DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Innovative Polyhydroalkanoates (PHA) Production with Microbial Electrochemical Technology (MET) Incorporation for Community-Scale Valorization

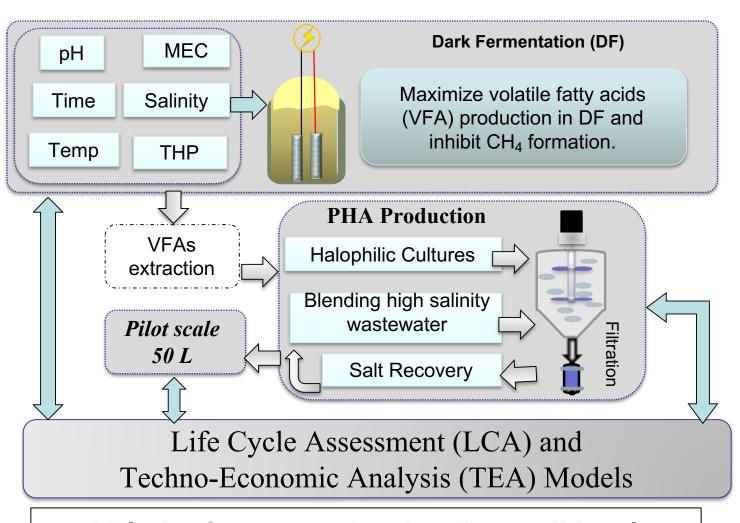
April 7, 2023 Organic Waste Session

Stephanie Lansing, Professor University of Maryland

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Project Overview

- 1) Initial verification (May 2021)
- 2) Maximize VFA production from waste for bioplastic polyhydroxyalkanoates (PHAs) formation.
- 3) Increase production of PHAs, specifically PHBVs, and enhance PHA separation using pure halophilic cultures and compare to PHA production from mixed cultures with enhanced PHA separation and salt recovery.
- 4) Incorporate techno-economic analysis (TEA) and life cycle assessment (LCA) into each process step.
- 5) Design a continuous reactor system (50 L) maximizing VFA production from food waste to produce PHA, while inhibiting CH₄ formation, for >100 hrs for each test.



Valorize food waste by shunting traditional anaerobic digestion processes and creating PHAs.

Project Overview: University of Maryland (Task 2)



Stephanie Lansing, PhD

- Professor
- Associate Department Chair
- UMD Research Leader Fellow

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Amro Hassanein, PhD

- Assistant Research Scientist
- Extension specialist

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- Expert in digestion and fermentation
- Microbial electrolysis and fuel cells
- Food resiliency and FEW nexus
- Life cycle assessments and Extension

- Expert in digestion and dark fermentation.
- Bio-hydrogen and electrochemical systems
- Life cycle assessments and nanotechnology
- Extension work in waste-based bioenergy



Naresh Kumar Amradi, PhD

- Postdoc Research Associate nareshka@umd.edu
- Expert in digestion and fermentation
- Biopolymer production
- Renewable chemicals and waste management

Project Overview: Virginia Tech University (Task 3)



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- Assistant Professor
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Xueyao Zhang

- PhD student
- B.S. and M.S. in Environmental Engineering

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- Expert in bioplastics and biogas fermentation
- Wastewater treatment and reuse
- Nutrient removal and recovery
- Solid waste management
- Bioprocess optimization

- Wastewater treatment
- Pure and mixed culture cultivation
- Graduated with B.S. and M.S. from Beijing University of Civil Engineering and Architecture in 2018 and University of Southern California in 2020

Project Overview: Idaho National Laboratory (Task 4)



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- Membrane separations
- Zero-liquid discharge
- Carbon capture and utilization
- Electrochemistry
- Techno-economic and life-cycle analysis

- Bioenergy feedstock handling and logistics
- Biomass preprocessing
- Microbial metabolism and biochemistry
- Enzymology
- Biomass characterization

Project Overview: Naval Research Laboratory (Task 2)



Matthew D. Yates, PhD

- Research Biologist
- Environmental Engineer
- Microbial electrochemical technologies and carbon

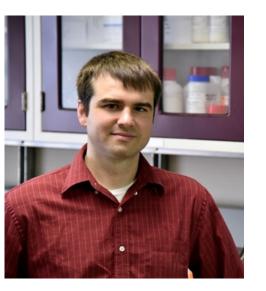
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- Research Biologist
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Brian Eddie, PhD

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- Research Chemist
- Characterizing materials
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Project Overview: Quasar Energy Group (Task 5)



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 Chief Innovation and Science Director
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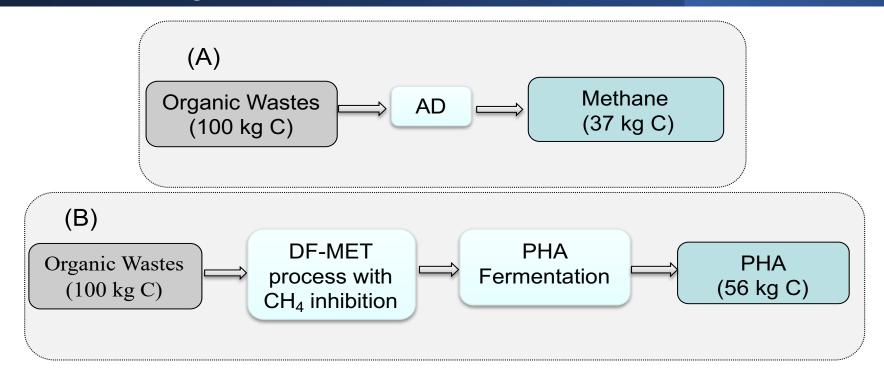
Xumeng Ge, PhD;

