

Using Effluent from a Hybrid Anaerobic Membrane Bioreactor Treating Fecal Waste for Hydroponic Fertigation of Pak Choi

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Challenges for future deep space ECLSS include providing potable water, supplying nutritious food, and managing wastes generated by the crew. With next to no readily available resources to sustain human life on the Moon and Mars, nothing can be considered a waste, and every resource, including all organic wastes generated by the crew (e.g., fecal, and inedible biomass), should be deemed for recovery and reuse to achieve closed loop systems. Fecal waste aboard the International Space Station (ISS) is currently treated as solid waste and not recycled in any capacity. The high-water content (fecal material being ~75% water), complexity, and the presence of pathogens make fecal waste difficult to stabilize and process. However, fecal material contains considerable fractions of carbon, nitrogen, phosphorus, and minerals which after stabilization, can be recovered and used in a downstream system. There is considerable research about growing food in Lunar and Martian greenhouses but a major limitation for plant growth will be continuously supplying fertilizer salts. Recognizing the need for a bioregenerative approach to fecal waste, an Organic Processor Assembly (OPA) unit was developed through collaboration between the University of South Florida and NASA's Kennedy Space Center. OPA is a physical-biological hybrid treatment technology that couples an anaerobic bioreactor with a tubular ultrafiltration membrane. OPA is designed to treat and recover resources from the solid organic (fecal) waste stream of a crew of four astronauts on an early planetary base. Aspects of OPA's long-term operations and water quality treatment analysis were presented at ICES 2022. This conference paper will present preliminary research regarding the downstream use of OPA's nutrient-rich effluent in supporting the growth of extra dwarf Pak Choi from germination to maturity. Overall, OPA is an enabling technology demonstrating its potential to offset fecal storage volume, assist in waste management, and potentially offset fertilizer demand.

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Nomenclature

<i>AnMBR</i>	= Anaerobic membrane bioreactor
<i>DO</i>	= Dissolved oxygen
<i>EC</i>	= Electrical conductivity
<i>ECLSS</i>	= Environmental control and life support systems
<i>g</i>	= gram
<i>h</i>	= hour
<i>ISS</i>	= International Space Station
<i>kg</i>	= kilogram
<i>KSC</i>	= Kennedy Space Center
<i>L</i>	= Liters
<i>NASA</i>	= National Aeronautics and Space Administration
<i>NFT</i>	= Nutrient film technique
<i>OPA</i>	= Organic Processor Assembly unit
<i>UF</i>	= Ultra filtration membrane
<i>USF</i>	= University of South Florida
<i>VFAs</i>	= Volatile fatty acids

I. Introduction

With the success of the Artemis 1 mission, human return to the Moon is rapidly approaching. Having a sustainable human presence on the Moon by the late 2020s will require an even more resilient and self-sustaining environmental control and life support systems (ECLSS) to better provide provisions (i.e., food and water) and improve ways of managing astronaut wastes on planetary surfaces. Most ISS solid wastes are disposed of using a vehicle that is incinerated during re-entry¹. Among the solids waste portions is the fecal waste generated by astronauts. The fecal waste is collected by the Universal Waste Management System in single-use gas-permeable bags made of a hydrophobic material that retains liquid water². After collection, crew members seal the bag and place it into a fecal holding canister. Once at capacity, the canisters are capped with an odor/ bacteria filter and stored before ejecting it with other waste. While this is practical for missions in low earth orbit, for missions to the Lunar and Martian surface, jettisoning metabolic wastes onto the Lunar surface will most likely be restricted due to planetary protections¹. There is a need to go beyond the current practice of merely storage and have strategies for degradation, pathogen inactivation, and resource recovery (e.g., water and nutrients).

Potential treatment, and recovery technologies have been highlighted regarding fecal waste for future missions including storage, thermal treatment (i.e., combustion, pyrolysis, gasification), and biological treatment¹. All methods have their strengths and weaknesses. The current option to store the fecal waste of a crew of four for a 30-month mission will require 400 fecal storage canisters³. This will require sufficient storage space and does not explore any resource recovery. Subjecting fecal waste to thermal treatment is an energy expensive process. These technologies can struggle to process water content beyond 25%, whereas fecal texture is often inconsistent and ranges anywhere from 65% to 85% water⁴⁻⁵. The kinetics of biological processes are slower when compared to the reaction of physical-chemical processes, which has been why biological systems have been typically overlooked for treating waste in space applications⁶. However, incorporating biological based systems, or bioregenerative life support systems, into deep space life support architectures serves multiple functions like processing wastes while generating energy, recycling water, and creating food, all the while undergoing self-repair⁷.

The median water content in feces is 75%⁸. This high-water content makes fecal a candidate to include in a resource recovery loop to assist in reaching the high-water recovery goals for deep space missions. It is projected that over the course of a 30-month mission one crew member will produce ~132kg (or 290 lbs.) of fecal waste¹. There is the potential to recover roughly 90 liters of water from one crew member. Fecal material also contains resources beyond water. Feces is made up of water, protein, undigested fats, polysaccharides, biomass, ash, and undigested food residues, with major elements, as percentage of wet weight, of oxygen 74%, hydrogen 10%, carbon 5%, and nitrogen 0.7%, this includes the hydrogen and oxygen present in the water fraction of the feces⁹. Fecal nitrogen varies with diet

but typically makes up 5–7% of the dried solids and is present in undigested protein, nucleic acids, and protein from bacteria^{9,10}.

