mission is completed and published in ICES 05. Based on the experimental results from the STAR research group, the paper documented the assumptions and methodologies that were used in estimating the ESM. Initial trade studies with similar solid waste treatment processes were also presented.

ESM estimation and comparison for LiFT
An initial ESM estimation for a LiFT process in the Mars surface mission is completed based on the preliminary study and experimental results. A trade study for LiFT and two competitive technologies, VCD and VPCAR, is performed with the respect of energy consumption. The comparison shows that LiFT is advantageous from an energy consumption point of view and can be a viable alternative for urine water recovery in ALS systems. Sensitivity analysis of the ESM provided design guidelines for the LiFT research group to further reduce the process ESM by improved process design.

Automated pH level control to reduce crew-time requirement for plant growth
A network ready multiplexed pH level control system for a hydroponic growth chamber was completed. Two conference papers are submitted for consideration, one is for potential presentation at the Habitation 2006 and the other is being reviewed for the 2006 American Control Conference. Significant reduction in crew time can be achieved, with a network closed-loop pH control system. The reduction in crew time has a direct impact in ESM estimation for plant growth chamber.

Future Research Directions:
System control and energy analysis
Model Predictive Control (MPC) formulation will be used to develop a subsystem level control strategy to maintain adequate life support capacity but minimize energy consumption or maximize subsystem level efficiency. A phenomenological dynamic model for the NSCORT water/air treatment systems will be developed based on existing data and estimations. This model will be used to formulate and design the MPC controller. The result of the investigation will provide capacity and efficiency requirements and the associated control strategy to maintain adequate air and water reclamation for the crew. It will also provide the necessary dynamic response for other systems that are directly connected to the air/water treatment systems.

Continue pH level control validation
Continue validating the effectiveness of the automated pH level control by comparing biomass production with and without automated pH control and identify the optimal pH time history based on plant growth physiology.

ESM sensitivity analysis
The ESM results from the integrated water/air treatment system will be examined and used to perform a sensitivity analysis. Sensitivity analysis can identify aspect of the system that will provide most significant improvement.

Trainees
YanFu Kuo, Mechanical Engineering, MSME
Jun Cai, Mechanical Engineering, MSME

Research Collaboration:
Molly Anderson at JSC is contacted for the dynamic modeling for NASA ALS technologies.
Julie Levri at AMS research center is contacted for the details of ESM conduction.

Publications and Presentations To-Date:
ICES05
Y.F. Kuo, D.R. Whitaker, G.T.-C. Chiu, and J.E. Alleman "System Level Design and Initial Equivalent System Mass Analysis of a Solid-Phase Thermophilic Aerobic Rector for Advanced Life Support Systems" Proceeding of the 2005 SAE ICEC.

Habitation 2006
M. Mukhtar, G. T.-C. Chiu, G. Massa, and C. A. Mitchell "pH Level Control of a Hydroponic Growth Chamber" Proceeding of the 2006 Conference on Habitation Research and Technology Development (in review).

2006 American Control Conference
M. Mukhtar, G. T.-C. Chiu, G. Massa, and C. A. Mitchell "Application of Switching based Nonlinear Model Predictive Control to a pH Neutralization Process" Proceeding of the 2006 American Control Conference (in review).

References:
Hanford, A. J., 2003, Advanced Life Support Research and Technology Development Metric – Fiscal Year 2002, CTSD-ADV-510, JSC 60313, National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston, Texas.

Levri, J. A., Drysdale, A. E., Ewert, M. K., Fisher, J. W., Hanford, A. J., Hogan, J. A., Jones, H. W., Joshi, J. A., and Vaccari, D. A., 2003, Advanced life support equivalent system mass guidelines document, NASA TM-2003-212278, National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California.

Stafford, K. W., L. T. Jerng, A. E. Drysdale, S. Maxwell, J. A. Levri, M. K. Ewert, and A. J. Hanford, 2001, Advanced Life Support Systems Integration, Modeling, and Analysis Reference Missions Document, JSC 39502, Revision A, National Aeronautics and Space Administration, Lyndon B. Johnson Space Center, Houston, Texas.

SYSTEMS MODELING OF ADVANCED LIFE SUPPORT

Principal Investigator Dr. Yuehwern Yih, PhD., Professor, Industrial Engineering, Purdue University

Project Goals and Objectives

- Investigate oxygen, carbon dioxide and water supply, storage tank and treatment capacities required for long term Mars surface mission
- Explore possible solutions to reduce the water storage tank ESM through what-if scenarios and sensitivity analysis
- Investigate and validate methodology based on Markov Principles, Catastrophe Theory, and possible use of BioSim to ensure a safe working and living environment for crewmembers in an enclosed environment for a given mission while minimizing ESM.

Cumulative Research Progress to Date

A dynamic simulation model is built to investigate the oxygen, carbon dioxide and water supply, storage tank and treatment capacities required for long term mission. 6 crewmembers and 15 years of Mars surface mission duration are assumed in this study. Several what-if scenarios are explored to evaluate the impacts of shipping all the supplies from Earth without regenerative technologies and crops, utilizing regenerative technologies, and growing crops on oxygen, carbon dioxide and water supply, storage tank and treatment capacities. 7-day safety stocks for contingency and different rates of leakage and technology inefficiency are also explored. Storage tank ESM is computed for every scenario. From this study, it is found that 1% of total loss (resulted from leakage and technology inefficiency) from the entire mission duration is roughly equivalent to 55 days of safety stock. In other words, leakage and technology inefficiency has a higher impact on storage tank ESM values than the safety stock for contingency.

The study also found that water demand and its corresponding storage tank ESM value are the highest, compared to oxygen and carbon dioxide. Therefore, the subsequent study focuses on the water subsystem only. Food subsystem is considered in this work. A linear program is built to determine the best crop combination for crewmember consumption based on crewmember energy requirement and their crop preference. The result affects the total crop area and water needed to grow the crops. Different scenarios are run to evaluate whether the total water supply, treatment and storage capacity in crewmember-and-crop scenario are the sum of water supply, treatment and storage capacity in crewmember-only and crop-only scenarios.

A framework has been built based on Markov Theory as a base for further studies. This framework outlines the interaction between crewmembers and plants in terms of oxygen and carbon dioxide exchange between these entities.

Three states are defined: Safe, Transient, Risky. These states are defined based on the level of carbon dioxide and oxygen in the enclosed area under scrutiny. The “Safe” state consists of sufficient CO2 and O2 for plants and humans, respectively; the “Transient” state consists of sufficient O2 for humans, but insufficient CO2 for plants, hence, leading to an insufficient O2 production when the plants eventually die; the “Risky” state can be broken down into two scenarios: 1) Insufficient O2 and insufficient CO2. Hence both humans and plants are both in danger. 2) Sufficient CO2 for plants but insufficient O2 for crewmembers. The immediate lack of O2 poses a great threat to the health of the humans.

Possible actions are defined for each state should one or more action(s) be taken as an effort to change the state of the system. These actions are common to all the states unless there is a need to define different sets of actions for different states. They are:

1) Do nothing
2) Release O2 from tank
3) Release CO2 from tank
4) Increase light intensity on plants
5) Decrease light intensity on plants

Probabilities of "success" are generated based on logical reasoning for the purpose of testing and simulation. "Success" refers to a change of state either 1) from "Risky" to "Transient", or 2) from "Transient" to "Safe". Each action (including do nothing) taken while the system is in the "Safe" state can either bring the system to "Transient", or remain at "Safe". Each action taken while the system is in the "Transient" state can bring the system back to "Safe", remain at "Transient" or to "Risky". Similarly, each action taken while the system is in the "Risky" state can either bring the system back to "Transient" or remain "Risky".