Lab Directorxge@quasareg.com

- 20+ years of experience in waste treatment and bioenergy and biomaterial production
- 100+ peer reviewed journal articles
- Editor of book series on Advances in Bioenergy, Elsevier

- 12+ years of experience in bioenergy and waste management
- 60+ peer reviewed journal articles
- Committee member for ASABE M-113 Engineering Concept of the Year

Project Overview: Baseline



- Project goal: increase the value (by 25%) and carbon conversion (by 50%) from 37% C conversion from anaerobic digestion (AD) of food waste through bioplastics (PHA) formation
- PHA production will displace CO₂ emissions of plastic manufacturing (3.8 kgCO₂.kg_{Plastic}⁻¹) and create a value-added product.

Project Overview: Relevance to BETO Goals

This project works towards BETO's goal to valorize wet organic waste streams, such as food waste, as potential feedstocks for the bioeconomy with the added benefit of managing wastes locally and creating processing systems for these materials at the sites where wastes are gathered, resulting in a profit from waste with increased sustainability.

Task Summary: Evaluate dark fermentation (DF) conditions (time, temperature, pH, and salinity), pretreatment (thermal hydrolysis) and adding MECs to increase VFAs and decrease CH₄ formation.

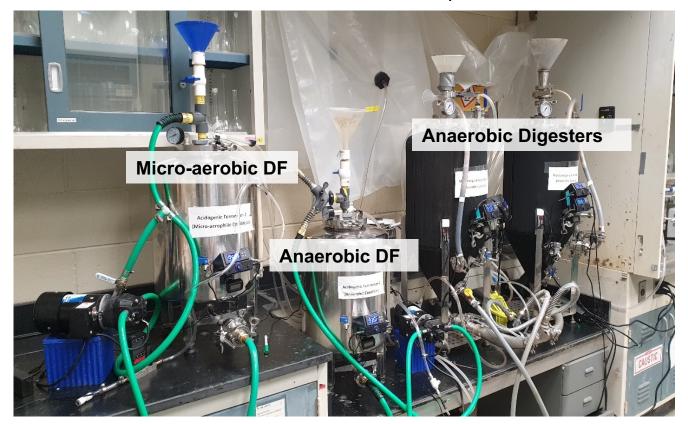


Fig 1: Semi-continuous dark fermentation reactors at UMD

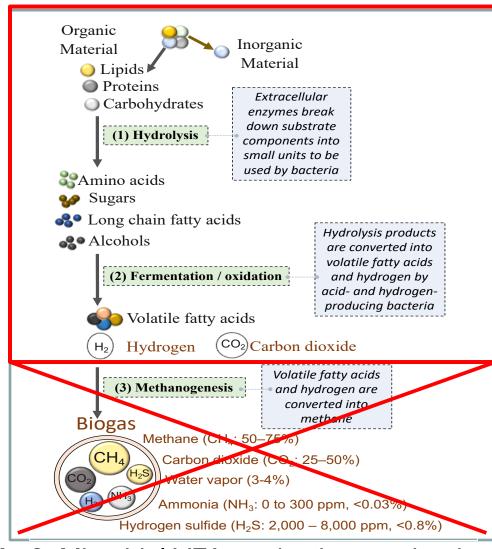


Fig 2: Microbial VFA production mechanism

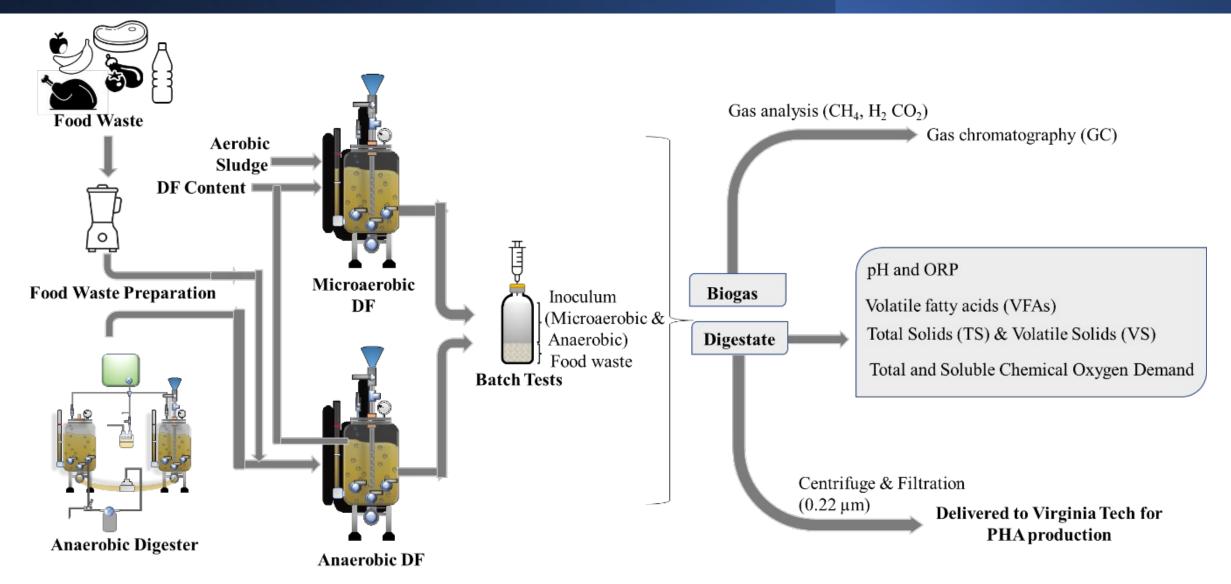


Fig 3: Overall experimental design for dark fermentation (Task 2)

