2015 NASA I² Internship Report – Asher Williams

Brief introduction/background of intern

I am a Trinidad and Tobago National Open Scholar and recently graduated summa cum laude with a B.S. degree in Chemical and Biomolecular Engineering from New York University Polytechnic School of Engineering. Outside of the classroom, I was involved in a variety of student run clubs and organizations while working as a Resident Assistant at the NYU Brooklyn dorms. Additionally, I worked in Dr. Jin Kim's laboratory as a protein engineering research assistant on a project aimed at increasing protein stability via insertional fusion. I was also a member of the Women in Science Scholarship Program at NYU, where we visited high schools and hosted outreach events to discuss our research projects and encourage young women to pursue careers in science. My research involvement spurred my decision to further my studies in Chemical Engineering and pursue greater levels of research, particularly to explore renewable energy options that are feasible in the Caribbean region. In the fall of 2015 I will be starting a Chemical Engineering PhD Program at Rensselaer Polytechnic Institute in Troy, NY.

Overview of the internship experience

For the 2015 NASA I² Internship Program, I was selected to work in Dr. John Hogan's laboratory on a Human Nutrient Production in Space (Bio-Nutrients) Project involving R&D in advanced microbial strategies for the production of nutrients within crewed spacecraft and habitats. Long-term space missions encounter the hurdle of substantial degradation of certain nutrients in food and supplements with time, potentially resulting in nutrient deficiency and serious health problems. The goal of the Bio-Nutrients Project is to enable rapid, safe, and reliable *in situ* production of needed nutrients using minimal mass, power, and volume. A platform technology is being developed to employ hydratable single-use packets that contain an edible growth medium and a food microbe engineered to produce target human nutrients. In particular, we examined the production of the carotenoids lutein and zeaxanthin in a spore-forming strain of the yeast *Saccharomyces cerevisiae*. Carotenoids are important antioxidants required for ocular health, a problematic area for some astronauts on long-duration ISS missions.

My work hours were 9 am - 5 pm, Monday to Friday, and the lab group held daily meetings or "scrums" to bounce ideas off each other, update the group on our individual progress, and figure out next steps. To meet the first-year milestones for the Bio-Nutrients project, my specific task was to design and run preliminary tests on a disposable bioreactor for *in situ* production of human nutrients in space.

Beyond learning laboratory techniques and gaining new perspectives during scrums, my internship at Ames allowed me to meet a diverse range of individuals who are incredibly smart and also very passionate about their research and space exploration; including Dava Newman, the Deputy Administrator of NASA, during a tour of our lab on her visit to Ames. The proposal and execution of a research project is a

very dynamic process and I had a firsthand view of it, as well as the opportunity to contribute my ideas to tackling the problem of nutrient degradation in space. Outside of the lab, one of my favorite aspects of the internship was the Ames Summer Series. At these seminars I was able to hear from a collection of subject leaders both from and external to NASA, spanning across multiple subject areas including science and technology, religion, science fiction, history, and exploration. At the NASA Lodge I interacted with other interns and took advantage of weekends to go traveling and sightseeing in the San Francisco Bay Area.

Tasks undertaken during the internship

During the first week of my internship I was primarily concerned with completing safety training modules and exams on NASA's online learning management system, as well as emergency and safety trainings specific to my building and lab. In addition, I worked with my mentors John Hogan, Hiromi Kagawa, and Aditya Hindupur to develop a Preliminary Internship Plan. My bag design had to meet the project objectives, which included:

- Achieving effective long-term storage and performance to eliminate the problem of nutrient degradation with time.
- Creating a user-friendly system of minimal mass, power and volume.
- Employing microbial production to create the desired nutrients.
- Consistently generating required compounds in desired quantities and quality.
- Reducing the likelihood of undesirable products or microbial contamination.

Experiments were designed and research and testing done to find answers to the following research questions:

- What is the most effective method of oxygenating the bag contents?
- How will activation/rehydration be carried out?
- What should the total bag volume be?
- How will the dry media be sterilized?
- How will gas exchange be facilitated in microgravity?
- Does the microbe need to be deactivated before consumption?