The success (or failure) for every effort made to cause a state change should be measured. A reward system has hence been defined. The Reward, Rij(k) is outlined as follows:

Rij(k) = +1 if action k causes the system to transit from Risky to Transient
= +1 if action k causes the system to transit from Transient to Safe
= +1 if action k causes the system to transit from Safe to Safe
= -1 if action k causes the system to transit from Safe to Transient
= -1 if action k causes the system to transit from Transient to Risky
= -1 if action k causes the system to transit from Risky to Risky
= 0 otherwise (if action k causes the system to transit from Transient to Transient)

Future Research Directions

- Sensitivity analysis is to be conducted to determine the impact of changing one variable on the water storage capacity
- What-if scenarios are to be explored to investigate possible alternatives to reduce the storage capacity and supply needed from Earth.
- A more rigorous definition of "states" to capture a comprehensive set of elements that can directly affect the health of the plants, the crewmembers, and hence the system of living entities. Look into the possibility of using Fuzzy Logic for state definition.
- Define different "time horizons" for simulation. Different types of actions may be taken depending on the mission length, or even control policies based on sampling rate.
- Include ESM calculations in the determination of "actions" to be taken during state changes.
- Besides ESM, include in the "cost" the effect of each action taken on the system in the long run.
- Further evaluation of BioSim to determine if it's the appropriate simulation software for this research.

Trainees

Chit Hui Ang, Industrial Engineering, Master's program
Tze Chao Chiam, Industrial Engineering, PhD program

Research Collaboration

- Dr. Yih, Chit Hui and Tze attended ICES 2005 in Rome, Italy.
- Work with Scott Bell from SKT Inc. and Dave Kortenkamp from Metrica Inc. to evaluate and investigate the use of BioSim for the research in Advanced Life Support Systems. Implementation, documentation, and additional features to be included in future versions of BioSim were also discussed.
- Information exchange and email correspondence with Dr. Eugeniy I. Trushliakov from National admiral Makarov University of Shipbuilding, Ukraine.
- Email correspondence with Molly Anderson in Johnson Space Center to obtain current storage tank technologies for ESM computation.

Publication

- Ang, C. H., T. C. Chiam and Y. Yih (2005). "Impact of Crewmember Schedule on System Performance," *International Conference on Environmental Systems*, SAE Technical Paper 2005-01-2918

Presentation

- Ang, C. H., T. C. Chiam and Y. Yih (2005). "Impact of Crewmember Schedule on System Performance," presented at the *International Conference on Environmental Systems*, Rome, Italy.

Education and Outreach Program
Executive Summary

The Department of Education has reported that eighty-two percent of our nation's twelfth graders performed below the proficient level on the 2000 National Assessment of Educational Progress (NAEP) science test. Not only are our children performing below the level of proficiency, but their skills are not increasing as they become older. According to the 1995 Third International Mathematics and Science Study, U.S. fourth graders ranked second among industrialized nations. By twelfth grade, they fell to 16th, behind nearly every industrialized rival and ahead of only Cyprus and South Africa.

According to National Science Foundation reports, there is a documented shortage of students pursing degrees in science, mathematics, and engineering (National Science Board, 1993, 2003, 2004). Eighty-two percent of our nation's twelfth graders performed below the proficient level on the 2000 National Assessment of Educational Progress (NAEP) science test (US Department of Education, n.d.). Our education system must address this lack in proficiency and interest.

In addition, there is ample research analyzing when youths' perceptions of science are solidified, what affect their attitudes play a role in their future career choice, and how their attitudes toward science affect their achievement levels in science (Joyce and Farenga, 1998; Simpson and Oliver, 1990 & 1984). It has also been shown that students' interest in science decreases as they progress from sixth grade to eighth grade (Simpson & Oliver, 1984). This data enforces the need to catch our students' attention early in their education.

NASA is faced with a deficient hiring pool to meet retirement and expansion needs. Sixty percent of NASA's yearly job openings are due to early retirement and the current ratio of employees over the age of 50 to those under the age of 30 is three to one (NASA 2005 Budget Summary). This problem has been heightened by the President Bush's initiative to advance U.S. scientific, security and economic interests through a robust space exploration program. It is believed that this problem is so great it may put, "future advancements in science, aeronautics and space at risk" (NASA 2005 Budget Summary). NASA is not only concerned with the declining population of science professionals, but it is also concerned with science literacy and education.

ALS/NSCORT Education and Outreach has designed two educational curriculum modules that were significantly disseminated nationwide in FY05 to address this crisis: 1) Mission To Mars for 5th-8th grade educators/students and 2) Equivalent System Mass Analysis: Will It Fly? for high school educators and students. Keeping with the *National Science Education Standards (NRC, 1996)*, *Inquiry in the National Science Education Standards* (NRC, 2000), and the *Glen Report*, these modules facilitate science, mathematics and engineering instruction that engages teachers and students in relevant, authentic ALS/NSCORT research driven experiences. The modules' activities captivate participants as they explore the complexity of long term space travel.

Julia Hains-Allen
Lead – Education and Outreach Focus Group

EDUCATION AND OUTREACH

Principal Investigator: Julia Hains-Allen, M.S.

BACKGROUND

The ALS/NSCORT Education and Outreach programs provide an avenue to engage and educate K-12 formal and informal educators/students in the center's investigations of the synergistic concepts and principles required for regenerative life-support in extended-duration space exploration. In 2005, Julia Hains-Allen, Education and Outreach Manager, successfully obtained $384,240.00 to fund the centers main two thrust areas in educational programming: 1) Mission To Mars and 2) Equivalent System Mass Analysis: Will It Fly?

Mission To Mars

Mission To Mars is a standards/inquiry-based learning module that introduces students to the interdisciplinary fundamentals of science, technology, and engineering that underlie Advanced Life Support research. The 200 page *Mission To Mars* module facilitates science instruction that actively involves students in experiences to promote scientific literacy while inspiring the next generation of explorers. The twelve module activities are based on cutting-edge research that is ongoing in the ALS/NSCORT. These direct links with research serve to build teacher expertise in cutting edge NASA science, while stimulating student interest by connecting learning to real-life challenges. *Mission To Mars* students use their knowledge of Earth systems and apply that knowledge to understand the issues/constraints of supporting humans in long term space missions.
In FY05, over five hundred educators were fully trained on the Mission To Mars module nationwide and hundreds more were trained on parts of the module. In addition, a thesis study was performed on four hundred and seventy two students completing the module in Indiana and New York.

Equivalent System Mass Analysis: Will It Fly?(Formerly Project Lead The Way)

Will It Fly? is a hands-on, interactive research program that engages teachers/students worldwide in Advanced Life Support (ALS) research in the high school classroom. High school students conduct classroom research that is being done in ground based research labs at NASA today – literally, using the same research hardware. ALS/NSCORT Education and Outreach disseminates the pilot tested high school learning module, which uses research prototype bioreactors and the Biomass Production Education System (BPES) and a NASA systems engineering model. Students design experiments that investigate variables needed for microbial and plant growth, efficiency of bioreactors and growth chambers along with design improvements to hardware. This program partners primarily with two pre-university programs of study, International Baccalaureate Program and Advanced Placement to increase the advanced science/mathematic content knowledge and higher-order problem solving/critical thinking skills of students by using cutting-edge ALS research tools and technology as its foundation

ALS/NSCORT EDUCATION/OUTREACH MISSION

To Inspire the Next Generation of Explorers

ALS/NSCORT EDUCATION/OUTREACH GOALS AND OBJECTIVES

1. Increase the number of educators and students who are involved in NASA related education opportunities.
2. Increase the scientific literacy of K-12 educators, students and informal education community, fostering public awareness and the earth benefits of NASA research.
3. Increase the number of educators and students directly engaged in NASA research.
4. Increase the number and diversity of students from underrepresented and underserved communities in NASA related STEM fields.