Table 1. Recoverable resources (in dry mass) on a high and low scale from the fecal generated by one crew member over the course of a 30-month mission. Of the 32kg of fecal solids that will be generated by one crew member, up to 3.6kg can be recovered as fertilizer like nutrients. Tabled adapted from Rose et al, (2015).

Recoverable Resource	(g/kg) High end	(g/kg) Low end	Highest Recovery from 30-month mission (g)	Lowest Recovery from 30-month mission (g)	Citation
Nitrogen	24.5	17.5	784.0	560.0	10
Phosphate	28.2	5.06	901.5	161.8	11,12
Potassium	20.5	5.09	654.6	161.8	13,14
Sodium	14.1	2.29	451.7	73.1	12,14
Calcium	12.2	7.66	390.4	245.0	12,15
Magnesium	8.2	3.8	261.5	121.6	13,12
Chlorine	1.7	1.7	54.9	54.9	12
Sulfur	2.5	2.5	79.5	79.5	12
Copper	0.02	0.02	2.6	0.6	12
Iron	0.57	0.57	18.3	18.3	12
Lead	0.02	0.0003	0.6	0.01	16,14
Zinc	0.193	0.139	6.17	4.4	15,14
Nickle	0.004	0.003	0.14	0.11	12,16
Chromium	0.003	0.001	0.08	0.03	14,16
Cadmium	0.018	0.001	0.58	0.02	14,16
Mercury	0.0001	0.0001	0.004	0.004	14
Total Recovery (kg):			3.6	1.5	

Reutilization of nutrients found in fecal matter can be used in downstream bioregenerative life support systems or can be utilized as fertilizer to grow food crops. NASA has long explored growing plants for consumption in space in systems such as Veggie and the advanced plant habitat. Pre-packaged processed foods alone will be unable to support the full range of diverse nutrients needed to support astronauts' health against risks associated with space travel such as, increased cancer risk from radiation, bone loss, muscle atrophy, and central nervous system health^{17,18}. However, if plants are used to generate food, they will require roughly 90kg of fertilizer to sustain production to support a 2500kcal diet of one crew member for one year¹⁹. The amount of fertilizer required will vary depending on the total planted areas and lighting conditions²⁰. The estimated requirements for small salad crop producing systems will require 1-2 grams of Nitrogen, 1-2 grams of Potassium and 0.1-0.2 grams of Phosphate to produce 250 grams of fresh weight of salad per person per day^{19,21}. Using common hydroponic chemicals this equates to about 15 g of fertilizer per person per day²¹. Ultimately, importing all fertilizer from earth will generate a large mass penalty on the mission¹. Recycling fecal derived nutrients could help to reduce the need to import all fertilizers from Earth. This conference paper examines a bioregenerative approach to the treatment of fecal waste, the recovery of fecal derived nutrients, and the feasibility of growing crops on fecal derived biofertilizer.

II. Material and Methods

A. Development of the Organic Processor Assembly (OPA) Unit:

The Organic Processor Assembly (OPA) unit was developed between the University of South Florida and NASA's Kennedy Space Center to bridge the gap between fecal treatment and resource recovery. The OPA is a bioregenerative life support system designed specifically for an early Lunar or Martian base habitat. OPA is a hybrid system consisting of a biological microbiome and tubular ultrafiltration (UF) membrane to make up this novel anaerobic membrane



Figure 1. Organic Processor Assembly unit. The biological portion (buffer tank, reactor 1, reactor 2) sits in the bottom of OPA. The tubular membrane sits in the upper right-hand corner where it is easily assessable.

and volume are based on the fecal solids content anticipated for the fecal and flush waste generated by a crew of four astronauts. This is estimated to be 2.5L/day of influent^{1,6}. Just as OPA treats 2.5L of wastewater a day, the system also generates 2.5L a day of product-water or permeate. OPA's second main objective is to recover nutrients from the fecal input within this permeate. Although OPA's permeate is not to drinking water standards, it is proposed that this permeate could be sent downstream for further treatment or instead of focusing on removal, use the nutrients within the permeate to feed into another bioregenerative system. The results from the long-term treatment of high solids content and chemical oxygen demand profile of OPA was presented at ICES 2022²⁵. After running the system on an actual (canine) fecal influent for over a year under continuous, semi-continuous, intermittent, and dormant stages, it was determined that OPA's permeate would be examined for the direct use to support the growth of crops.

B. Water Quality Analysis

Beginning June 2021, samples of each treatment stage of OPA (e.g., buffer tank, reactor 1, reactor 2) and its permeate were taken. On these samples collected, water quality analyses are performed to monitor treatment and overall performance of the system. Oxygen reduction potential (ORP) and pH serve to monitor the anaerobic digestion process over the phases of treatment. Chemical oxygen demand, total suspended solids, and volatile suspended solids monitor the treatment of the waste when comparing the influent to the permeate. Testing for nutrient concentration (total nitrogen, ammonia, and phosphorous) provides data on recovery. Ion chromatography was done to provide nutrient recovery data in the permeate beyond the range of weekly water quality testing. The transmembrane pressure (TMP) is recorded as an indicator of the membrane's condition, ideally staying below 1 bar.