Conducted seven round (193 triplicate reactors) of batch experiments to optimize VFA production; operated two (37.8 L) semi-continuous reactors for 438 and 367 days, respectively.

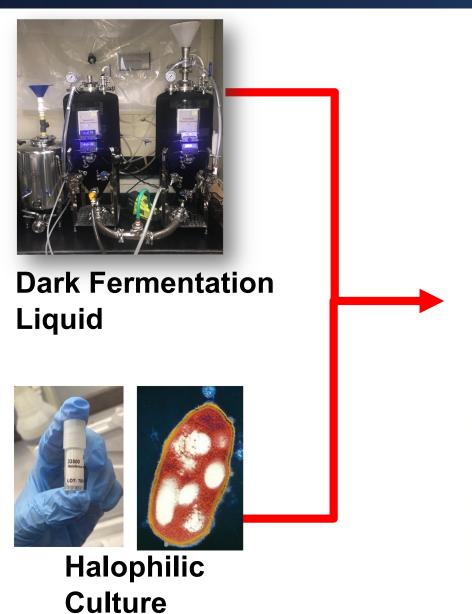
Variables included:

- 1. Anaerobic and microaerobic microenvironments
- 2. Food waste and high salinity food waste as substrates
- 3. Organic loading rate
- 4. pH adjustment (5.5, 7.0, and 10)
- 5. Residence time (0 to 20 days)
- 6. Thermohydrolysis pretreatment (120 and 150 °C for 15 and 30 min)
- 7. Reactor volumes (250 mL, 500 mL, and 37.8 L).
- 8. Temperature effect (35°C and 55°C)
- 9. Effect of salinity in food waste (0 to 150 g/L NaCl)
- 10. Residual solids for VFA production (collected after VFA filtration)
- 11. Residual solids for methane production (collected after VFA filtration)





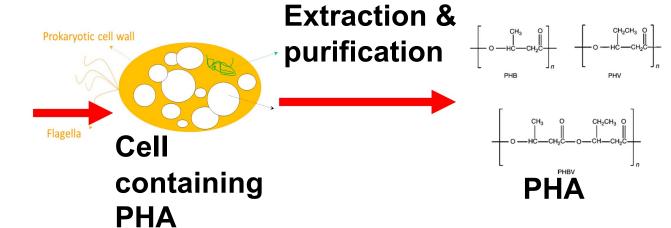
Fig 4: Food waste and batch reactors

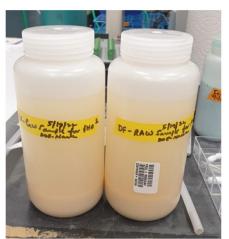






Task 3: Verify PHA fermentation using pure halophilic cultures (*HM*). Determine PHA recovery process, use high salinity waste, and recover salt.





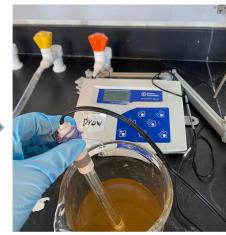
UMD DF liquid



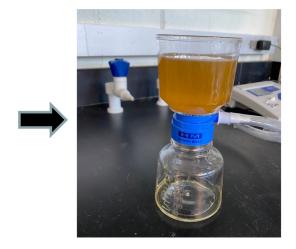
Centrifuge: keep supernatant



Mix macro & micro nutrients, NaCl, and fermented waste



pH adjustment



0.22μm filtration



Inoculation in biosafety cabinet



Training Co.

Batch and Sequencing batch reactors

Conducted eight rounds (59 different mediums) of batch experiments at 37 °C to optimize parameters for *HM* growth and PHA production.

Variables included:

- 1. Medium substrate types (synthetic and fermented waste from UMD)
- 2. Salinity concentration (156 and 250 g/L NaCl)
- 3. Initial pH (original pH of fermented waste at 5-6 and adjustment to 7)
- 4. Macronutrient type and concentration (with and without addition)
- 5. Micronutrient type and concentration (yeast extract and SL-6 trace solution)
- 6. Initial COD loading of substrate (dilution factor)
- 7. VFA species type and concentration
- 8. Aeration rate (250ml flask and 500ml flask).
- 9. C:N and C:P ratios