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ALS/NSCORT EDUCATION OBJECTIVES/CORRESPONDING PROGRAM DELIVERABLES

1. Increase the number of educators and students who are involved in NASA related education opportunities.
   ALS/NSCORT Deliverables
   - Mission To Mars
   - Mission To Mars 4-H Program
   - Mission To Mars Key Learning Community
   - Will It Fly?
2. Increase the scientific literacy of K-12 educators, students and informal education community, fostering public awareness and the earth benefits of NASA research.
   ALS/NSCORT Deliverables
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   - Mission To Mars Key Learning Community
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3. Increase the number of educators and students directly engaged in NASA research
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   - Mission To Mars
   - Mission To Mars 4-H Program
   - Mission To Mars Key Learning Community
   - Will It Fly?
4. Increase the number and diversity of students from underrepresented and underserved communities in NASA related STEM fields.
   ALS/NSCORT Deliverables
   - Mission To Mars 4-H Program
   - Key Learning Community
   - Will It Fly?

EDUCATION AND OUTREACH PROGRESS

ALS/NSCORT Education and Outreach has grown significantly in FY05. Programs were in their infancy stage at the beginning of FY05 and each has completed extensive pilot testing, assessment, and revisions. Dissemination of the two thrust programs is worldwide to date, including educators in 11 states and Tegucigalpa, Honduras.

MISSION TO MARS

Using the Mission To Mars module, three separate programs received external funding and have been developed and/or expanded in FY05; 1) ***Mission to Mars Program*** introduces 5th-8th grade educators and students to the complex issues involved with living on Mars, stressing the interdisciplinary fundamentals of science, technology and engineering that underlie Advanced Life Support research. This standards/inquiry-based, hands-on activities module completed pilot testing in classrooms throughout Indiana and New York followed by the initiation of national dissemination, 2) ***Mission To Mars 4-H Collaborative Project*** trains Extension Educators on the *Mission To Mars* curriculum, providing programming for formal education classrooms as well as after-school programs and summer camps. A pilot program, Mission To Mars 4-H Project, will begin October 2005 in Indiana. This program will provide the assessment data needed to offer *Mission To Mars* as a 4-H project nationwide. 3) ***Key Learning Community Project*** is a full collaborative partnership with a K-12 inner city school in Indianapolis, Indiana. This partnership was expanded to include professional development for the 50 Key teachers on *Mission To Mars* and provide on-going support for the implementation of programs within the school

1. Mission To Mars Program

The Mission to Mars module explores the factors involved in creating a habitat on Mars along with the issues/constraints presented by the Mars environment. The multidisciplinary module includes laboratory exercises revolving around the study of plant growth, ecosystems, water and waste treatment, recycling and food production in a space environment. Earth systems exploration is central to the *Mission To Mars* module. Investigations include survival necessities on Earth and issues that will challenge survival in the Mars habitat. Students explore Earth benefits of NASA research as they investigate how new research will allow us to live in a space environment and simultaneously improve our lives on Earth.

The *Mission To Mars* module is a collection of twelve hands-on, inquiry based activities, each addressing an average of seven (7) National Science, Mathematics and Literature Standards. An integral part of a professional development workshop on *Mission To Mars*, is instruction on integrating these activities into the existing curriculum, replacing activities that are inadequate with *Mission To Mars* activities that have proven effective on teaching the standards for each lesson.

The *Mission To Mars* module stimulates student interest in STEM content material by connecting learning to the real life challenges of Advanced Life Support research along with allowing them hands-on opportunities with authentic, relevant, and standards based inquiry experiments that are directly linked to the research in the ALS/NSCORT. Module activities also incorporate NASA educational materials from NASA ARC, ASU Mars Education Program, JPL, and JSC.

DELIVERABLES - PILOTED AND DISTRIBUTED TO EDUCATORS NATIONWIDE IN FY05

The two hundred-page *Mission to Mars* module includes an introductory PowerPoint, teacher and student editions of 12 laboratory activities, background science/mathematics content material and a pre/post assessment tool in addition to assessment tools for each individual activity.

Mission To Mars module activities include:

- The Big Question: How are we going to live on Mars? What are the problems and challenges?
- Recycling In Space – Why do we need to recycle during long-term space missions?
- Mars Ecosystem: Ghost Shrimp – What is an ecosystem? Lets build an ecosystem!
- AstroVenture – What are the survival needs? www.astroventure.arc.nasa.gov
- Chemystery- What is research? Why is research important before we travel to Mars?
- Microbe Column – Why do we need microbes? How will they help us survive on Mars?
- Explore Mars Now – How much space will we have in our habitat? www.exploremarsnow.org
- Growing Plants in a Habitat – How will we grow plants in the Mars habitat?
- Density Straws – How will we know our water is clean in the Mars habitat?
- Cleaning Water on Mars – How will we clean our water in the Mars habitat?
- Is It Alive? – What will we eat in the Mars habitat? What will we cook?
- Off To Mars –Why bring environmentally friendly things with us to Mars?

New Additions to *Mission To Mars* module in FY06

- MarsBound - A current NASA ASU/JPL self-contained activity in which students use realistic techniques to plan an UNMANNED mission to Mars. In collaboration with ASU/JPL, a new MANNED mission version of MarsBound will be designed by ALS/NSCORT.
- Houston Keep Us Alive – An activity written by Marybeth Edeen will be adapted for ALS, engaging students in the engineering design of biological/physical/chemical air and water treatment systems. In the final stages of development, it will be piloted in the fall FY06.
- Foul It Up – An activity directly linked to ALS/NSCORT Removal of Dissolved Wastes research group at Howard University. Students will use a simulated contaminated water sample to discover the concept of membrane fouling. In the final stages of development, it will be piloted in the fall FY06.

Dissemination

A pilot tested, 2-day professional development workshop is the delivery tool to educators nationwide. By participating in the workshop, educators will acquire the knowledge and information needed to integrate the *Mission To Mars* module into their curriculum. The workshop will include rigorous science/math content instruction, hands-on experience with the twelve individual, standards-based activities in the module, use of assessment materials, curriculum integration information, and a full resource base via NASA Education Headquarters.

The professional development workshops are conducted nationwide via school corporation workshops, museums/science center workshops, and national/regional teacher association conferences. Evaluation materials were given to all educators participating in professional development programs. Educators were asked to rank all the activities within the *Mission To Mars* module using two indicators: 1) whether they were applicable to the

classroom and 2) contained science content they could apply to the standards. Data obtained from the evaluation clearly demonstrates that the *Mission To Mars* program is: a) highly applicable to their classroom and b) contains a large amount of standards/based science content they can easily integrate into their curriculum.

