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Final Technical Report (FTR)

Cover Page

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c. *Project Title*	Innovative Polyhydroxyalkanoates (PHA) Production with Microbial Electrochemical Technology (MET)	
d. *Recipient Organization*	University of Maryland	
e. *Project Period*	*Start:* 10/1/2020	*End:* 3/31/2025
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2. Disclaimer:

3. Executive Summary:

The project “Innovative Polyhydroxyalkanoates (PHA) Production with Microbial Electrochemical Technology (MET)” addressed food waste disposal challenges by successfully converting food waste to bioplastics (known as PHAs). The novel process created by our team of researchers from universities, national labs, and industry substantially enhanced overall carbon conversion efficiency of food waste processing (> 50%), while reducing disposal costs (> 25%). The project showed economic viability potential at community scale through pilot-scale demonstration at a relevant scale (50 L reactor volume) with more than 100 hours of PHA production using realistic conditions.

The project goal was to valorize food waste by shunting traditional anaerobic digestion processing and creating a value-added PHA processing route that improves the economics and sustainability of local, community-scale, wet organic waste treatment. First, the food waste undergoes microbial-based, dark fermentation to break down the food to small carbon chains known as volatile fatty acids (VFAs). Instead of microorganisms converting the VFAs into methane using normal anaerobic digestion processing, our innovative process inhibits methane production. This preserves the produced VFAs for extraction and use by a novel *Haloferax mediterranei* (*HM*) archaea, which effectively converts the VFAs to bioplastics. The project added microbial electrochemical cells (MEC) to the dark fermentation process to enhance the VFAs produced and optimize the type of bioplastics formed (see Zhang et al., 2025).

The project overcame past challenges with use of high salinity waste in anaerobic digestion system by creating a pathway for this waste to PHAs, which increases profit margins and decreases the salt content needed for the halophilic *HM* bacteria. The carbon conversion efficiency of transforming of food waste using anaerobic digestion is only 37% with 1.5 g/L of VFA produced. Landfilling food waste has greenhouse gas emissions of 5 kg CO_{2eq}/kg product and a cost of $75/ton. Our novel PHA process had a higher carbon conversion efficiency (56.4%), with 8 to 33 g/L of VFA produced to create PHAs at an efficiency of 55 to 70% PHAs per dry carbon matter. PHA production with high salinity food waste showed large reductions in greenhouse emissions (only 0.26 kg CO_{2eq}/kg product) compared to landfilling at a cost of $33.9/ton PHA produced.

The project exceeded expectations, with our robust bacterial consortium creating high VFA concentrations (> 30 g/L) over long-term operation (> 2 years), exceeding our 3 g/L target. Application of 1.5 V to the fermentation reactor via application of MEC technology increased the production of VFAs (propionic and valeric fractions) desired for PHA production, while also increasing the more desirable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) concentration. The use of a novel *HM* archaea effectively created extractible PHAs, with a maximum PHBV content of 70% wt/wt and 3-hydroxyvalerate (HV) content of 12% wt/wt, exceeding our combined 30% target. We maintained high PHA performance for over a year without contamination (see Zhang et al., 2024), which far exceeded the 60-day target. It was shown that 93% PHA can be recovered using only water-based methods (no solvent use), surpassing the 90% target. Ten iterations of the pilot-scale PHA production validated the lab scale testing results, totaling 2,376 operational hours, well above the 100-hour target. The techno-economic analysis and life cycle assessment showed PHA production can reduce costs through lower transport distances, higher efficiencies, and create a highly marketable bioplastic product that uses food waste; a substrate that is traditionally an environmental burden.

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5. Background:

5.1 Dark Fermentation (DF)

Food waste decomposition in landfills contributes 58% of methane emissions annually from municipal solid waste (USEPA, 2024). Dark fermentation can decompose food waste into small carbon chains, known as volatile fatty acids (VFAs), which can be converted into valuable bioproducts, such as bioplastics (Agnihotri et al., 2022). Food waste is an abundant source of organic material with a high carbon content. Slezak et al. (2017) and Swiatkiewicz et al. (2021) showed that a lower organic loading rate (5 g VS/L-day) based on volatile solids (VS) loading and a longer retention time (10 days) resulted in higher VFA production (22.3 g/L), while higher loading rates (12.1 g VS/L-day) and shorter retention times (4 days) decreases the pH below 5.5 and limited VFA production (9.81 g/L). This previous work used food waste comprised of 44% vegetables, 30% fruits, and 25% other biomaterials (tea, coffee, rice, and pasta). Food waste can be comprised of various amounts of proteins, carbohydrates, lipids, and acidity that impact fermentation conditions for VFA production.