The PresSure-Lok System was discovered after researching bag designs (available from US producers) that could meet our needs, while considering the unique limitations and requirements of our desired system. PresSURE-Lok is an ideal solution for controlled release of fluids from a lightweight, easy-to-use package. It contains a controlled-dispensing technology that delivers a unique all-in-one solution by combining a flexible pouch with a flexible and self-contained fitment, eliminating the need for a separate dispensing component. A user would simply squeeze the flexible pouch to open the seal, allowing a liquid or gaseous product to flow into the dispensing chamber. The package then automatically reseals itself for future use. This sealing mechanism is designed to allow the flexible package to lie on its side, or even upside down, without unintentional dispensing. This feature makes it suitable for a

microgravity environment, as there will be no risk of spillage. After reaching out to the VP of Sales and Development at TechniPac, developer of the PresSURE-Lok System, a teleconference was scheduled. During this meeting we were able to gain more information about their product and arrange for a few sample bags to be sent to Ames to run trial experiments.

Design Experiments

The PresSure Lok System was tested alongside a regular Tetra Pak bag as a control. Each bag contained 10 ml of our cell culture and 40 ml of YP5D media. They were both placed in a 30° C shaking incubator overnight. The result was that the PresSURE-Lok System vented the CO_2 produced and remained intact, but the Tetra Pak bag ruptured due to increased gas pressure.

Figure 1: PresSure Lok and Tetra Pak bags before incubation.

Figure 2: PresSure Lok and Tetra Pak bags after incubation.

The design of the bag with the PresSURE-Lok bag was then altered by heat sealing, to remove the gusset at the base and create a slant at the top to facilitate more effective CO_2 release. When incubated overnight with shaking, it was observed that a kink formed at the top of the bag, inhibiting gas escape. Additionally, some of bag's contents spilled due to the incubator's vigorous shaking.

The next experiment was run with the same bag, but containing only half of the previous volume of contents (5 ml of cell culture and 20 ml of YP5D). A makeshift side support was also added to the bag using a pipette and tape to prevent the kink from forming. It was incubated overnight at 60° C without shaking. The result was that the bag became tightly inflated with CO_2, causing the tape to unstick and the kink to return.

Figure 3: PresSure Lok bag with makeshift side support before incubation.

Figure 4: PresSure Lok bag and side support after incubation.

The experiment was repeated with a more sturdy side support, consisting of a metal clamp system that was custom-made for the bag. This proved to function effectively, as the bag was slightly inflated with CO_2 at the end of the experiment and a kink did not form. The optical density of the end product was found to be 17.35.

Figure 5: PresSure-Lok bag with metal clamp before incubation.

Figure 6: PresSure-Lok bag with metal clamp after incubation.

Repositioning the bubble to the center of the bag, rather than having it off to one side, can also prevent the problem of kinking during CO_2 production.

Growth Experiments

With a new bag and the metal clamp system, the growth of vegetative cell and dry media (for 25 ml of solution) was tested by overnight incubation at 30° C without shaking. Again, the bag functioned effectively for this experiment. The optical density of the end product was 17.35, showing that vegetative cells rehydrated with dry media grew just as well as the cell culture and liquid media in previous experiments.

In the next set of experiments the growth of dried spores rehydrated in dry media was observed. To keep the spores separate from the media, two types of capsules were tested for possibly storing the spores. A cellulose capsule dissolved in a beaker of water after 2 hours, while a gelatin capsule was still intact after 24 hours in the water. The spores were placed into cellulose capsules sterilized with ethanol and UV radiation then put into new bags with dry media. The bags were purged with nitrogen gas, heat sealed, and rehydrated with 25 ml of autoclaved water. This process also involved the growing of a fresh batch of spores, microscopy, centrifugation, sonication, and lyophilization.

The last set of bag experiments completed before my internship ended were aimed at comparing the growth of spores stored in water, spores stored in a cryogenic protectant, vegetative cells stored in water, and vegetative cells stored in a cryogenic protectant. This required the growth, freezing, and lyophilization of a new batch of vegetative cells. Dried spores and vegetative cells were added to Tetra Pak bags (with caps adapted to allow CO_2 release) containing dry media and rehydrated with 25 ml of autoclaved water. Growth curves were created by recording the optical density of the contents of each bag every two hours during work hours. Additionally, the percentage increase in dry mass of each bag was determined and the results were compared. Microscopy was used to confirm that there was no contamination or foreign organisms growing in the bags alongside the yeast cells.