In addition to the initial training workshops for the module, ALS/NSCORT offered a *Mission To Mars II* Professional Development workshop in FY05. All participants had previously attended a Mission workshop AND used the curriculum in their classroom/informal education setting. These educators represented urban, inner city and rural classroom teachers. The workshop agenda included module editing and implementation ideas for meeting the needs of the three groups. In addition, educators created an individual assessment tool for each activity within the module. Changes to the module and assessment tools will be piloted in FY06.

Macon Fish Beck Thesis Study

ALS/NSCORT graduate student (now ALS/NSCORT Outreach Coordinator) Macon Fish Beck initiated a thesis study in the fall of 2004, followed by an accepted thesis defense in June 2005. This study involved eleven teachers in Indiana and New York. The inclusive thesis question was, "How does '*Mission to Mars*' impact the classroom?"

The goals of the thesis study were: 1) Evaluate and analyze student attitudes toward science after completing the *Mission to Mars* program using an attitudinal survey, 2) Evaluate and analyze the educational effectiveness of the *Mission to Mars* program on covering Academic Science Standards using a content assessment tool, 3) Evaluate and analyze the ease of use of the 'Mission to Mars' program by teachers using an open ended survey.

The attitudinal and content assessment tool was developed specifically for *Mission To Mars.* The development of this tool coincided with the development of the curriculum and was evaluated in a previous study completed with Extension Educators in Indiana.

One teacher from eleven (11) chosen school corporations participated in the thesis study. All teachers were required to attend a 2-day professional development workshop in September 2004 at Purdue University. During the program, teachers received intense instruction on the *Mission To Mars* module, science content instruction, supplies for their respective classrooms and an assessment tool to be used in a pre/post test setting. Educators signed an agreement to complete all 12 lessons in the module and administer a previously piloted pre/post assessment tool to participating students. The assessment tool consisted of 13 content knowledge questions based on each lesson and the Academic Science Standards. In addition, a 12 question attitudinal survey was given. Students were individually assigned an ID number to be used during the pre and post testing period.

Each participating teacher administered the pre-test content and attitudinal surveys to students in late September. Following the pre-testing, each student in the study participated in the 12 hand-on learning activities with the *Mission To Mars* module during October - December. Post-test content and altitudinal surveys were given to all students in the study before December 18, 2004.

The raw data has been analyzed. A 2.67 average increase in student scores from the content knowledge pretest to the content knowledge posttest was shown. The paired T-test adjusted with Bonferroni revealed a significant change from the pre- test to the post-test.

Analysis of raw data from the attitudinal survey indicates that the overall attitude of the students from the pre-test to the post-test positively increased by 1.5 on a 5 point scale. This is based on a normal Likert type scale where 1=strongly disagree, 2=disagree, 3=undecided, 4=agree, 5=strongly agree. Student average score was analyzed using a matched pairs t-test, and categorical comparisons were made using ANOVA.

In addition to the student data, participating teachers were asked to complete a qualitative survey upon completion of the *Mission To Mars* module. Nine of the eleven teachers returned the survey. The data from the survey indicates that the goals for the teacher part of the program

were met. Teachers were relatively well prepared to teach the lessons, the lessons addressed most of the science standards and the activities were relatively easy to follow.

Evaluation

Evaluation materials have been developed for the module in collaboration with the School of Curriculum and Instruction at Purdue University, pilot tested in FY04 and reevaluated. The refined evaluation tool was used in the thesis study previously reported. The tool includes an attitudinal study along with a full assessment to evaluate students' knowledge gained in STEM content, experimental design, and critical thinking skills as a result of completing the *Mission To Mars* module. In FY06, educators will report pre/post results via www.spacelife.org in a designated section. In addition, a Mission To Mars II Workshop resulted in an evaluation tool for each of the 12 lessons. This tool will be disseminated to all teachers participating in the program and data collection via www.spacelife.org. New evaluation tools will be developed for additional FY06 deliverables. Professional development workshops' on-going formative evaluation (including interviews, observations and questionnaires) will provide constant feedback for use in redesigning and modifying the *Mission To Mars* module and professional development program based on the results. Additional metrics include the number of professional development workshops, participants, and the number of students reached via trained teachers.

Partnerships and Collaborations

- School Corporations and Science Museums nationwide
- Arizona State Mars Education Program/JPL are partners in the development of a "manned mission" version of MarsBound to be added to Mission in FY06.
- AstroVenture – ARC program is used in Mission To Mars as one of the activities.
- JSC provides content for Mission To Mars along with dissemination support.
- Indiana Technical College will leverage funds in FY06 to provide network and support for distance learning professional development workshops.

2.Mission To Mars 4-H Collaborative Project

As a land grant university, Purdue has a strong presence in 4-H Extension Education throughout the state of Indiana. Extension Educators assist teachers in every county in Indiana, bringing academic materials to rural youth. In the early stages of *Mission To Mars*, 4-H Extension Educators in Indiana participated in a pilot program. This pilot resulted in valuable assessment materials to be used in revising *Mission To Mars* along with the development of an assessment tool to be used in classrooms. Since its inception, the collaboration with 4-H has increased drastically to include professional development trainings on Mission To Mars for over 35 Extension Educators in Indiana along with a new collaborative project in FY05 that has adopted *Mission To Mars* as an official 4-H project for Indiana. The 4-H project will be pilot tested in Indiana during the 2005-2006 4-H season.

Building on the collaboration with Indiana 4-H, ALS/NSCORT has developed another strong collaborative partnership with 4-H Youth Development in North Carolina. ALS/NSCORT was invited to participate in a NASA Explorer Institute involving NASA's Exploration Systems Mission Directorate (ESMD), NASA Langley's Center for Distance Learning, and the North Carolina 4-H. The NEI's goal was to form a

collaborative group that would be an instrumental force for inspiring young people in North Carolina to pursue careers involving STEM and to diffuse and utilize NASA education products within North Carolina. ALS/NSCORT trained NC Extension Educators on the *Mission To Mars* module during the NEI, held in late January 2005 in North Carolina. Among the 50 states, North Carolina 4-H is considered to have one of the top 4-H organizations in the country. Each year, over 27,000 young people and adults participate in programs sponsored by the North Carolina 4-H.

The results of these two collaborative efforts have lead to extensive dissemination of the *Mission To Mars* module. In Indiana, this module is taught in classrooms throughout the state by Extension Educators. Throughout North Carolina, 4-H Extension Educators are using the module in classrooms as well as a foundation for summer camps and after school programs. The *Mission To Mars* module was also used in a 4 day Latino Leadership Conference held in North Carolina for 250 Hispanic youth in June 2005.

In addition, thirteen 4-H Extension Educators from Indiana and North Carolina, working in collaboration with ALS/NSCORT, met for an intensive 2-day professional development workshop to collectively design and development a new version of *Mission To Mars* to be utilized as a 4-H project for FY 06-08. This 4-H project will be strategically positioned to reach Hispanic/Latino and rural youth in the participating states.

Partnerships and Collaborations

- Indiana and North Carolina Department of Youth Development partner and collaborate on the development and design of 4-H programs involving Mission To Mars
- 4-H Extension Educators are trained in professional development workshops and take the program into schools and 4-H programs throughout Indiana/North Carolina.
- NASA Langley Research Center partners and collaborates with ALS/NSCORT to bring programming into the Latino Community in North Carolina.

3.Key Learning Community Project

In the fall of 2003, the ALS/NSCORT Center and Key Learning Community in Indianapolis formed a coalition to enhance the learning of students in Indianapolis. The coalition has designed a rigorous course of action to address the problem that many underrepresented students in Indianapolis are not pursuing careers in science, technology and engineering.