Our study used previous research to establish key parameters to optimize fermentation for food waste, with initial testing based on the average household food waste in the US (Buzby et al., 2014). The food waste comprised of potatoes (19.81%), mixed veggies (19.81%), water (17.65%), white bread (10.67%), apple (7.70%), chicken (6.09%), pork (6.09%), banana (4.57%), cheese (4.57%), and cooked rice (3.04%). Our study tested residence time, temperature, thermohydrolysis (THP), pH, salinity, and microbial electrolysis cell inclusion, which had not previously been tested. Previous studies used batch experiments to determine the optimal conditions for fermentation. Our study progressed prior research by utilizing both batch and semi-continuous experiments at bench-scale (0.5 L), lab (37.8 L), and pilot-scale (100 L) with traditional food waste and industrial high salinity food waste that is traditionally expensive to treat.

Challenges found in previous studies were addressed in our study. For example, mono-fermentation of food waste can cause extreme acidity with high organic loading (Elbeshbishy et al., 2017). Our work progressed these limitations by investigating lower organic loads and co-fermentation with alkaline, high salinity food processing waste to buffer lower pH conditions. Organic waste with high salinity, often derived from food processing and biodiesel by-products, pose additional challenges, such as disruption of cellular osmolarity and reduced microbial diversity (Wang et al., 2023; Zhang et al., 2016). Our study co-fermented traditional food waste with high salinity food waste at various ratios in a semi-continuous bench-scale experiment. Additionally, previous work used sodium chloride (NaCl) to evaluate the effect of salinity on VFA production (Huang et al., 2022; Liu et al., 2017). However, high salinity food processing and biodiesel waste contains additional organic compounds, in addition to salt, which affects the fermentation processes and downstream PHA production. Our study used high salinity industrial organic waste directly to account for both inhibitory organic compounds in the waste and high salinity in our fermentation and downstream PHA production experiments. Overall, our study investigated various pretreatment methods and operating conditions to process household food waste and high salinity food waste different scales and evaluated the efficiency of condition. The results of this study provide a strong foundation for the design and operation for a full-scale system.

5.2 Microbial Electrolysis Cells (MEC)

Other biotechnologies can be incorporated to optimize fermentation output, such as microbial electrochemical technologies (METs). METs utilize microorganisms that can electrochemically interact with (either oxidize or reduce) solid surfaces as part of their metabolic process to derive energy. Different iterations of METs have been developed to produce electricity, hydrogen, and desalinated water, among other products. The incorporation of a microbial electrolysis cell (MEC) into the dark fermentation is particularly interesting because the cathode in the electrolysis cell consumes protons to produce hydrogen (Call and Logan, 2008) or other products, including volatile fatty acids (Batlle-Vilanova et al. 2015), which could enable increased control over the reactor pH to decrease the amount of chemicals required. The addition of an MEC could enable enhanced production of the desired end products of the fermentation process. Further, there has been recent interest in the concept of electro-fermentation (Villanoet al., 2017; Shanthi et al., 2018), whereby application of a voltage to an electrode provides an external electron source or sink to a microorganism (Virdis et al., 2022). That extra oxidizing or reducing power enables the cells to shift the end product of their metabolism toward something potentially more desirable. The combination of dark fermentation and MEC technologies are an intriguing prospect to increase the productivity of fermentation by applying a low potential (ca. 1.5 V) to electrodes integrated into the system. This project was the first to test and prove this concept.