C. Permeate Collection and Analysis

Upon inoculation and OPA's initial feeding, permeate generation was recorded daily and cumulatively. Because of the hybrid design and the UF membrane, OPA begins generating permeate immediately upon startup.

bioreactor (AnMBR). The microbiome is made up of an anaerobic sludge which is primarily bacteria and archaea working together to make up the process of anaerobic digestion. This microbiome breaks down the complex organic matter from the fecal wastes. This process is attractive for space for several reasons, the low energy input (as it does not require aeration) and the potential for energy harvesting²². Well known outputs for anaerobic digestion are biogas, mainly composed of CH₄ and CO₂, biosolids for fertilization, and when combined with a filtration membrane, a nutrient laden product water or permeate. The UF membrane serves to separate the solubilized nutrients from the microbiome of the reactor. It does so by allowing water and ions to filter through while rejecting the solids portion back to the reactor preventing biological washout and ensuring some pathogen rejection²³.

The total treatment volume of OPA is 60L, made up of three sequential 20L reactors. OPA is fully automated, the control system is based on a programmable logic controller, which can incorporate safety factors. This system monitors the liquid levels of each phased bioreactor within OPA; buffer tank, reactor 1, reactor 2, and permeate collection tank. This is done through capacitance liquid level sensors attached to the outside of the tanks. The system is phased for optimal treatment, and it is hypothesized that the microbiome of each individual 20L reactor cultivates microbial diversity and environmental conditions favorable to the fermentative bacteria (reactor 1) followed by methanogenic archaea (reactor 2), whose activity can be suppressed by low pH of fermentative conditions²⁴.

OPA has two main objectives, the first is to continuously treat a high strength and high solids fecal waste stream. OPA's size

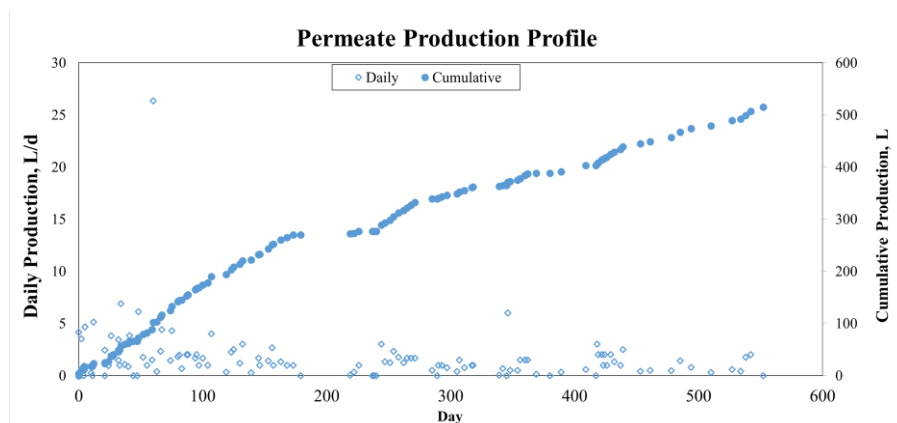


Figure 2. OPA’s daily permeate production and cumulative production through day 552. The permeate production profile includes dormancies, intermittent operations, hardware updates, maintenance, and troubleshooting (days 180-222; 318-339; 389-410). The system has cumulatively produced 515 liters of permeate.

The permeate filtered directly from the membrane has been monitored weekly since the beginning of operation for conditions that reflect the environment and treatment of the bioreactor. This includes pH, oxygen reduction potential (ORP), and ammonia levels. Permeate collection began July 2022 and stored in 20 L carboys at room temperature until a backstock of 140 L was collected. Anaerobic digestate liquid effluent can have limitations as a fertilizer due to the primary nitrogen source being ammonia, which can damage plants in high concentrations²⁶. The permeate’s main nitrogen form is ammonia averaging 283 mg/L. Freshly filtered from the membrane, before storage, the average permeate pH from over 500 days of operation is 7.0. The permeate leaving the system is anoxic (depleted of dissolved oxygen) averaging -147 mV. Oxygen deficiency can also damage plants. It was anticipated that storage would influence the conditions of the permeate. To examine if the stored permeate could be conditioned for increased dissolved oxygen and potential nitrification, storage tanks 1,2, and 3 were aerated at 3 L/min for 14 days. While storage tanks 4,5,6, and 7 were unconditioned and stored stagnant. An analysis of the conditioned and unconditioned permeate was conducted for nitrogen forms (e.g., ammonia, nitrite, nitrate), pH, dissolved oxygen (DO), and electrical conductivity prior to downstream hydroponic use. In addition to testing for nitrogen concentrations, OPA’s permeate was analyzed on an ion chromatography instrument to measure essential plant macro and micronutrient concentrations.