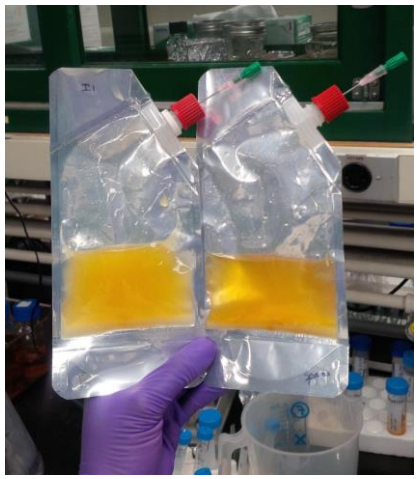

Figure 7: Two of the four bags used for experiments comparing the growth of spores and vegetative cells

Ergosterol vs. Shaking

A hypothesis to be tested was whether or not shaking of the bag could be replaced by the addition of ergosterol, a sterol found in fungi cell membranes that protects membrane integrity. This was done by comparing the optical density and dry mass of yeast cells from three sets of bags under different conditions: incubated without shaking and containing no ergosterol, incubated without shaking and containing ergosterol, and incubated with shaking and containing no ergosterol.

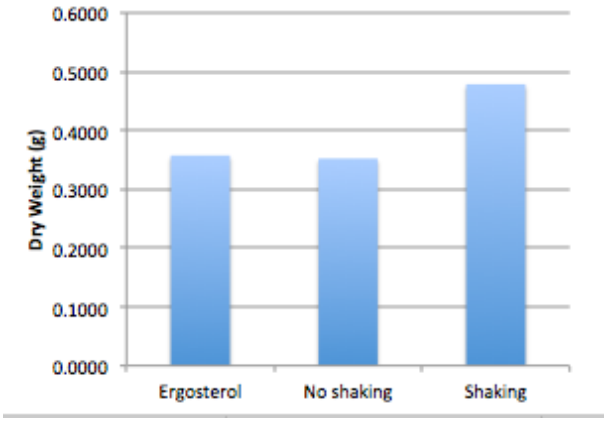

Figure 8: Effect of ergosterol and shaking on dry mass of yeast cells.

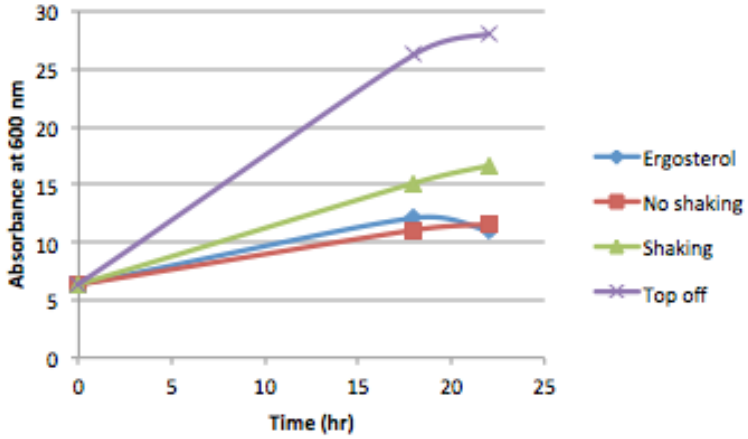

Figure 9: Effect of ergosterol and shaking on OD of cell culture.

The results (Figures 8 and 9) showed that shaking was more effective for aeration than adding ergosterol.

Aeration

Since the importance of aeration was highlighted, research was done into various methods for oxygenating the bag contents. Possible ways to do this include:

- A carbonation stone type straw system that will bubble oxygen into the bag's contents.
- A membrane oxygenator with at least one hydrophobic microporous membrane, having a gas side and a liquid side.
- Use of chemical compounds that produce oxygen – peroxides, chlorates, perchlorates, and inorganic superoxides *(safety issues likely invalidate this option).*

The bag can possibly be connected to an artificial gravity (centrifugal) system to aid gas exchange.

Media Sterilization

To reduce the likelihood of undesirable products or microbial contamination, the conditions and contents of the bag must be kept sterile. Sterilization of the dry media before adding it to the bags was another issue that had to be tackled. A concentrated solution of YP5D containing 40% glucose was used for the drying experiments described below.

The first trial was carried out by placing samples of the concentrated media in a beaker, a 15 ml Falcon tube, and an autoclave sterilization bag, in a 60° C oven. After three days, the media formed a thick, caramelized substance that was unsuitable for our purposes.

Figure 10: Media samples in 60° C oven initially.

Figure 11: Media samples in 60° C oven after two days.

For the second trial, a sample of the media was placed in a beaker in a vacuum oven at 60° C. Again, the media formed a thick, caramelized liquid after two days.

Figure 12: Media sample after two days in a 60° C vacuum

For the third trial, the concentrated media was frozen at -80° C in 15 ml Falcon tubes and then lyophilized. After two days, the product was observed to be a caramelized liquid with some sporadic crystallization.

Figure 13: Media samples after two days of lyophilization.