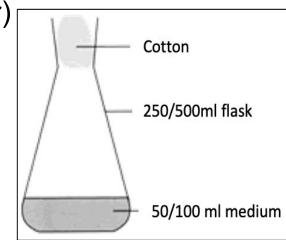
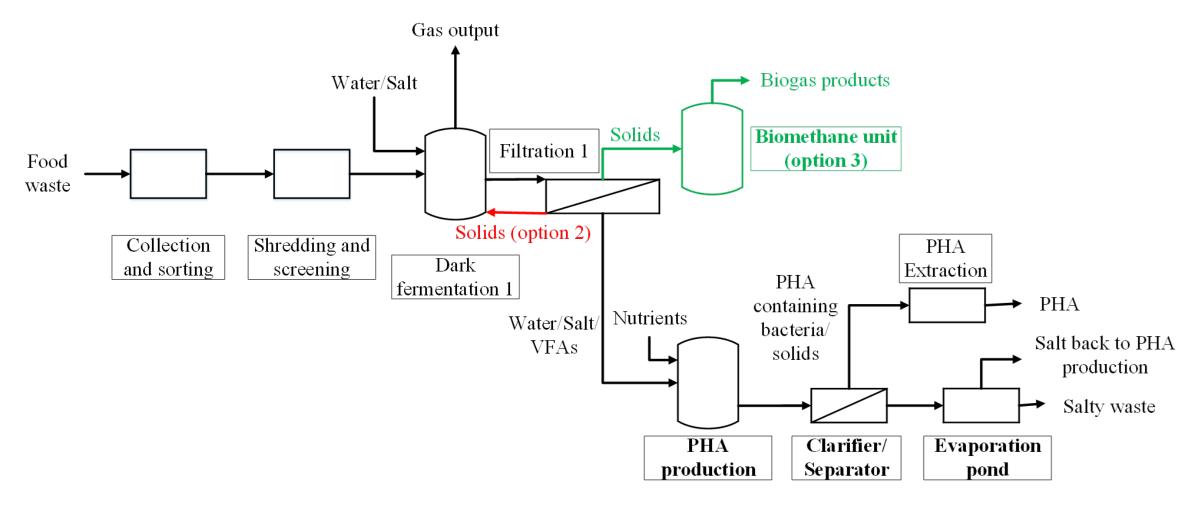




Fig 5: PHA production batch reactors

Conduct TEA/LCA on VFA and PHA production operational parameters with targets of increasing carbon conversion efficiency >25% and reducing disposal cost by 10% after BP2.



INL built an excel-based TEA model

Licensing issue associated with Aspen Technologies

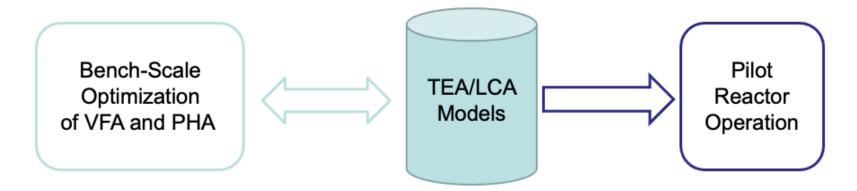
Approach taken for TEA:

- Design each unit operation needed for the process with choice of material
- Choose the place of installation and have installation factor separate
- Project capital-cost required and breakeven cost of PHA production was calculated
- Energy cost (electricity and heating) required for the system was calculated
- 10% Return on investment and 6% annual interest rate assumed

TEA Assumptions

- Model size: 10,000 kg/h of waste-food, with other processing sizes evaluated
- Stainless steel used for continuous stirred tank reactors (CSTR)
- Equipment manufacturing and concrete for the base assumed
- Processing plant life: 20 years

- Approach taken for LCA:
 - GREET database was used for the analysis
- System boundary, specific approaches for each unit operation:
 - Distributed US Mix was the source of electricity
 - Natural gas heat source was considered for heating
 - Stainless-steel Injection Molding database was used for equipment used
 - Plant construction was not considered for the calculations
 - Chemical reaction balance was used to create a database for something that was not available in GREET



System Integration in Pilot Reactor

 Integrate dark fermentation with PHA fermentation for continuous operation for 100 hours at the 50 L scale.

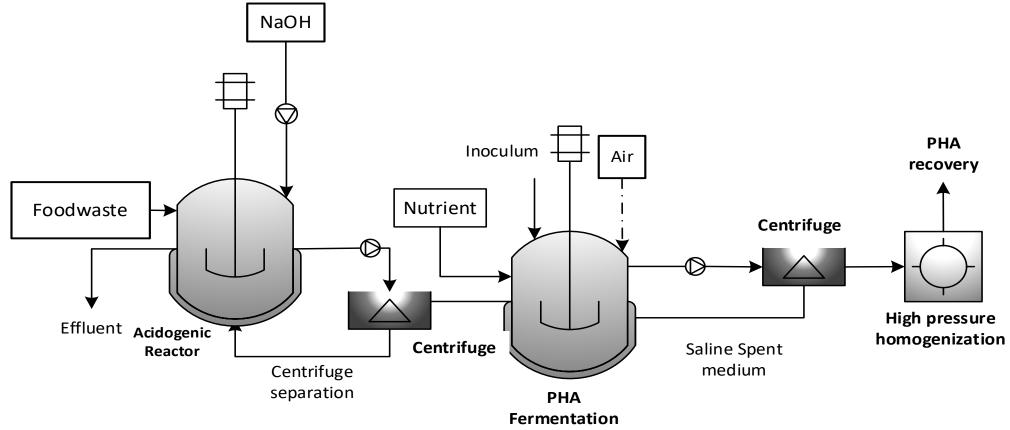


Fig 6: Pilot reactor process diagram

1 – Approach: Challenges Encountered

Challenge: Development of a MEC electroactive biofilm was more difficult than expected for enhanced VFA production.