MECs have been incorporated previously into food waste anaerobic digestion processes. For most of these systems, the main goal was to increase the amount of hydrogen (Hassanein et al., 2017; Kim et al., 2024) or biogas production (Wu et al., 2021; Deb et al., 2025; Zhao et al., 2025). One strategy implemented MECs to facilitate an increased consumption of the VFAs produced during hydrolysis, alleviating conditions that inhibit biogas formation (Xiao et al., 2024). Another strategy that has been developed is to use a two-stage process to increase the overall yield of hydrogen from the food waste fermentation by consuming excess VFAs (Sivagurunathan et al., 2018; Magdalena et al., 2023). To date, studies have not operated over long periods of time (> 30 days) while tracking VFA production and changes in VFA profile from a MEC integrated in fermentation to maximize the use of VFAs as an intermediate for downstream conversion to more valuable products, such as PHAs. Previous studies have explored long-term batch testing (Xiao et al. 2024) or short-term, batch-fed testing (Shanthi et al., 2018) utilizing food waste in an anaerobic digester with an integrated MEC. Here, we characterized the effect of integration of a MEC into dark fermentation with a specific focus on tracking VFA content and VFA profile over an extended (60 day) period in fed-batch reactors. Furthermore, the food waste substrate was comprised of a real food mixture with no addition of salts or buffers. Our results indicate that the integrated fermentation-MEC system significantly increased the concentration of VFAs and shifted the profile toward increased propionate and (iso)valerate for the first 30 days of the experiment compared to the control. These results add to the body of literature that supports integration of technologies to optimize conversion of food waste into useful products.

5.3 Polyhydroxyalkanoates (PHA)

PHAs, particularly poly(3-hydroxybutyrate-co-3-hydroxyvalerate) known as PHBVs, have gained global attention as biodegradable bioplastics with mechanical and thermal properties comparable to petroleum-based plastics, yet their widespread commercialization remains constrained by high production costs associated with carbon substrates, sterilization requirements, and solvent-based downstream recovery (Kusuma et al., 2024). Recent advances in bioprocessing have sought to reduce costs by utilizing waste-derived feedstocks and extremophilic microbes such as *Haloferax mediterranei* (*HM*), which naturally accumulate PHBV without requiring 3-hydroxyvalerate (HV) precursors and thrive in hypersaline environments that enable unsterile operation and osmotic lysis-based recovery (Longo et al., 2024; Zhang et al., 2025). Wang and Zhang (2021) and Wang et al. (2022) reported the feasibility of using *HM* and low-cost food waste for PHA production for cost saving. However, most studies were limited to flask-scale, single-batch experiments, reported only modest HV content, and did not identify or mitigate inhibitory compounds present in real food waste.

To build on these foundational studies and overcome their key limitations, this project systematically investigated and addressed the bottlenecks in PHA production from food waste. Prior research by Wang (2022) suggested that undiluted or minimally diluted food waste digestates inhibit microbial growth and PHBV accumulation, but the specific inhibitory compounds remained unidentified. Building on this, we first optimized dilution factors to improve PHA production, then investigated inhibitory compounds and their mitigation. While Ferre-Guell and Winterburn (2018) demonstrated that synthetic propionic acid (C3) and valeric (C5) VFAs enhanced HV content in PHBV, we aimed to extend this strategy to real waste streams by investigating the integration of MEC with aAD as a approach to enrich digestate with C3 and C5 VFAs for subsequent use in *HM*-based PHBV production. In terms of reactor configuration, continuous stirred-tank reactors (CSTRs) have been explored for *HM*-based PHBV production using synthetic feedstocks, as demonstrated by Parroquin-Gonzalez and Winterburn (2023), but typically resulted in low PHBV production, likely due to low substrate concentration. To address these limitations, we adopted a sequencing batch reactor (SBR) configuration using real food waste digestate, which maintains higher substrate concentrations, minimizes operational downtime and facilitates auto-dilution by replacing only a portion of the working volume in each cycle (Li et al., 2014; Yang & Sha, 2019).

Previous studies have investigated downstream processing to reduce the use of costly solvents and the wastewater that results from PHA extraction using solvents. These previous studies indicated the potential for water-based PHA recovery in halophilic systems due to the natural osmotic sensitivity of halophilic cells (Koller et al., 2013). However, these studies often lacked detailed quantification of operating parameters, such as lysis time, centrifugal force, and resulting product quality, making it difficult to assess their industrial applicability. Building on this foundation, this project investigated detailed methods for water-based PHA recovery and evaluated the resulting recovery efficiency and product purity. Given the knowledge gap in scaling food waste-to-bioplastic conversion, this project directly leveraged our lab-scale results to guide the design and operation of a pilot-scale system, with operation of pilot-scale PHA processing over time using real food waste and high salinity processing waste.