To meet the needs/mission of Key Learning Community, the *Mission To Mars* module was expanded to include other discipline (linguistics, social studies, visual arts, bodily-kinesthetic and music) activities, captivating students as they explore the issues/constraints of long term space travel. In addition to stimulating student interest in STEM content material, the interdisciplinary connection of the *Mission To Mars* module through other subjects will connect multi-subject content learning to real life challenges of living on Mars. This module fits perfectly into the mission of Key Learning Community. This K-12 school teaches using a

"thematic" approach, whereby teacher and student exploration of three interdisciplinary themes direct instruction for all grades at Key Learning. The *Mission To Mars* module serves as a "theme" for the 2005-2006 school year and instruction in all K-12 classrooms is revolving around this topic. A three day "Mission To Mars Key Community Professional Development" program was held in July on the Purdue University campus. Instruction was individualized to include NASA materials applicable for all K-12 educators. Session included 1) intensive training on the *Mission To Mars* module; 2) presentation of other NASA materials by Bonnie McClain; 3) Mission To Mars Art; 4) Mission To Mars Music; 5) Cape Canaveral History; 5) NASA websites; 6) Mission To Mars Elementary/Middle School Literature; 7) Mission To Mars Mathematics and 8) Implementing a school-wide project "Marsville". Instructional specialists joined the ALS/NSCORT team in the workshop to provide the Key Learning Community teachers with a foundation for the interdisciplinary K-12 use of *Mission To Mars* at Key Learning Community during the 2005-2006 school year.

Future Directions for MISSION TO MARS in FY06

Mission To Mars received ESMD funding for FY06 for nationwide dissemination, including school corporations, museums, national conventions and 4-H. Dissemination mechanism will be professional development workshops via the traditional on-site setting along with distance learning programs. Over 400 educators alone will be trained in formal workshop environments. In addition, the development of the 4-H project "Mission To Mars" will exponentially increase the reach and Train the Trainer workshop will be held using ESMD funding, allowing for other individuals across the country to provide professional development workshops in their area. The Key Learning Collaborative project will be evaluated as a pilot program for the integration of *Mission To Mars* in multi-disciplinary classrooms.

Educators participating in FY05 professional development workshops will administer the thesis tested assessment tool as a pre/post evaluation of the module. Data will be collected and analyzed by ALS/NSCORT, to be used for future revision and adaptations.

EQUIVALENT SYSTEM MASS ANALYSIS: WILL IT FLY? PROGRAM

The thrust of the ALS/NSCORT center rests on design systems that provide for the required components of successful space travel with minimal mass. A system engineering model, "Equivalent System Mass (ESM)", is an integral part of all space system designs and is used by NASA engineers and researchers as a standard to quantify effective and efficient life support components. An ESM value represents the sum of the life support system mass and supporting system masses, including volume, power, cooling, and crew time. (NASA JSC/SIMA)

ALS/NSCORT's research-driven program, *Equivalent System Mass Analysis: Will It Fly?*, addresses the nation's crisis in education and NASA's future challenge of recruiting the top individuals and building a workforce from a decreasing pool of highly qualified individuals.

Project Design

In a collaborative effort for FY04, NASA Advanced Life Support joined with Orbital Technologies to design Biomass Production Educational System (BPES), a research grade plant growth chamber that can be used in high school classrooms across the country. ALS/NSCORT Education and Outreach designed a learning module integrating the BPES and ESM.

Pilot Study

Initial pilot testing of the ALS/NSCORT developed curriculum began at McKenzie Career Center, MSD Washington Township School Corporation, Indianapolis in the fall 2004. This pilot was lead by Jeff Martin, a pre-engineering teacher at McKenzie. Twenty students presented their research findings to a panel of scientists, engineers and educators from NASA during a symposium at Purdue.

> A national merit scholar high school student in the McKenzie program had the following comment about the pilot program: **"**The program provides students with valuable lessons that go beyond the academic teachings of mathematics and more conventional science courses. This program allows students to be involved in real-world applications of research." Charlie Myers Carmel, Indiana.

Equivalent System Mass Analysis pilot program attracted the interest of top NASA administrators in the Systems, Integration, Modeling, and Analysis program, or SIMA, which provided students with key research materials. "The students' solutions may be novel ideas for NASA," said Tony Hanford, deputy lead for SIMA at the NASA Johnson Space Center in Houston.

Seven additional BPSE systems were delivered allowing a second pilot testing period to begin in January 2005. This testing period included nine teachers throughout Indiana and a Challenger Learning Center. Two of the nine teachers were piloting this program for International Baccalaureate. Upon completion of the second pilot period, the pilot data was analyzed by an assessment team at Purdue University. This team, consisting of pilot teachers, ALS/NSCORT researchers and staff members, developed an assessment tool for the new curriculum and recommend changes for curriculum revision. Curriculum revisions were completed by the ALS/NSCORT team in August 2005 and the module *Equivalent System Mass Analysis: Will It Fly?* was the result.

The Module

The *Equivalent System Mass Analysis: Will It Fly?* curriculum module incorporates the following student activities:

- **CALCULATING ESM VALUES FOR EXPERIMENTAL SYSTEMS USING PLANT GROWTH CHAMBER DATA AND THE FORMULA**:

$$ESM = M + (V * V_{EQ}) + (P * P_{EQ}) + (C * C_{EQ}) + (CT * D * CT_{EQ})$$

— *M, V, P, C* and *CT* are the mass, volume, power, cooling and crew time needs. Mass includes growth chamber infrastructure + daily input.
— *Veq, Peq, Ceq*, and *CTeq* are the volume, power, cooling and crew time mass equivalency factors.
— *D* is mission segment duration. (NASA JSC/SIMA)

- Using the Biomass Production Education System (plant growth chamber), students design and conduct experiments to determine plant varieties and species that will produce the lowest ESM values with optimum growth.
- Using the Biomass Production Education System, students design and conduct experiments to determine media and lighting systems that will produce the lowest ESM values with optimum growth.
- Using the Biomass Production Education System, students design and conduct experiments to determine baseline values for plant health.
- Students create design plans for adapting the Biomass Production Education System to provide information needed for an effective plant growth chamber based on ESM calculations.
- Using ESM calculated values on their experimental designs; students compare their results (ESM values) with ALS research to determine the feasibility of their experimental designs.
- Data analysis and interpretation resulting in a report of final ESM value for plant growth scaled to useable edible mass.
- Website communication of research/experimental results/ ESM values/ applications in collaboration with other schools and eventual nationwide dissemination.
- Final research paper presentation making recommendation for lowering ESM, decision on which plants "will fly" and defense of the recommendation.

Will It Fly September 20-21 Professional Development Workshop

The second pilot period for *"Will It Fly"* began in September with an intensive 2-day professional development training provided by the ALS/NSCORT team. Funding from ESMD Education allowed the purchase of 15 new chambers and funds for travel and implementation expenses incurred by the educators. Using the revised curriculum materials, 10 educators participated in the training. Participating educators represented International Baccalaureate programs (one in Tegucigalpa, Honduras), Indiana School for the Deaf, Advanced Placement Biology, Imagination Station Science Museum and middle school mathematics programs. After completing instruction on ESM calculations, data analysis, research document use and evaluation/assessment, these educators will pilot the revised curriculum during the 2005-2006 school year. A pre/post assessment tool will be used to evaluate student content knowledge gained and attitudinal changes. Additional revisions and adaptations to *Will It Fly* will be made in FY06 using this data in preparation for nationwide dissemination of the module.