Solution: Lengthening the enrichment time at the beginning of the experiment and altering the conditions to select for electroactive organisms was conducted.

Challenge: Testing accuracy for high salinity food waste due to interference.

Solution: Instrument change (HPLC for GC); drying time increased for solids.

Challenge: Contamination encountered during PHA cultivation

Solution: More strict sterilization; inoculation from agar plate; 0.22 µm filtration

1 – Approach: Risk Mitigation

Risks	Anticipated Mitigation
pH drop in the DF reactors	pH can be adjusted to 7 with potassium hydroxide
Low VFA Production	Change retention time, organic loading rate, or inoculum
Sampling or analysis error	Analyses performed in triplicates, with statistics applied
Excess solids accumulation in DF reactors	DF reactors could be restarted with new inoculum and use lower organic loadings rates
Culture contamination	A new biosafety cabinet was secured to mitigate this problem, with positive controls used and testing prior to incubation with the singular organism.

1 – Approach: Go and No Go Decisions

Budget Period 2 Go/No-Go Decision Point:

Increased carbon conversion efficiency >25% and decreased waste disposal costs >10% based on LCA/TEA. These metrics will be achieved from a combination of VFA enhancement of >100%, PHA production >30% wt/cell wt with >8% PHBV content, and >90% PHA recovery compared to baseline values.

Critical Decision: Approve Interim Verification (DOE) to begin Pilot scale. The successful Intermediate Verification occurred on Feb 22nd, 2023.

Task 6.0: Final Verification

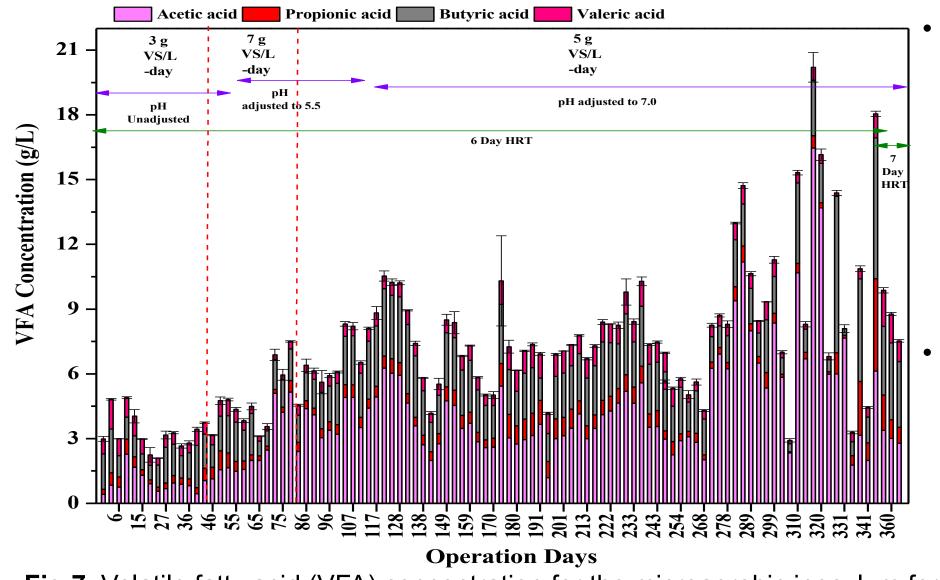
Task Summary: Final verification of the process and deliverables of >50% carbon conversion improvements and >25% decrease in disposal fees.

Budget Period 3 Go/No-Go Decision Point: Final Report Delivered to DOE.

1 – Approach Diversity, Equity, and Inclusion

- Project PI serves as the Chair/Co-Chair of Department and College-level DEIR committees
- Four diverse students working on this project:
 - African American male undergraduate student majoring in Material Science Engineering
 - Latina female undergraduate student majoring in Biochemistry.
 - Latina female high school student intern
 - Female Summer Opportunities in Agricultural Research and Environment (SOARE) student; NSF-funded initiative to give diverse undergraduates research experience
- At UMD, there are 5 female students directly involved in this project, and 1 female at NRL.
- At Virginia Tech, a female PhD student is funded and won the 2022 WaterJAM 2022 Fresh Ideas Young Professional Poster Contest and the 2022 AWWA Student Water Challenge.
- Stakeholders/Advisory Group: MD Env Services (Tomczewski), Hampton Roads Sanitation (Wilson) as well as funded partners at Quasar Energy (Li and Ge)

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- Max VFA (20.2 g/L) in microaerobic DF seed reactor (Day 317); largely acetic acid (16.2 g/L), with VFAs higher than the anaerobic DF reactor (max of 15.2 g/L) but less consistent.
- Maximum FW to VFA conversion (415 g VFAs/kg FW) was reached on Day 320 with OLR of 5 g VS/L-day and pH 7.0.

Fig 7: Volatile fatty acid (VFA) concentration for the microaerobic inoculum fermenter.