5.4 Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA)

There is a greater need than ever for plastics materials that are renewable and recyclable due to the growing concerns over the pollution caused by conventional plastics, such as nano-plastics and PFAS contamination. Conventional petrochemical-based plastics materials cost 0.8-2 $/kg plastics (Gundlapalli & Ganesan, 2025) and emit 3.4 – 5.2 kg CO_2/kg plastics (An et al., 2022), which can be considered a major contributor to greenhouse gas emissions. Bio-based plastic production provides an opportunity to reduce costs and carbon emissions of fossil fuel-based plastic materials that are renewable and biodegradable.

Bioplastics are considered alternatives to petroleum-based plastic materials. PHAs are a promising class of bioplastics that can be produced from bio-based raw materials. (Park et al., 2024) They are widely regarded as renewable, with a high degree of biodegradability and a diverse range of applications. Derived from renewable biological sources like plants, algae, and bacteria, PHAs offer a sustainable alternative to petroleum-based plastics. The production of bioplastics also has a significantly smaller environmental footprint, as they do not rely on fossil fuels, resulting in a reduced carbon footprint (Naser et al., 2021).

However, commercializing bioplastics comes with several challenges. One of the main hurdles is the high cost of production, both in terms of operational and capital expenses. Most bioplastics are priced between $6 and $20 per kilogram, which is substantially higher than the price of conventional plastics (Bohlmann, 2007). This high cost of production remains a significant barrier to the commercialization of bioplastics (Zytner et al., 2023). Furthermore, the resource-intensive nature of the fermentation process used to produce PHAs adds to the high operational costs. Processes like aeration, temperature control, solvent use, and nutrient supply require substantial energy, which can increase both production costs and the overall environmental impact of PHA manufacturing (Leong et al., 2021; Leong et al., 2017).

The sustainability of feedstock also presents a critical issue in PHA production. (Sirohi et al., 2020) Currently, many feedstocks for PHAs are derived from crops, such as sugarcane, corn, and wheat. Large-scale cultivation of these crops can have adverse environmental impacts, including deforestation, soil degradation, and water scarcity (Meereboer et al., 2020). The use of fertilizers and pesticides can further contribute to pollution. Moreover, relying on food crops creates competition between industrial and food markets, raising both costs and ethical concerns (Dietrich et al., 2017).

While alternative feedstocks, such as agricultural waste (e.g., corn stover or rice husks) or industrial byproducts (e.g., glycerol from biodiesel production), show promise, they also present challenges (Pydimalla et al., 2023). These challenges include inconsistent quality and the need for pre-treatment and post-PHA production solvent-based extraction processes. The use of sustainable, non-food-based carbon sources that do not contribute to environmental degradation remains an ongoing challenge.

One potential solution to these issues is the creation of biodegradable, waste-based bioplastics (Yu et al., 2018). By utilizing food waste that would otherwise end up in landfills and produce large amounts of biogas that contribute to atmospheric emissions, this approach can reduce the environmental impact of PHA production. Moreover, it presents an opportunity to improve the cost-efficiency of bioplastics while also mitigating associated carbon emissions.

6. Project Objectives:

The project outcome is marketable bio-based plastics in the form of PHAs and reduced waste to increase the profitability of community-scale treatment facilities. This will be the first effort to create a sustainable PHA production system with full industry integration to process organic wastes and include processing of high-salt food waste, where previous treatment options have been limited. Our sustainable PHA production process combines innovative technologies (dark fermentation and microbial electrolysis cells) with salt recovery to create PHAs from any wet organic waste, with feedback from TEA/LCA models incorporated into each step of the production process. We will produce PHAs from VFAs using the most economical processing methods, purify the PHAs using an innovative approach to cell lysis and product recovery, and prove the technology using continuous processing at the 50 L scale. This project will address PHA production limitations through using *HM* to build a continuous processing system for turning waste into PHAs, with a high fraction of PHBV produced and new PHA extraction techniques that reduce solvent costs and end of life impacts.

This is a novel design process created by our team with specific expertise in food waste to VFAs, MEC inclusion, PHA production, LCA, and TEA feedback. The impact of this work will be increased profitability for community-scale treatment facilities and transforming traditional anaerobic digestion technology into bioplastic productions to maximize beneficial use of waste and reduced fossil fuel use associated with plastics manufacturing. This potential pathway for low cost PHA production will bring new solutions to solving existing challenges faced by the waste industry in handling food waste, including high salinity waste, and the bioplastics industry to find a lower cost process to produce and extract PHAs without contamination concerns and use of expensive solvents, which results treating additional, costly PHA wastewater streams.