Partnerships and Collaborations

Numerous changes have occurred within this program in FY05. Project Lead The Way (PLTW) collaborative partnership dissolved due to copyright issues. New partners for FY05 emerged rapidly for the *"Will It Fly?"* program

- JSC Systems Integration and Modeling group in the use of NASA research documents.
- International Baccalaureate Program – Will It Fly will be piloted in FY06 in three IB schools, two in Indiana and one in Tegucigalpa, Honduras
- Advanced Placement Biology – Will It Fly will be piloted in FY06 in two AP Biology classrooms.
- Challenger Learning Center and Imagination Station Science Museum – Will It Fly is presently being used in one Challenger Center and will be piloted at Imagination Station in FY06.
- Indiana School for the Deaf – Will It Fly will be piloted at the School for the Deaf in FY06.
- Tippecanoe County School Corporation – Will It Fly will be piloted in 8th grade mathematics classes in FY06.

Future Directions for WILL IT FLY in FY06

"Will It Fly" has expanded to incorporate research from another focus group in the ALS/NSCORT. Learning modules are presently being developed to integrate ESM analysis and prototype bioreactor wastewater research. Using results from a research grade prototype bioreactor located in the classroom, students will investigate topics such as:

- Factors that effect microbial growth in bioreactor waste water treatment
- Design improvements to bioreactor prototype
- Efficiency of bioreactor in removing surfactant contaminants in gray water
- Implementation of factors for ESM reduction

The culmination of this module for each student will be the creation of a traditional style research paper of the entire experimental process. This expanded module will be pilot tested at McKenzie Career Center in FY06

Pending future funding, Will It Fly? curriculum modules of Biomass Production Education Systems and Wastewater Bioreactors will be disseminated nationwide via partners in this program.

PUBLICATIONS

Hains-Allen, J., M. K. Banks, S. Sharvelle, M. Fish (2005). "ALS/NSCORT Education and Outreach." *International Conference on Environmental Systems*, Paper # 2005-01-3107

PROFESSIONAL DEVELOPMENT WORKSHOPS/PRESENTATIONS FY05:

- Hains-Allen, Julia, Jeff Martin, Symposium: ESM Will It Fly, December 2004
  Teachers: 1 Students: 20
- Hains-Allen, Julia, Presentation: Mission To Mars - Continuing Education Directors IVY Tech, December 2004
- **AWARDED $56,740 Indiana Commission for Higher Education Grant for Mission To Mars, December 2004**
- Hains-Allen, Julia, Beck, Macon, Workshop: Mission To Mars - NASA Explorer Institute North Carolina, January 2005
  Extension Educators: 35 Teachers : 175[1] Students : 9625
- Stafford, Shandra, Classroom Integration: Mission To Mars - Alabama, January-June 2005
  Teachers: 3 Students: 90
- Hains-Allen, Julia, Presentation: Mission To Mars – Lafayette Elderhostel, February 2005
- Hains-Allen, Julia, Presentation: Mission To Mars – Indianapolis Garden Club, March 2005
- Hains-Allen, Julia, Beck, Macon NASA Booth National Science Teacher Association, Dallas, TX, March 2005 (reach is approximately 1000 teachers)
- Hains-Allen, Julia, Presentation: Mission To Mars – Teachers National Science Teacher Association, Dallas TX, March 2005
  Teachers: 45 Students: 2,475
- Martin, Jeff NASA Booth National Council for Teachers of Mathematics, Anaheim, CA April 2005 (reach is approximately 500 teachers)
- **AWARDED $174,500 ESMD Education for Mission To Mars and Equivalent System Mass Analysis for FY05 May 2005**
- Beck, Macon Workshop: Mission To Mars – North Carolina Extension Educators, May 2005
  Extension Educators: 30 Teachers: 150[1] Students: 8250
- Hains-Allen, Julia, Presentation: Equivalent System Mass Analysis – International Baccalaureate Program teachers, Valparaiso IN, May 2005
  Teachers: 20

- Hains-Allen, Julia, Workshop: Mission To Mars – Colorado Springs CO, June 2005
  Teachers: 15 Students: 825
- Beck, Macon, Workshop: Mission To Mars - Agriculture Teachers, Purdue University, June 2005
  Teachers: 30 Students: 900
- Oware, Euridice, Workshop: Mission To Mars – ScienceScape Camp, Purdue University June 2005
  Students: 30
- Hains-Allen, Julia, Presentation: Mission To Mars – ScienceScape Camp, Purdue University June 2005
- Hains-Allen, Beck, Macon, Workshop: Mission To Mars – Ft. Wayne IN, June 2005
  Teachers: 20 Students 1100
- Hains-Allen, Julia, Beck, Macon Workshop: Mission To Mars II – Brownsburg Challenger Center, June 2005
  Teachers: 35[2] Students: 1925
- Martin, Jeff, Presentation: Equivalent System Mass Analysis - International Baccalaureate Conference, Jacksonville, Florida, June 2005
  Teachers: 60
- Gillman, Ivelisse, Latino Leadership Conference: Mission To Mars - North Carolina June 2005
  Students: 250
- Hains-Allen, Beck, Macon ALS/NSCORT staff, Workshop: Mission To Mars – Key Learning Community, July 2005
  Teachers: 45 Students: 500
- Hains-Allen, Julia, Presentation: ALS/NSCORT Education – ICES Rome, Italy, July 2005
- North Carolina Extension Educators, Spacapalozza Summer Camp - North Carolina, July 2005
  Teachers: 14 Students: 66
- Penley, Ned, Summer Camp: Mission To Mars - Tribal Reservations Montana July 2005
  Students: 200
- Hains-Allen, Julia, Workshop: Mission to Mars - Encouraging Technology and Hands-On Science Organization, August 2005
  Teachers: 30[2] Students: 1650
- Martin, Jeff, Presentation: Equivalent System Mass Analysis - North Chicago Suburban Educators, September 2005
  Teachers: 55
- Hains-Allen, Julia, Beck, Macon Workshop: 4-H Project Mission To Mars - Imagination Station Lafayette, IN September 2005
  Extension Educators: 13 Teachers: 65[2] Students: 3675
- **AWARDED $153,000 ESMD Education for Mission To Mars for FY06 September 2005**
- Hains-Allen, Julia, Beck, Macon, Workshop: Mission to Mars - Buffalo Grove IL September 2005
  Teachers: 10 Students: 300
- Beck, Macon, Presentation: 4-H Project Mission to Mars - Indiana Extension Educators September 2005
  Extension Educators: 30
- Beck, Macon, Presentation: 4-H Project: Mission to Mars - 4-H Foundation, September 2005
- Hains-Allen, Julia, Presentation: ALS/NSCORT Research - Teacher Awareness Workshop September 2005
  Teachers: 75
- Hains-Allen, Julia, Workshop: Mission to Mars - Fort Wayne IN September 2005
  Teachers: 30 Students: 1650
- Beck, Macon, Workshop: Mission to Mars - Kansas Cosmosphere, September 2005
  Teachers: 35 Students: 1925

[1]Numbers are based on potential reach of educators trained. Extension Educators have the potential for the largest reach as they each work with approximately 5 teachers, primarily in elementary school. Therefore, the number of teachers reached through the Extension Educators is significant.
[2]Master teachers were trained in these workshops. Each master teacher works with approximately 5 classroom teachers. Therefore "teacher" numbers reflect this multiple.