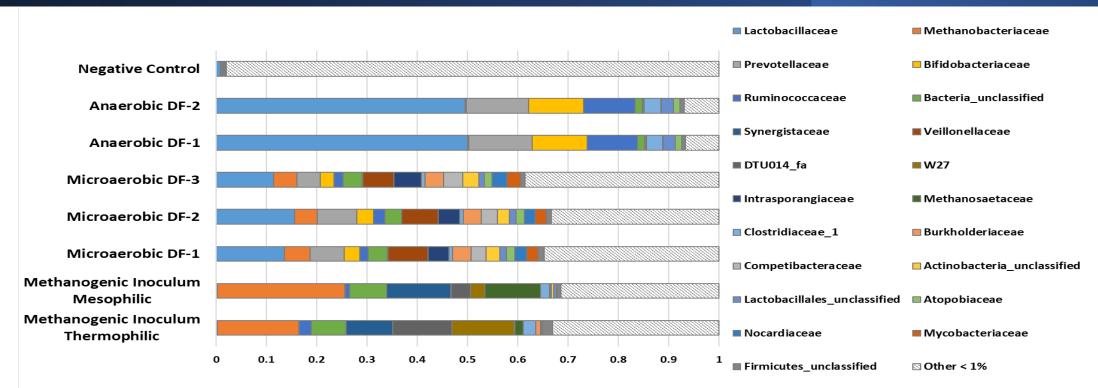


Fig 8. Genus-level breakdown of abundance based upon 16S rRNA amplicon analysis.

- Organisms identified with differences based on reactor type (e.g., methanogens only in the methanogenic reactors and not present in anaerobic and microaerobic DF reactors).
- Low variability between technical replicates indicate that one sample is sufficient to describe the reactor community.

Subtask 2.1: Effect of **temperature and time** on VFA concentration and CH₄ inhibition. **Milestone:** VFA increase of >35% from 1.5 g/L to 2.025 g/L with temperature and optimal time frame established compared to our baseline. **Achieved:** Maximum VFA of 30.4 g/L (1,929% increase from 1.5 g/L baseline) at 55°C over 12 days. No CH₄ production.

Subtask 2.2: Identify the effect of **Thermohydrolysis pretreatment (THP)** on VFA production. **Milestone**: VFA increase >40% from 1.5 g/L to 2.1 g/L VFA. Achieved: THP (120°C for 30 min) had 22.6 g/L VFAs, not statistically different from no THP (18.37 g/L).

Subtask 2.3: Effect of pH and Salinity on VFA production.

Milestone: VFA increase >40% from 1.5 g/L to 2.1 g/L. Achieved: Microaerobic DF had a 1922% increase in VFAs (30.3 g/L) when adjusted to pH 7. High salinity food waste (HSFW) at pH 7 had a 1,558% increase in VFAs (24.9 g/L).

Subtask 2.4: Effect of **MEC** voltages (0.9, 1.5, 3.5 V) on VFA production and CH₄ inhibition. **Milestone:** Increase VFAs >100% 1.5 g/L to 3.0 g/L and identify microbial communities. Achieved: VFAs at 27 g/L with MEC applied; microbial communities identified.

- The highest VFA achieved from all experimental analyses was 30.43 g/L using the microaerobic DF seed with food waste (FW) at an ISR of 1:4 at 55°C for 12-days.
- The pilot reactor operational conditions recommendations:
 - Blend of food waste (FW) and high salinity food waste (HSFW) as substrates
 - Microaerobic DF inoculum used as seed
 - Operate initially at thermophilic temperature (55°C) with 6-day retention time
 - Adjust pH to 7.0; may not be necessary with blended FW (pH 5.5) and HSFW (pH 10).
 - No thermohydrolysis applied and MEC electrodes used initially for surface area only.
- Community analysis protocols and procedures were validated that show distinct communities from different reactors and that a reactor community can be accurately described from one sample.

Subtask 3.1: Halophilic Pure Culture PHA Production (note: PHBV & PHV are PHAs)

- Milestone: 3.1.1: Achieve the cellular PHA content > 30% wt/wt.
- Milestone: 3.1.2: Achieve HV content of PHA > 8% wt/wt.
- Milestone: 3.1.3: Achieve COD (chemical oxygen demand) removal efficiency > 60%.
- Milestone: 3.1.4: Stabilize the halophilic fermenter for more than 60 days

Achieved: Under a 130-day continuous reactor, PHBV max of 65% wt/wt and PHV max of 10% wt/wt, greater than batch testing of 61.09 % wt/wt PHBV, with 72.1% COD removal.

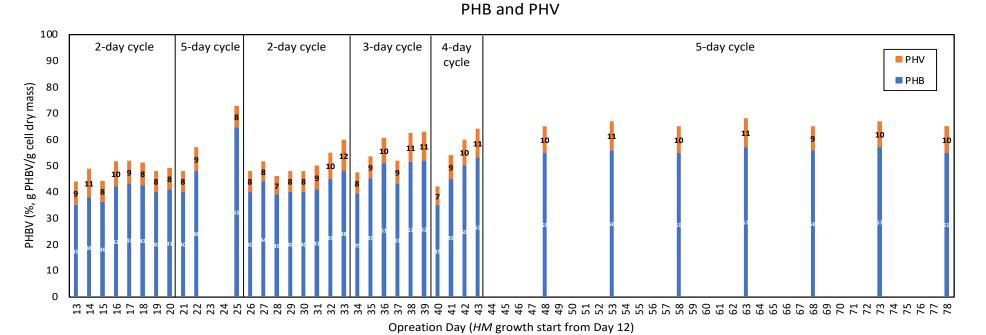


Fig 9: PHB and PHV in continuous reactors

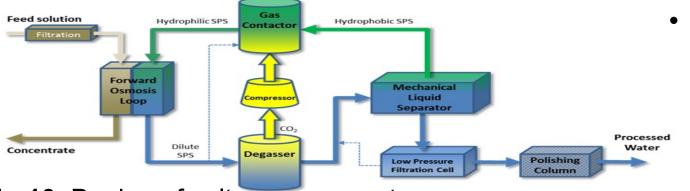
Subtask 3.2: Separation for Salt Recovery