Our technology represents an innovative approach to carbon conversion to more valuable bioplastics with less overall CO_2 emissions. Using *HM* as our PHA production platform will enable us to use complex and variable waste sources (including high salinity wastes), which has not been done before at any scale. This novel system offers significant economic, technical, and environmental advantages for industry-generated waste streams and pre-and post-consumer food waste diversion from landfills by: 1) enhancing profitability for industries by increasing value-added products and reducing disposal costs and waste volumes; 2) providing a promising application for sustainable bio-based plastics from waste; 3) promoting potential work and economic opportunities for local communities by providing more jobs. We will improve carbon conversion efficiency by >50% and reduce the disposal costs of the target feedstocks by > 25%.

To date, there has been limited production of PHAs at industrially relevant scales from soluble waste-derived precursors. Considering the high selling price of PHA ($5,000-6,000/ton), one major limiting factor preventing scaled waste-to-PHA processes is that high yield PHA production (70% wt PHA/wt cell) requires pure cultures of wild-type or genetically modified microorganisms (30% wt PHA/wt cell for mixed cultures). Our project will transform the PHA market by tapping into the vast waste resources transformed by dark fermentation. Moreover, the novel method of using halophilic cultures to achieve high PHA yields from fermentation effluents will enable new avenues to handle and valorize high salinity wastewater, a technical challenge for industry, resulting in increased environmental and financial sustainability.

6.1 Summary of Tasks from Statement of Project Objectives (SOPO):

Task 1.0: Initial verification of the carbon conversion efficiency.
Task 1.1 Food waste anaerobic digestion (AD) to produce VFAs using mixed cultures. Carbon conversion (37% C to CH_4) and disposal cost ($75/dry ton) shown.

- *Milestone 1.1.1:* Baseline carbon conversion for AD of food waste (37%), and baseline VFA concentrations in food waste (1.5 g/L) based on previous research.

Task 1.2: PHA Production with mixed cultures. The VFAs (Task 1.1) will be fed into SBRs with activated sludge as mixed culture to produce PHAs with no process controls.

- *Milestone 1.2.1:* Baseline carbon conversion for PHA production indicate cellular PHA content <30% w/w with minor HV content when real waste streams used.

Task 1.3: TEA and LCA for Baseline Costs and Carbon Conversion. TEA/LCA for baseline disposal costs ($75/dry ton) and carbon conversion for AD (37%) presented.

- *Milestone 1.3.1:* Baseline carbon conversion (37% C conversion) and food waste disposal costs ($75/dry ton) developed with AD of food waste being the baseline.

Budget Period 1 Go/No-Go Decision Point: Report Delivered to DOE.

Task 2.0: Maximize Volatile Fatty Acids (VFA) Production in Dark Fermentation. In Months 4-18, we will evaluate DF conditions (fermentation time, temperature, pH, and salinity), pretreatment (thermal hydrolysis) and adding MECs with voltage pulsing to increase VFAs and decrease CH_4 formation.
Subtask 2.1: Residence Time and Temperature: Identify the effect of temperature and time on VFA concentration and methanogenesis inhibition and determine competitive drivers between VFAs and CH_4.

- *Milestone 2.1.1:* VFA increase of >35% from 1.5 g/L to 2.025 g/L to with temperature and optimal time frame established compared to our baseline.

Subtask 2.2: Thermohydrolysis pretreatment (THP): Identify the effect of THP prior to DF on VFA production and CH_4 inhibition.

- *Milestone 2.2.1:* VFA increase >40% from 1.5 g/L to 2.1 g/L.

Subtask 2.3: pH and Salinity Control: Identify the effect of pH and salinity on VFA production and CH_4 inhibition.

- *Milestone 2.3.1:* VFA increase >40% from 1.5 g/L to 2.1 g/L.

Subtask 2.4: MEC Inclusion: Identify the effect of varied voltages (0.9, 1.5, 3.5 V) on VFA production and CH_4 inhibition.

- *Milestone 2.4.1:* Show that inclusion of MEC, temperature, and thermohydrolysis into the system increases VFA content by >50% from 1.5 g/L to 2.5 g/L.
- *Milestone 2.4.2:* Further refinement of applied voltages in complete system will to increase VFAs >100% 1.5 g/L to 3.0 g/L and identify microbial communities.