Note: Teachers trained in the ALS/NSCORT programs varied in grade level; therefore the student projected reach is calculated as an average of 55 students per teacher trained. Middle school teachers have approximately 100 students in their classrooms, while elementary teachers average 25 students in their classrooms.

CONCLUSION

ALS/NSCORT education and outreach programs have experienced substantial growth over the last year. To accommodate the growth and future nationwide dissemination of Mission To Mars and Equivalent System Mass Analysis "Will It Fly?, funds were obtained and leveraged to allow the hiring of two full time outreach coordinators in June 05. The entire Education and Outreach staff will provide numerous workshops throughout the United States in FY06 on all programs within ALS/NSCORT Education and Outreach. The scope is nationwide with the primary audience consisting of 5-8th grade educators/students for *Mission To Mars* and 11th-12th grade educators/students in *Equivalent System Mass Analysis Will It Fly?*.

ALS Center News of Interest

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AMERICAN SOCIETY FOR ENGINEERING EDUCATION
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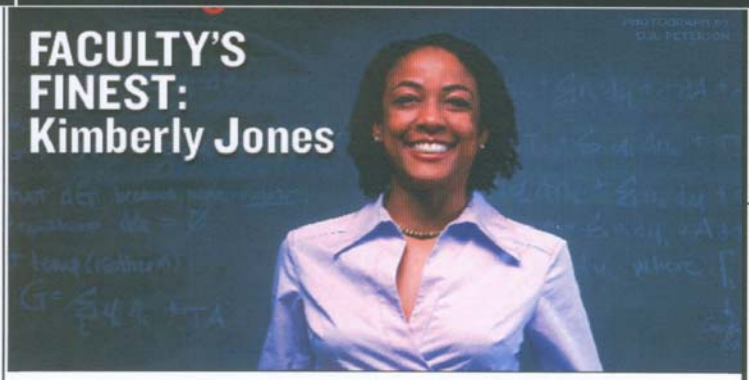
VOLUME 14, NUMBER 8

teaching toolbox >>>

FACULTY'S FINEST: Kimberly Jones



OUT OF THIS WORLD: KIMBERLY JONES HOPES HER RESEARCH MAY SOME DAY HELP TO MAKE LIVING ON MARS POSSIBLE.

If Kimberly Jones has her way, future space travelers will be drinking their own shower water. Well, after it's run through a few filters, of course. Jones, a professor of civil engineering at Howard University in Washington, is working with researchers from Purdue University and Alabama A&M University at the NASA Specialized Center of Research and Training for Advanced Life Support. The center's mission is to create space colonies whose inhabitants will live on crops they grow themselves and recycle all their waste. Her work on purifying wastewater by running it through filtering "membranes" is vital to creating that environment.

In addition to her research, Jones for the past seven years has taught the Introduction to Engineering course. Run by five professors, one from each of the engineering disciplines taught at Howard, the course is mandatory for all freshman engineering majors.

Jones didn't always know she wanted to be an engineer. "I sketched a lot as a child and thought I would be an architect," she says. "But I liked math and science, too." Jones needed a guidance counselor to "suggest civil engineering as a way to combine the two."

TOP ^

U.S. Space & Rocket Center®

May 5, 2005

Caula A. Beyl, Ph.D.
Director, Institutional Planning
Research & Evaluation
Alabama A&M University
P.O. Box 272
4900 Meridian Street
Normal, Alabama 35762

Dear Caula:

Our mission has always been to leverage the journey of space exploration to inspire learners of all ages and interests in the study of math, science and technology. In addition to training the workforce needed for the success of the long-term exploration program, we must develop the innovative technologies, knowledge, and infrastructures both to explore and support decisions about the destinations for human exploration.

The U. S. Space & Rocket Center is embarking on an exciting new exhibit for our museum to highlight the President's new initiative for Space Exploration. The exhibit will focus on multiple challenges related to space exploration such as those to be overcome as we return to the Moon, go to Mars and beyond. Further, the exhibit will feature the benefits citizens will reap from such exploration.

As one of the most exciting challenges of the new century, NASA's new vision for space exploration is fast becoming the primary focus of our museum and world renowned SPACE CAMP® training programs. The emergence of these new programs will allow this recognized center to serve as a showcase and distribution point for information and immersive training in the area of long duration flight and space exploration. Furthermore, we will strive to highlight the spatial technologies and basic science that are so closely related to this endeavor. The convergence of technology applications here on Earth coupled with a renewed vision for space exploration provides a unique opportunity to engage the public in a journey that will shape the course of human destiny.

As we develop the concept for this exhibit, to be known as "Moon, Mars, Earth ~ Living in a Challenging Environment", a strong presence of current research is needed in the area of controlled environment methodologies and technologies. We also need your creative thinking in presenting the materials in a comprehensive manner. To that end, we will hold a wide-ranging **meeting on Tuesday, May 17, 2005,** to further develop this multi-faceted hands-on exhibit and share our mutual vision for the content and future of the exhibit space. Our aim is to bring together a few of the most creative minds in the controlled environment research arena for this effort.

University & Industry Consortium

May 1, 2005

Dr. Cary Mitchell
Professor of Plant Physiology and Horticulture
Department of Horticulture & Landscape Architecture
Purdue University
West Lafayette, Indiana 47907-2010
Dear Dr. Mitchell:

Thank you for presenting at the 2005 Spring University & Industry Consortium Meeting. Members found your presentation on the human habitation in space absolutely fascinating. I know I had not considered all of the substantive issues of environmental hazards such as wind or meteor storms.

I learned much about the concepts of sustainability of habitat and requisite sustainability of life. In addition, your presentation really added to the whole concept of exploration of science and frontiers of space, especially with the U.S. Space Center visit the night before. Your conversational delivery style was very captivating to the audience and resulted in greater learning.

We were glad that you were able to attend other portions of the meeting as well. Members enjoyed interacting with you and we look forward to seeing you at a future meeting.

Again, thank you for a job well done.

Sincerely,

Ralph Douglas Tisher
Chairman

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PURDUE
UNIVERSITY

DEPARTMENT OF FOOD SCIENCE

April 27, 2005

Nicole Castrale
745 Agriculture Mall Drive
West Lafayette, IN 47907

Dave Kotterman
Operations Manager ALS-NSCORT
745 Agriculture Mall Drive
West Lafayette, IN 47907

Dear Dave,

Thank you so much for all of your help and support in putting on the Food Science Club's symposium. You were a great person to know when finding speakers and advertising. The symposium committee would like to sincerely thank you for helping us get connections to Jerry, Karen, and Michele. Thank you for sponsoring Michele as well. You and ALS-NSCORT were a major part of our symposium and I think you had a big part in helping it succeed.

Thank you for video taping the symposium as well. We are sending the link to Jerry, Karen, and Michele so they can watch it as well.

Thank you again for all that you did in making the symposium successful.

Sincerely,

Nicole Castrale

Nicole Castrale
Vice President, Food Science Club

Department of Food Science
745 Agriculture Mall Drive ■ West Lafayette, IN 47907-2009 ■ (765) 494-8256 ■ Fax: (765) 494-7953 ■ www.foodsci.purdue.edu

Space Research Gets Front-Page Publicity by ABC

In December, the astronauts on the International Space Station were depending on a Christmas delivery to avoid a possible food shortage. The December 25 trip took 2.5 tons of food, supplies, and presents to the crew. Though the situation was not dire, the mission to resupply the space station became headline news. Purdue Food Science became a contributor to the story and Amanda Onion, writer for ABC News, interviewed Dr. Lisa Mauer. The resulting story ran on the front page of the ABC News web page.