Milestone 3.2.1: Salt recovery of > 50% and purification of PHAs > 90%.

50% of salt is recycled after clarification; 50% sent to evaporation pond for drying

Subtask 3.3: Blend high salinity waste to enhance halophilic PHA production viability Milestone 3.3.1: Displace 10% of salt utilized by adding high strength saline wastewater Milestone 3.3.2: Displace 50% of salt by adding high salinity food waste.

 Toxic compounds in high salinity FW (HSFW) may inhibit HM growth. Blending of FW with HSFW will be used to reduce inhibition, as FW inclusion exceeded our PH production Task 3 milestones and dark fermentation of HSFW was successful in Task 2.



Feasibility study proved membrane separation was infeasible (practically and economically) when NaCl >10 wt%. Current process = 15.5% NaCl.

Fig 10: Design of salt recovery system

Milestone 4.1: Conduct TEA/LCA to compare VFA and PHA production to baseline conditions with targets of increasing carbon conversion efficiency >25% (to 46% from 37% in baseline) and reducing disposal cost by 10%.

Scenario	DF1	DF2	Biogas	\$/kg PHA	kg CO₂/kg PHA	Carbon conversion efficiency (%)
1	Yes	No	No	2.76	5.27	47.9
2	Yes	Yes	No	2.37	4.53	60.6
3	Yes	No	Yes	2.73	5.26	47.9

Selling price, \$/kg PHA	Total revenue (\$K year-1)	Total cost (\$K year-1)	% Profit
1	9,352	21,594	-57
2	18,704	21,594	-13
2.3	21,594	21,594	0
3	28,056	21,594	30
4	37,408	21,594	73
5	46,760	21,594	117
6	56,112	21,594	160

- Carbon conversion was >46% for all PHA scenarios as well as PHA production with digestion of residual solids after VFA extraction.
- Disposal cost: \$4 MM/yr for 10,000 kg/h food waste.
- Our analysis showed there was **NOT** a PHA production cost, but a net revenue generated from PHAs: **\$16.6 MM year**-1

2 – Progress and Outcomes: BP3 and Task 5

The BP3 tasks shown below will begin April 1, 2023 when BP3 begins.

Milestone 2.5.1: Increase C conversion by 50% (from 37% to 56%) and decrease costs >25% through increased VFA production and further identify microbial communities.

Milestone 3.4.1: Achieve a cellular PHA content > 50% wt/wt to increase C conversion >50% and decrease costs >25%, with successful dewatering/salt removal of >40%.

Milestone 4.2.1: Build integrated model and conduct TEA/LCA analyses based on pilot-scale operation and design scenarios to increase economic and carbon conversion viability.

Milestone 5.1.1: Set up and operate pilot scale process capable of PHA production.

Milestone 5.1.2: Increase C conversion >25% and decrease costs >15% in pilot system.

Milestone 5.1.3: Increase C conversion >50% (from 36% to 56% C conversion) and decrease costs >25% in the pilot system.

3 – Impact

IMPACT

- Our PHA production shows a large profit can occur from using food waste (FW), with higher VFA
 production and PHA production efficiencies achieved than previous work in the literature.
- The high profit margin shown could drive the impact of collecting and processing FW, thereby, reducing GHG emissions from FW going in landfills and fossil fuel-based plastic production.
- The high salinity food waste (HSFW), which has limited availability but a high tipping fee, will be
 mixed with residual FW, which with a high availability and lower value.
- Complete integration of industry in our process, including landfill/compost facilities (MES), waste treatment (Hampton Roads), waste to energy facilities that use HSFW, and PHA commercialization process (led by Quasar).
- Numerous presentations have been given and publications are ready for submission from the BP2 phase.

Summary

- State of the art expectations in dark fermentation (DF) has been exceeded, with very high VFA concentrations from converting food waste (FW) to organic acids and then to PHAs, with long-term operation already shown for DF (>1 year) and PHA (130 days).
- A robust bacterial consortium was created for DF, and the use of a novel *Haloferax mediterranei* (*HM*) bacteria was shown to effectively create extractible PHAs.
- This project has already increased the value (by >100%) and carbon conversion (by >45%) of food waste through bioplastics (PHA) formation compared to traditional, low-value anaerobic digestion processes.
- The PHA production was shown to displace CO₂ emissions of plastic manufacturing (3.8 kgCO₂.kg_{Plastic}⁻¹) and food waste emissions and create a value-added product.
- Pilot-scale integration in BP3 will be the first integration of the entire process ever conducted at the 50 L scale over 100 hours of operation.