Task 3.0: PHA Production: Verify PHA fermentation using halophilic cultures compared to mixed cultures. Determine optimal PHA recovery process, source of salinity, and salt recovery systems.
Subtask 3.1: Halophilic Pure Culture PHA Production. The DF leachate produced from Task 2 will be fed to SBRs. The halophilic culture will be inoculated to start the pure culture PHA fermentation. The hydroxybutyrate (HB) and hydroxyvalerate (HV) contents in PHA will be analyzed to assess the PHA production performance.

- *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 removal efficiency > 60%.
- *Milestone: 3.1.4:* Stabilize the halophilic fermenter for more than 60 days.

Subtask 3.2: Separation for Salt Recovery: Economical salt recovery will be important to maintaining salinity in the SBR sufficiently high to avoid microbial contamination.

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

Subtask 3.3: Blending high salinity wastewater to enhance the economic and environmental viability of the halophilic PHA production**:** Blending ratios of high salinity wastewater and DF leachate will be investigated to test its effect on PHA production and requirements for salt separations and reuse technology identified in Subtask 3.2.

- *Milestone 3.3.1*: Displace 10% of salt utilized by adding high strength saline wastewater, while maintaining performance metrics.
- *Milestone 3.3.2:* Displace 50% of salt utilized by adding high strength saline wastewater derived from a food waste source.

Task 4.0: Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA): TEA and LCA analyses will be conducted for Tasks 2 and 3 to determine optimal PHA culture conditions and necessary VFA enhancements.

Subtask 4.1: TEA/LCA for VFA and PHA Generation: The model outputs will be used to determine optimal conditions moving forward in Tasks 2 and 3 and compared to the baseline model (Task 1). We will begin developing ratios for combined PHA-MEC-AD scenarios (further developed in Task 4.2).

- *Milestone 4.1.1:* Conduct TEA/LCA to compare VFA and PHA production to baseline conditions with targets of increasing carbon conversion efficiency >25% and reducing disposal cost by 10%.

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.

Budget Period 3: Pilot-Scale Evaluation with Lab-Scale Contributions.

Subtask 2.5: Optimization of VFA production: Determine pre-treatment and DF conditions for VFA product based at the lab-scale to assist and troubleshoot the pilot-scale system. Complete microbial community analysis of the DF reactor conditions to understand underlying processes. Determine effect of incorporating high-salinity food waste and explore inhibition mechanisms on the mixed microbial community. Clarify the role of MEC inclusion in VFA production increases, including the surface area effect, surface type, and using MEC brush electrodes (poised & unpoised) on VFAs increases.

- *Milestone 2.5.1:* Increase C conversion by 50% from 37% to 56%, with >25% less cost (from $75/dry ton), identify microbial communities.
- *Milestone 2.5.2:* Determine optimal ratios for high salinity food waste blending and account for any microbial inhibitions occurring.

- *Milestone 2.5.3:* Clarify the role of MEC incorporation in VFA production and the effect of the MEC surface.

Subtask 3.4: Optimization of PHA production: Determine optimal conditions to integrate PHA fermentation, with co-product formation from DF; integrate dewatering using coagulants to guide pilot-scale operation; give insights into the lysis mechanism during fresh water PHA extraction, and explore ways to mitigate the inhibitory effect of high salinity food waste inclusion on bacterial survival and PHA production.

- *Milestone 3.4.1:* Achieve a cellular PHA content > 50% wt/wt while increasing carbon conversion >50% and decrease costs >25%, successful dewatering of HM cells to >40% dryness using coagulants, and clarification of lysis mechanism and high salinity food waste inclusion effects on PHA production, and give insights into HM cell lysis mechanism, and explore ways to mitigate high salinity food waste inclusion effects on PHA production.

Subtask 4.2: TEA and LCA for PHA enhancement: A complete process model will be developed to determine optimal product ratios (PHA, H_2, CH_4) based on various input scenarios, including feedstock source characterization, use of high salinity food waste, feedstock disposal fee, co-product market prices, and current/future tariffs and policy changes. The models will be refined based on outputs from Tasks 2.6, 3.4, and 5.1.

- *Milestone 4.2.1:* Conduct cost/benefit analysis of using high-salinity food waste. Techno-economic scenario will take into consideration higher tipping fees available for this waste and its impact on salt demand for PHA fermentation, PHA yield/quality, and salt solution disposal costs.
- *Milestone 4.2.2:* Build an integrated model and conduct TEA/LCA analyses on the operation of pilot-scale reactor and design scenarios to increase economic and carbon conversion viability.