Dr. Mauer, member of the Advanced Life Support center at Purdue called the NASA Specialized Center of Research and Training, discussed the mission to mars project and some of the difficulties of providing food in space. "Running into a situation where food is running out would be catastrophic on a mission to Mars", said Dr. Mauer. Another concern is ensuring food safety without refrigeration. This leads to the NSCORT project in which they seek to develop systems to grow, harvest, and process the foods during extended missions in space. These missions would include trips and colonies on the moon or Mars.

The goal for a round-trip mission to Mars is to create foods that will last and be preserved over a five-year period. "The goal is to prepare foods for the six- to eight-month flight out and then have a good supply with a five-year shelf life for the return trip," said Dr. Mauer. Currently, the packaged meals used have a maximum shelf life of only one year.

Also interviewed was Dr. Michele Perchonok, a food scientist at NASA's Johnson Space Center in Houston. Dr. Perchonok works with the NSCORT project and visited Purdue to participate in the Food Science Club Symposium that is described on page 17.

Middle School Students Get Lab Expereince

Dr. Lisa Mauer's lab is quite diverse. Through an agreement with an Indianapolis middle school, she has assisted several of their students who have a firm grasp on science with science fair projects. "Students of that age are looking for ways to apply the science they are learning in the classrooms," says Dr. Mauer. "A good science fair project gives them the 'hands on' work they need to keep them excited about science." Indeed! Many of the students who have worked in her labs have gone on to be very competitive, if not win, their local science fairs.

"I had a seventh grader, Lauren Stephens, who did some very nice work with FT-IR spectroscopy. She is presenting a poster at IFT this year!"

Lauren was recently interviewed by the Discovery Channel for her winning the biological sciences division of the State Fair competition.

CE Interim Head Appointed August 15, 2005

It is an honor to serve as Interim Head of the School of Civil Engineering for the upcoming year. These are exciting times for the civil engineering profession. Public demand is high for a safe and effective infrastructure while maintaining a clean environment. Purdue's School of Civil Engineering is responding to this need by initiating an active phase of educational growth and innovative research. Our school currently has 57 faculty members, 300 graduate students, and 530 undergraduate students. The School of Civil Engineering's educational programs rank among the nation's top, with an undergraduate ranking of 7th in civil engineering and 17th in environmental engineering, and a graduate ranking of 5th in civil engineering. Purdue's Civil Engineering research program is strong, with a budget of over $12M in faculty directed research projects this year. To address the multidisciplinary nature of engineering research, our school has been active in Purdue's signature area program. In 2003 and 2004, our school added several faculty positions in two of the engineering signature areas, Intelligent Infrastructure Systems and Global Sustainable Industrial Systems. Thanks to our former Head, Professor Fred Mannering, we are well-positioned for future success.

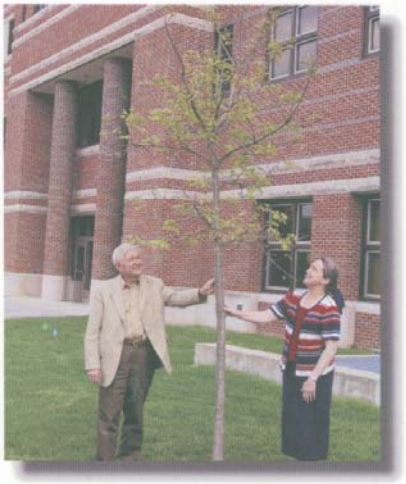
We currently are searching for a Head of Civil Engineering and a Head of Construction Engineering and Management to lead our school. The upcoming year will be an exciting one of new beginnings, with interviews and discussions of a variety of future paths to continued excellence. Please feel free to contact me if you would like more information about our School's activities.

M. Kathy Banks
Professor and Interim Head

Department News

Trees Arrive at Food Science Building

In April, the Department of Food Science received a special gift from Purdue's Ground Maintenance Department -- trees. Though official landscaping has not begun, the University has funds for "street trees" for general beautification of the West Lafayette-campus. Trees were planted along the front and two sides of the building. In the picture to the right, Dr. Phil Nelson and current Head, Dr. Suzanne Nielsen, admire the trees after delivery.



Graduate Student Accepts Position with Purdue's Office of the Provost

Dr. Kauline Davis, 2005 Food Science Ph.D. graduate, has become the special assistant to Provost Sally Mason. Her role will be working with the Provost to organize Purdue's efforts with regard to diversity across the University. She also will work with the Indiana Commission for Higher Education on Purdue's statewide efforts in diversity. She will prepare and present Purdue's diversity issues to the Board of Trustees.

Faculty Receives Tenure

Dr. Bruce Applegate has been promoted to Associate Professor with tenure, effective July 2005. Dr. Applegate has been with the Department since 2000.

Date: August 3, 2005
From: Julie A. Levri, NASA Ames Research Center
Subject: Commendation Letter for Dawn Whitaker

To Whom It May Concern:

The purpose of this letter is to commend Dawn Whitaker in her work on the NASA Advanced Life Support (ALS) On-line Project Information System (OPIS).

I have had the great pleasure of having Dawn as a Graduate Student Associate on the OPIS Project for the past two and a half years. Dawn works on OPIS year-round, with the majority of her assignments occurring in the summer months.

Dawn's first summer at Ames Research Center involved the development of critical questions to ask of ALS Principal Investigators who develop waste processing-related hardware. The work involved an aggressive schedule of drafting forms, holding daily meetings to critique form content, and initiating resultant edits. Dawn readily stepped up to these demands with ease and clear train of thought. She always presented options in a humble but proficient manner and responded to team member suggestions and questions with great respect. She also displayed the indispensable ability to consider the practical implementation of reporting requests that would otherwise go unchecked. Because of these qualities, I requested that Dawn return to Ames the following two summers. Fortunately, she has joined us for both of those summers.

With each returning summer, Dawn's level of technical experience has escalated. Because of her thirst for learning, she has easily applied the hardware development and laboratory analytical experience gained during the school year to continuously improve OPIS form content. Currently, in addition to the waste processing area, Dawn is responsible for content and example development of six additional areas of ALS focus. Each time Dawn returns to Ames for the summer, she has made the existing OPIS form content more elegant, practical and technically accurate. Her work is extremely professional and incorporates a valuable degree of life experience. Dawn is focused, self-motivated and reliable. She finishes tasks ahead of schedule and even takes it upon herself to find additional, useful tasks to achieve.

The OPIS development process requires many long meetings with multiple team members, each with a different perspective. Dawn's patience and respect play major roles in getting the team to a workable solution. She is highly skilled in working with multiple team members with a variety of personas, and she has come up with countless, practical ideas for improving the reporting content that the entire team has embraced. I feel very fortunate to have her on the OPIS Team and look forward to working with her at any opportunity in the future.

Sincerely,
Julie Levri
NASA ALS OPIS Principal Investigator
Bioengineering Branch (SLB)
NASA Ames Research Center
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Indiana State Fair August 2005

ALS NSCORT had an Indiana State Fair exhibit on display entitled “The Mission to Mars” designed emulating a circular, pod-type surface lander that kids and adults could walk through reading multiple large panels describing and illustrating the NASA research objectives and earth benefits being conducted by the Center. Complete with a hydroponic growth chamber growing real dwarf tomatoes and basil, and pinball-tilting table game of “Surviving Mars” an estimated 300,000 attendees experienced the exhibit during this 14 day period.