Quad Chart Overview

Timeline

Project start date: Oct 1, 2020

Project end date: Sept 30, 2024

	FY22 Costed	Total Award
DOE Funding	\$481,688 (UMD- only; does not include federal labs: INL or NRL)	\$1,985,230 (UMD and federal labs)
Project Cost Share	\$174,664	\$496,306

TRL at Project Start: TRL-3 TRL at Project End: TRL-6

Project Goal:

Valorize food waste by shunting the traditional anaerobic digestion process towards value-added bioplastics (polyhydroxyalkanoates [PHAs]) to improve the economics of community-scale systems treating wet organic wastes.

End of Project Milestone:

The project goal is to increase the value by 25% and carbon conversion by 50% (from 36% to 56%) of food waste and mixed organic waste through bioplastics (PHA) formation compared to traditional anaerobic digestion of food waste.

Funding Mechanism

 FY20 Bioenergy Technologies Multi-Topic FOA: DE-FOA-0002203; Subtopic 2b: Optimizing Community-Scale Wet Organic Wastes.

Project Partners

- Funded: Drs. Lansing and Hassanein at University of Maryland (lead);
 Dr. Wang at Virginia Tech University; Drs. Li and Ge at Quasar Energy Group; Drs. Wahlen and Adhikari at Idaho National Lab (INL); Dr. Yates at Naval Research Lab (NRL)
- Non-Funded: Mr. Tomczewski at Maryland Environmental Services (MES); Mr. Wilson at Hampton Roads Sanitation

Additional Slides

Responses to Previous Reviewers' Comments

- The project has not been previously reviewed at at BETO meeting.
- Passed Initial Verification: "met the Go/No-Go decision point by experimentally demonstrating the baseline values for carbon conversion efficiency (36.2 wt% of carbon in food waste to methane) and production of VFAs from food waste plus inoculum equating to 3.9 g VFAs/kg food waste. Baseline disposal costs (\$75/kg) were established using literature." Suggested to "consider potential mass transfer limitations and its implications as reactor geometries."
- Passed Intermediate Verification: "achieved all objectives outlined for BP2 via exceeding most targets, with the exceptions being (1) that the high-salt wastewater displacement of salt supplementation was unsuccessful and (2) the results that MEC inclusion did not significantly increase VFA content relative to controls. Investigations into the source of toxicity from the high-salt FW continue to possibly resolve this hurdle, and the unexpected finding with MEC efficacy, however, offers opportunities to elucidate fundamental questions about where this approach is useful. The verification team recommends a "Go" decision to proceed into BP3."

Publications, Patents, Presentations, and Awards

Presentations

- Amradi, N.K., Hassanein, A., Lansing, S. Biological Conversion of Food Waste to Bioenergy and Bioplastics. AGNR Cornerstone, University of Maryland, Oct 12, 2022.
- Amradi, N.K., Hassanein, A., Lansing, S. Dark Fermentation for Volatile Fatty Acids to Produce Bioplastics from Food Waste. American Ecological Engineering Society Annual Symposium, Baltimore, Maryland. June 20-23, 2022.
- Amradi, N.K., Hassanein, A., Lansing, S. Volatile Fatty Acids and Bioplastic Production from Food Waste using Dark Fermentation. Northeast Agricultural and Biological Engineering Conference (NABEC-2022), Edgewood, Maryland, July 31- August 3, 2022.
- Amradi, N.K., Hassanein, A., Lansing, S. Biological Conversion of Food Waste to Bioenergy and Bioplastics. Postdoc Research Symposium, Maryland, September 23, 2022. Hassanein, A., Lansing, S., Naresh K. Amradi., Innovate Technology: Food Waste to Energy and Value-Added Products. International Conference on Green Climate and Smart Waste Management, Cairo, Egypt, Oct 11-12, 2022.
- Hassanein, A., Lansing, S., Food Resiliency and Circular Economies: Food Waste to Bioenergy and Value-Added Products, Urban Food Security and Food System Resiliency, AGNR Cornerstone, University of Maryland, Maryland. Oct 12, 2022.
- Lansing, S., Hassanein, A., Naresh K. Amradi, N., Wang, Z., Zhang, X., Wahlen, B., Adhikari, B., Yates, M.D., Ge, X., Li, Y. Valorizing Food Waste through Dark Fermentation to Bioplastic Production. Symposium on Biomaterials, Fuels, and Chemicals. Society Industrial Microbiology and Biotechnology (SIMB). New Orleans, LA. May 2 4, 2022.
- Zhang, X., Hassanein, A., Amradi, N.K., Lansing, S., Wang, Z. Potential of Polyhydroxyalkanoate Production from Food Waste by Haloferax Mediterranei. ASABE-American Society of Agricultural and Biological Engineers, Houston, Texas. July 17-20, 2022.

Other:

- Accuweather Prime TV, 2021 (video). Team of researchers studying how to turn trash into energy. Air date: November 21, 2021. Available at: https://www.accuweather.com/en/videos/team-of-researchers-studying-how-to-turn-trash-into-energy/39Gf58k0
- Bacon, Auzinea, 2021 (online article). UMD researchers use food waste to create bioplastics cleaner energy. The University of Maryland Diamondback Newspaper. Print date: December 7, 2021. Available at: https://dbknews.com/2021/12/07/umd-research-food-waste-biofuel-bioplastics/
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