D. Germination and Microgreens Growth Feasibility Study

A germination study was conducted to determine if the current nutrient composition of OPA permeate would have any phytotoxic effects on the germination of extra dwarf Pak Choi seeds. OPA stored permeate (aerated) was used as a comparison against the control, ½ concentration of Hoagland’s nutrient solution. ½ Hoagland’s nutrient solution was used as it is a common liquid nutrient solution used at the Kennedy Space Center in plant growth experiments. Distilled water was used as a negative control. Nine grams of seeds were germinated on stone wool substrate (6 in. x 12 in.) for 13 days then harvested and weighed. A full spectrum Agrobrite high output light was used for a photoperiod of 16/8hr calibrated to 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

E. Hydroponic Growth Feasibility Study

Extra dwarf Pak Choi seeds were germinated on netted coconut core seed starters using, in separate seed beds, ½ Hoagland’s, OPA permeate, and distilled water. After germination and appearance of the first true leaves (12 days), six Pak Choi seedlings from each treatment were transplanted into vertical nutrient film technique (NFT) hydroponic system from Zipgrow (<https://zipgrow.com/zipgrow-towers/>). Each tower contained a matrix media and polyester wicking strip that the seedlings were sandwiched into at six inches apart. Each hydroponic solution was pumped at 10 L/ hour from a nutrient sump containing 10L to the top of the towers where it percolated through the matrix and recirculated back into the respective sumps²⁷. Conditioned OPA permeate from storage tanks 2 and 3 (mixed) were used throughout the grow out. pH, DO, and EC were monitored daily. Plant growth was measured 3 times a week. The ideal pH of extra dwarf Pak Choi ranges from 6.0-7.0 and the optimal EC ranges from 1.8-2.4mS²⁹. Both the microgreens and hydroponic feasibility studies were conducted in a laboratory where the temperature minimally fluctuated from 20-23°C.

III. Results and Discussion

A. Permeate Collection and Analysis

To compare the nutrient composition of OPA permeate to ½ concentration Hoagland’s, an ion chromatography comparison was done, shown in Figure 3. It is improbable that OPA’s permeate will be completely analogous to Hoagland’s. It was anticipated that the permeate could have some minor nutrient deficiencies such as, potassium, sulfate, and iron while having excess of other such as calcium, magnesium, sodium, and chloride. What is recoverable in the permeate is dependent on the influent of OPA. Since fecal material is highly dependent on diet, it is likely that canine food is less nutritious than that of a human diet.

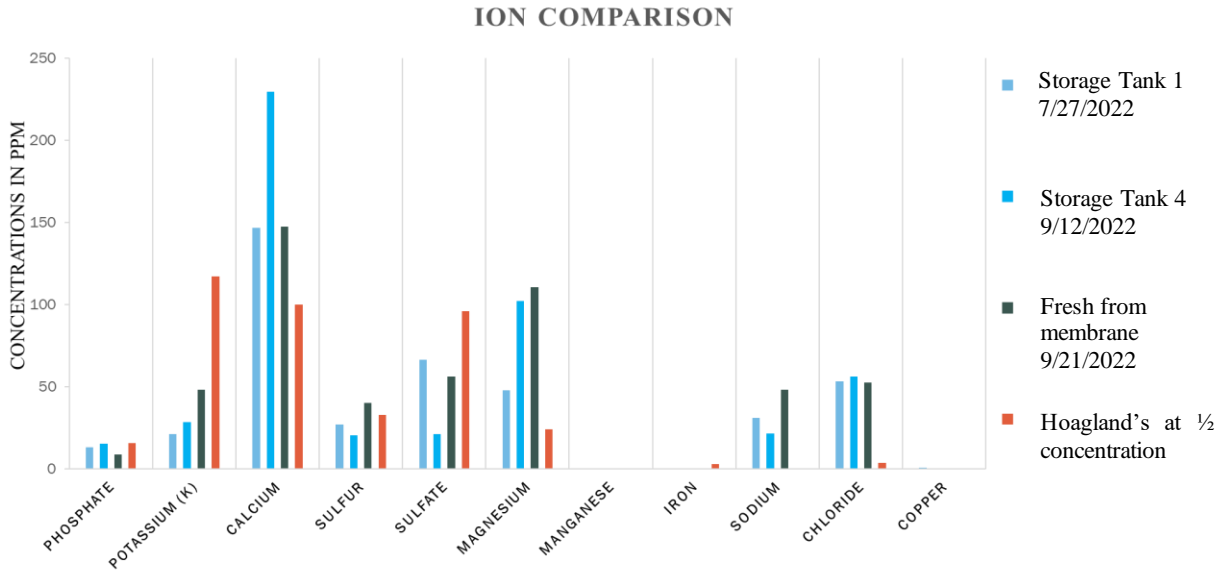


Figure 3. Three samples of OPA’s permeate from different storage scenarios analyzed on an Ion Chromatography instrument to compare nutrient composition of the permeate to ½ concentration Hoagland’s nutrient solution. Each sample was unconditioned/ unaerated. Nitrogen ranges of ½ Hoagland’s ½ is 105mg-N/L while OPA averages 293 mg-N/L. The variation between OPAs sample is likely due to the variation found in the fecal influent.

Table 2. The averaged nutrient concentration in mg/L found in OPA permeate and total recovered nutrients. The total recovered is based on the averaged ion chromatography data, water quality data, and the total volume of permeate produced throughout the 552-day semi-continuous treatment on canine fecal influent.