Lastly, samples of the concentrated media were placed in eppendorf tubes in a speed vacuum at low heat. A hardened top layer with a gooey liquid underneath was observed in each tube after one day.

Figure 14: Media samples after one day in a speed vacuum at low

None of these sterilization methods proved to be successful. A spray dryer might work; otherwise the best way to sterilize the dry media could be by irradiation.

Sizing

An excel template was developed for calculating the bag size when values for certain variables were provided. The constants were the target amounts of each product - 2 mg of zeaxanthin per day and 10 mg of lutein per day. To determine what the average volume of each Bio-Nutrient packet should be, the required data included:

- Amount of lycopene (beta-carotene/alpha-carotene) produced per gram of dry yeast
- Target amount of lycopene desired per packet (based on conversion efficiency to the required amounts of lutein and zeaxanthin)
- Amount of powdered media that needs to be added
- Dry mass of spores that needs to be added
- Volume of water required to adequately hydrate the spores and media and create the desired consistency
- Dissolving factor of spores/media (how much the volume changes upon hydration)
- Headspace required to allow for bag expansion and gas exchange
- Amount of desired nutrients initially present in spores before germination

Stoichiometry of the biosynthesis pathway:

$$C_{40}H_{56} \text{ (lycopene/beta-carotene)} + 2NADH + 2H^+ + 2O_2 \rightleftharpoons C_{40}H_{562}O_2 \text{ (lutein/zeaxanthin)} + 2NAD^+ + 2H_2O$$

Looking at the metabolic pathway, stoichiometry of the actual reaction, suggestions for theoretical maximum yield, as well as yields obtained from experiments described in various research papers, a very wide range of conversion efficiencies and expected yields were found. This made it difficult to enter accurate values into the template and pinpoint a reasonable range of possible bag sizes.

Lessons learnt from the internship experience - both from an academic/ research perspective, as well as from a personal perspective

I consider myself vey lucky to have had the opportunity to work in Dr. Hogan's lab. My mentors were always very helpful and approachable, and seamlessly integrated me into the lab. They pushed me to think of solutions and next steps concerning my project, and answered any questions I had, while challenging me with questions of their own.

During my time at Ames I learned to use laboratory equipment that I was previously unfamiliar with, such as a lyophilizer, hemocytometer, inverted microscope, anaerobic chamber, clean hood, speed vacuum, and vacuum oven. Since the risk of cross contamination is high while working with yeast cells I was taught sterilization techniques such as working around a Bunsen burner or in a clean hood. Along the way I learned a few tips for preventing contamination in autoclaved solutions and even a clever method for balancing a centrifuge without using a water balance. I also had the chance to glean some procedural advice and recommendations for increasing product yield in bacterial transformations.

Working on the bag design highlighted the importance of experimental controls for allowing data to be correctly interpreted and testing if a system is behaving as expected. If something goes wrong, a control makes it possible to pinpoint any

experimental errors that should be rectified in future experiments. Research is a very iterative process where experiments often need to be repeated based on previous results or improved methods. Results may not be very reliable unless they are obtained consistently from experiments run multiple times under similar conditions. Additionally, experiments rarely work out perfectly but valuable lessons can be learned from each attempt.

I was not only engaged in carrying out experiments and recording data, but also interpreting that data, clearly presenting it in tables and graphs, and using the results obtained to plan ahead for future experiments. The proposal and execution of a research project is a very dynamic process and I had a firsthand view of the background logistics and brainstorming that go into such a process, as well as the opportunity to contribute my ideas to the project. Our meetings taught me a different way of thinking and to not be afraid of approaching problems in unconventional ways.

The way forward - the relevance of the research to society and its impact on the future

Next steps for the project would include improving the current design through extensive ground testing, then moving on to flight-testing. Some of NASA's life support projects are perhaps applicable on earth, particularly since the degenerative effects of microgravity on the body are somewhat similar to the effects of aging on earth. The Bio-Nutrients project is focused on the production of the carotenoids lutein and zeaxanthin, but this technology can be expanded to other vitamins and supplements that are producible in microbial systems.

Making these compounds only when they are needed eliminates the need to keep them preserved at the expense of nutrition. In addition, storing pre-made nutrients takes up precious space and energy that can be conserved and allocated to more important things if they are produced in space instead.

The scope of the project concept may also be expanded in the future to engineering multiple nutrients to be produced in a single packet, or the production of other small-quantity compounds such as pharmaceuticals. The Bio-Nutrients project can serve as a template for on-demand, configurable production of a multitude of needed compounds and in situ production of organisms wanted for unexpected needs in space.