Nutrient	Average mg/L	Total Grams recovered
Total Nitrogen	297	153.0
Phosphate	12.2	6.3
Potassium	32.5	16.7
Calcium	174	89.9
Sulfur	29.0	14.9
Sulfate	47.8	24.6
Magnesium	86.9	44.7
Manganese	0.03	0.0
Iron	0.26	0.1
Sodium	33.5	17.2

Chloride	53.9	27.7
Copper	0.27	0.1

OPA produces 17.5L of permeate a week and to collect a large backstock for the series of hydroponics experiments, earlier collected permeate aged for up to six months over the collection period. As stated above, when the permeate leaves OPA it is anoxic, to condition the permeate to better support plant growth concerning dissolved oxygen (DO) levels, storage tanks 1 thru 3 were aerated while tanks 4 thru 7 remained stagnant. The pH, EC, and DO of the conditioned and unconditioned permeate were measured after conditioning to compare how aeration would affect the permeate as can be seen in figure 3. After aeration, the DO increased in tanks 1, 2, and 3 while remaining near zero in the unconditioned storage tanks.

Table 3. Each storage tanks' date of collection and any form of conditioning. Permeate collected for the hydroponic study was collected for a minimum of once a month the best represents the nutrient compositions over long term operations.

Conditioning	Storage tank	Date Collected	Volume (L)
Aeration	1	7/27/2022	20
Aeration	2	8/3/2022	20
Aeration	3	8/12/2022	20
None	4	9/12/2022	20
None	5	10/3/2022	20
None	6	11/14/2022	20
None	7	1/20/2023	13

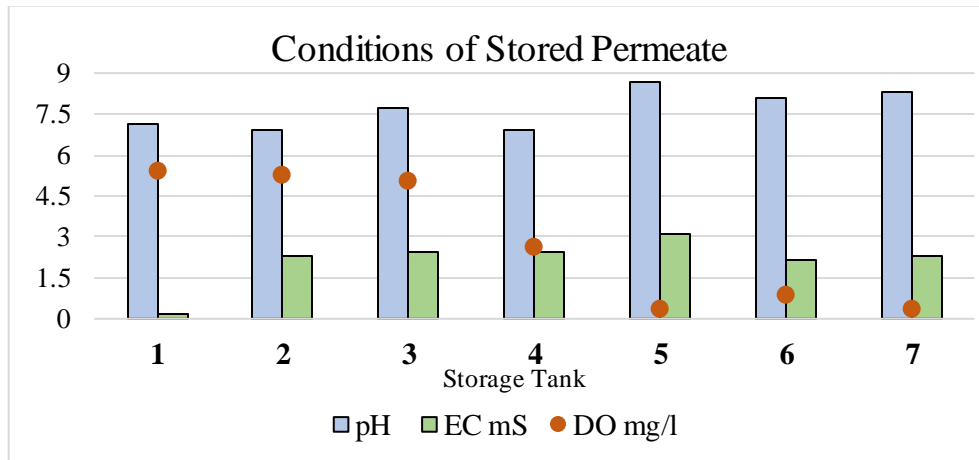


Figure 4. The storage tank's pH, EC, and DO. Taken after conditioning, if received. The conditioned tanks (1, 2, and 3) saw an increase in DO. Although storage tank 4 did not receive any aeration, it is likely that as it ages, the permeate interfaces with oxygen from the atmosphere passively increasing the DO. The variation in EC is due to the variation of fecal influent. The fluctuating pH ranges is likely attributed to any biological activity. Samples were taken before the growth experiments.

Nitrogen form is important to plant growth, each tank was measured for concentration of ammonia, nitrite, and nitrate before use as a nutrient solution for Pak Choi seeds. Ammonia oxidation and nitrification, at various stages in each individual tank as can be seen in figure 4 below. It should be noted that the storage tanks were never inoculated with any bacterial culture

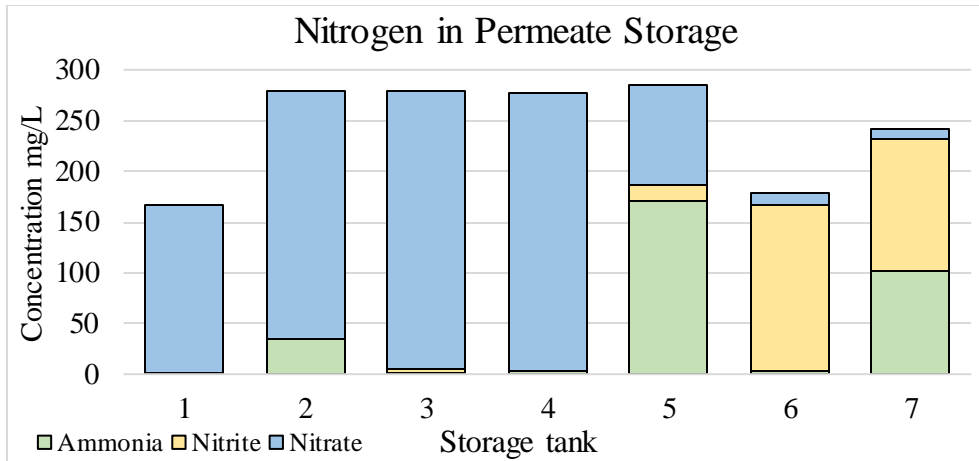


Figure 5. Concentrations of ammonia, nitrite, and nitrate within each stage of the aged storage tanks. As previously mentioned, ammonia is the primary form of nitrogen as the permeate is filtered through the membrane with an average of 283 mg/L. Storage tank 4 is an outlier and appears to have undergone nitrification, without active aeration, whereas the tanks that did not receive conditioning contain mostly ammonia and nitrite.

Although storage tank 4 did not receive any aeration it contains nitrate level comparable to the aerated tanks (1,2, and 3) and is an outlier among the tanks. This could indicate that through merely storage alone, permeate could become better suited for plant growth by biological means. Further testing is required to make this assumption. All tanks continue to be monitored for nitrogen forms.

B. Germination and Microgreens Growth Feasibility Study

After determining that OPA's permeate had comparable macro and micronutrient nutrient composition to ½ Hoagland's nutrient solution, identifying any phytotoxicity effects the permeate may exhibit on germination was necessary. Extra dwarf Pak Choi seeds were germinated over the course of several days and observed for any abnormal growth (e.g., coloration, root development), when compared to ½ Hoagland's. During the imbibition phase, germination could have been negatively affected by slightly higher than usual salinity of OPA permeate. Germination could also have been inhibited by ammonia concentration, but by utilizing the conditioned permeate from tank 2 ruled this possibility out. Volatile fatty acids (VFAs) are a byproduct of anaerobic digestion and could additionally be an inhibitor of germination. Despite this, the OPA permeate germinated seeds all exhibited radicle elongation and overall, no inhibitory effects were observed. The overall biomass of microgreens produced when compared to Hoagland's was similar, shown in Table 5.

Table 4. Nutrient solution conditions before seed inoculation. OPA permeate from tank 2 was used.

Conditions	pH	Electrical conductivity (µS)
½ concentration Hoagland's	6.16	980
OPA permeate	6.7	2300
Distilled water	6.5	5

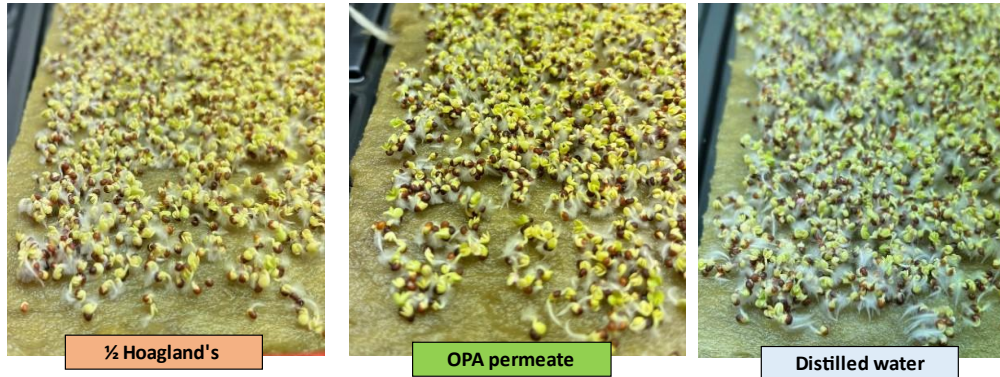


Figure 6. Day two after plating – December 6, 2022. Germination and root hair growth on all three nutrient solutions. Permeate from only tank 2 was used.

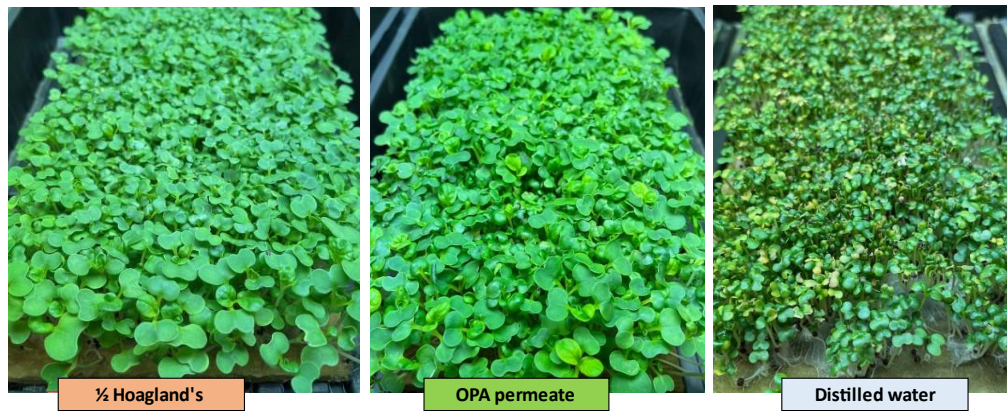


Figure 7. Day 12 of a 13 day grow out period. True leaves began to sprout from Hoagland’s and OPA permeate. Distilled water begins to show signs of stress.

Table 5. Final volumes of the nutrient solutions used and the final mass of microgreens. The volume of distilled water was lower than Hoagland’s and OPA perm as the microgreens in this control stopped absorbing water because of their lack of growth.

Solution	Total volume (L)	Mass (wet) of microgreens (g)
Hoagland’s	1.8	121.7
OPA permeate	1.8	139.6
Distilled water	1.6	45.1

C. Hydroponic Growth Feasibility Study

After observing no phytotoxicity effects during the germination and growth into microgreens, a new round of extra dwarf Pak Choi seeds were sown. After the appearance of the first true leaves (12 days), six seedlings were transplanted into vertical hydroponic towers.

Table 6. A timeline of any added volume (distilled water or nutrient solutions), pH control, or refreshing of out the nutrient reservoir. Distilled water was added to maintain the volume of the nutrient reservoirs between 8 and 10L.

Day	Volume added (L)	Distilled water	Nutrient solution	pH control
3	1	X		
8	1	X		
10	2	X		
			Swap for fresh solutions (OPA)	

14	10		permeate: Tanks 2 and 3 in combination)	
17	1	X		+ 200 μ L of 85% phosphoric acid
20	1	X		+ 200 μ L of 85% phosphoric acid
22	1	X		
23	1	X		

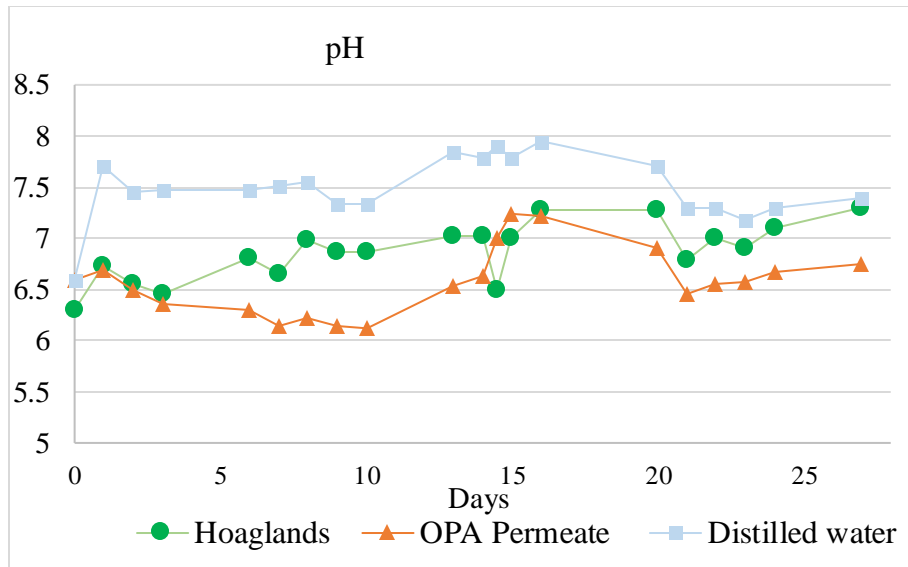


Figure 8. Tracking the pH throughout the 28 days grow out. Day 14 marks the switch to fresh nutrient solution.

The pH of OPA permeate and ½ Hoagland’s began at similarly and followed the same downward trend for the first few days after transplanting. On day six however, ½ Hoagland’s pH began to increase as plants began to absorb nutrients. OPA permeate continued to decrease until day thirteen. This could possibly indicate the elevated EC levels may have blocked nutrients from being absorbed as rapidly when compared to ½ Hoagland’s, as pH typically increases as nutrients are absorbed. OPA’s permeate is biological and there is potential for activity to lower the pH. On day fourteen, the solutions of all three treatments were completely refreshed. OPA permeate and ½ Hoagland’s pH responded quickly with a rise in pH, requiring pH control on days 17 and 20. The general response of OPA permeate and ½ Hoagland’s pH after the refresh in solution was a rise. The distilled water’s pH rose quickly in response to the complete lack of nutrients.

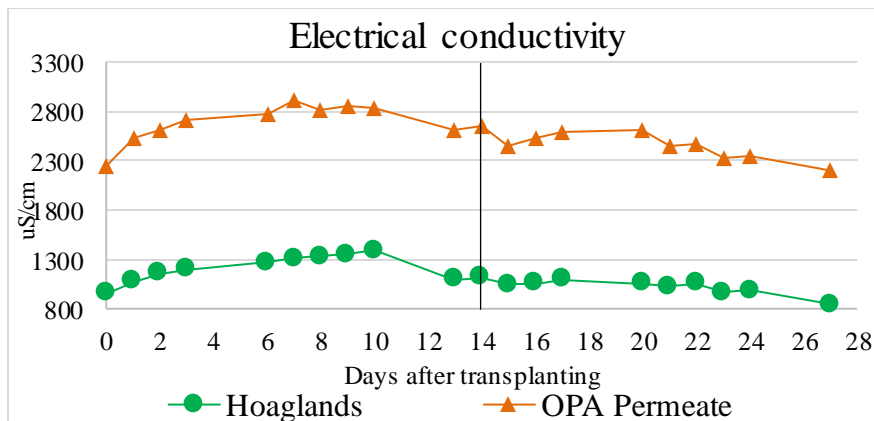


Figure 9. Tracking EC throughout the 28 days grow out. The distilled water (not shown on this plot) EC fluctuated between 11 and 88 $\mu\text{S}/\text{cm}$ throughout the growth period.

OPA's permeate is nutrient rich and began with a higher EC than the tailor-made 1/2 Hoagland's. Observations that were pertinent to the OPA plants were any physiological indication of osmotic stress, due to the slightly higher sodium levels, or any ion toxicity/ deficiency. Upon transplanting into NFT towers, both 1/2 Hoagland's and the OPA permeate EC increased. Indicating that the plants were taking up mostly water until day thirteen, where the slight drop in EC on both solutions possibly indicated a nutrient up take. After the solutions complete refresh on day fourteen, 1/2 Hoagland's maintained a balanced uptake until day twenty-three. OPA permeate hovered in and out of a balanced uptake, signifying that solubility and availability of nutrients were likely affected by the addition pH control implemented on days 17 and 20.

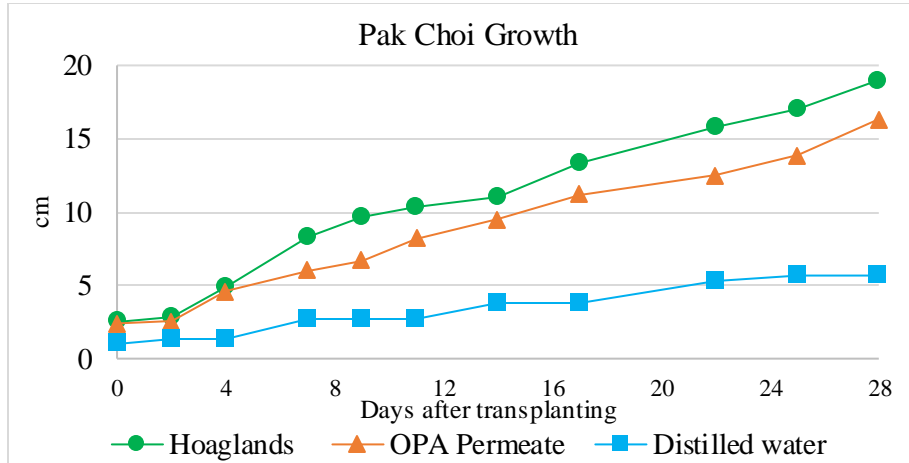


Figure 10. A time series plot of the averaged width of the six extra dwarf Pak Choi plants within each treatment.

Table 7. The total edible biomass produced from each treatment.

Edible biomass (wet weight)			
	1/2 Hoagland's	OPA Permeate	Distilled water
Total (g)	175	76	6.79

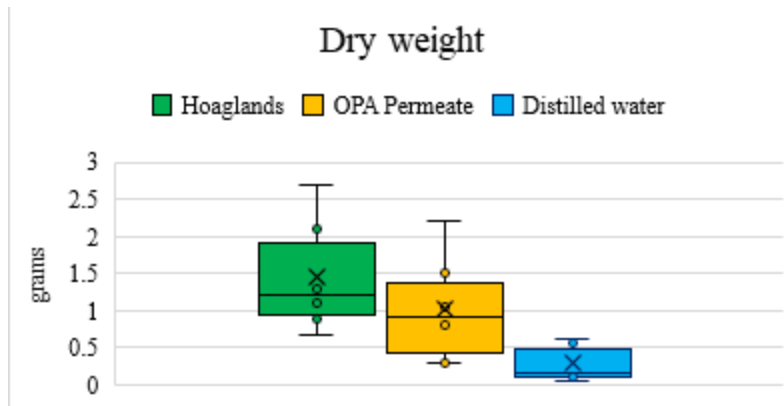


Figure 11. Comparison of the dry weight (not including the roots) of each treatment. Each treatment consisted of two separate towers each housing three plants (n=6).

Although the plants grown on OPA permeate had a lower overall edible biomass than those grown on $\frac{1}{2}$ Hoagland's the average dry weights are similar. The dry weight refers to the plant's constituents with the absence of water and provides information regarding growth performance in terms of nutrition and photosynthesis. $\frac{1}{2}$ Hoagland's mean dry weight is 1.5 grams while OPA's is 1.0 gram.



Figure 12. Photo of harvested extra dwarf Pak Choi plants from each treatment (6 plants per). A: distilled water. B: OPA permeate. C: $\frac{1}{2}$ Hoagland's.

IV. Conclusions and Recommendations for Future Research

To conclude, fecal waste will need to be treated for planetary missions regardless of if there is a need to recover resources from fecal. However, approximately 3.6 kg of fertilizer nutrients can be recovered from one crew member over the course of a 30-month mission. A capable bioregenerative technology to stabilize fecal solids and recover resources in a liquid state is in development at USF. Though the OPA's permeate is not an absolute perfect match to $\frac{1}{2}$ Hoagland's nutrient solution, it can provide nutrients within a comparable range. There is potential for OPA's permeate to supplement or even replace chemical fertilizers in crops with a short lifecycle and low nutrient demand such as extra dwarf Pak Choi. OPA's permeate is an organic permeate and is biologically dynamic, but through passive or active stabilization practices there lies ability to perfect its usage for crop growth. When OPA's conditioned permeate was used at full strength in comparison to $\frac{1}{2}$ Hoagland's to germinate microgreens, the mass of microgreens produced was comparable. OPA permeate was additionally used to support Pak Choi growth to maturity and had similar growth trends regardless of higher than normal EC ranges. Overall, OPA's preliminary data suggests that this technology can stabilize the problematic fecal solid waste stream, overall reducing the fecal material's solids, and recovering resources through a liquid permeate that can then be utilized in other areas downstream.

For future research, OPA's architecture also could allow for the possibility of other byproducts besides a traditional liquid fertilizer solution for plant growth. The biological basis of OPA is an anaerobic digestion. A well-known byproduct during the stages of anaerobic digestion is the production of VFAs, such as acetate, which can serve as a carbon source for algae, fungus, and potentially plants. OPA could be investigated for the harvesting of VFAs which then could be used to grow food crops independent of photosynthesis, as Hann et al (2022) did, by cultivating organisms on acetate in the dark³⁰. OPA's byproducts could additionally be studied to cultivate edible filamentous fungus such as Uwineza et al (2021) investigated³¹.

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