Task 5.0: Pilot-scale Reactor: Design and operate a pilot-scale reactor (50 L) under continuous feed for production of PHAs and test over a period of six months in >100 hour runs and evaluate for: 1) treatment efficiency, 2) PHA, production, 3) energy efficiency, 4) separation and dewatering efficiency, 5) LCA and TEA, with analysis of the effect of both fossil fuel-based and carbon-free energy sources used to supply energy to a theoretical scaled-up system.

Task 5.1 Pilot-scale Reactor: Conduct >10 iterations of co-product formation ratios using triplicate runs of >100 hours. Test for product formation (PHAs). nutrients (N, P), PHA type (PHB, PHBV), gas production and composition (CO_2, H_2, and CH_4), separation and dewatering efficiency. Inputs of carbon-free energy for MEC electricity will be tested to determine effect on LCA and TEA outputs.

- *Milestone 5.1.1:* Set up & operate pilot scale process capable of PHA production.
- *Milestone 5.1.2:* Increase carbon conversion >25% in pilot-scale system and decrease costs >15% in the pilot system at Month 26.
- *Milestone 5.1.3:* Increase carbon conversion >50% (from 36% to 56% C conversion) and decrease costs >25% in the pilot system at Month 33.

Task 6.0: Final Verification: 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.

7. Project Results and Discussion

7.1 Dark Fermentation (DF) and Microbial Electrolysis Cells (MEC)

Task 2.0: Maximize Volatile Fatty Acids (VFA) Production in Dark Fermentation. Evaluate DF conditions (fermentation time, temperature, pH, and salinity), pretreatment (thermal hydrolysis) and adding MECs with voltage pulsing to increase VFAs and decrease CH_4 formation.

Table 7.1.1: Summary of milestones, achieved results, and methodologies for Task 2.0.

Anticipated project outcomes	Achieved results	Methodologies
Milestone 2.1.1*:* VFA increase of >35% from 1.5 g/L to 2.025 g/L to with temperature and optimal time frame established compared to our baseline.	VFAs reached 30.64 g/L in batch trials and 26.4 g/L in semi-continuous trials.	Semi-continuous (2 years) and batch scale (0-9 days) residence time tested. Two temperatures (35 and 55°C) tested at batch scale (0-9 days).
Milestone 2.2.1: VFA increase >40% from 1.5 g/L to 2.1 g/L.	THP resulted in VFAs yields of 21.25 g/L at 150 °C for 30 minutes.	Thermohydrolysis pretreatment (THP) at batch scale (0-9 days) using two THP temperatures (130 and 150°C) and lengths (15 and 30 min).
Milestone 2.3.1: VFA increase >40% from 1.5 g/L to 2.1 g/L.	VFAs increased by 1,900% from 2.1 g/L VFA to 42 g/L in the anaerobic inoculum through high salinity food waste co-fermentation.	pH and salinity testing at batch scale (0-9 days) using unmodified pH values (5.5) and pH of 7 and 10. Salinity levels of 0 – 150 NaCl were tested.
Milestone 2.4.1: Show that inclusion of MEC, temperature, and thermohydrolysis into the system increases VFA content by >50% from 1.5 g/L to 2.5 g/L.	MEC increased VFA 4.3x over the baseline to 6.5 g/L.	Test the effect of varied voltages (1.5 V, OCP, and no electrode) on VFA concentration and profile**.**
Milestone 2.4.2: Further refinement of the applied voltages in the complete system will be used to increase VFAs >100% 1.5 g/L to 3.0 g/L and identify microbial communities.	MEC increased VFA 4.3x over the baseline to 6.5 g/L. Microbial communities were significantly different for aAD with and without electrodes.	Test the effect of varied voltages (1.5 V, OCP, and no electrode) on VFA concentration and profile and identify microbial communities**.**
Milestone 2.5.1: Increase C conversion by 50% from 37% to 56%, with >25% less cost (from $75/dry ton), identify microbial communities.	C conversion from 47.9% to 60.6% with residual solids refermented. Microbial analyses showed domination by *Lactobacillus*.	Microbial community analysis of fermentation reactor operated under different organic loading rates and at different salinities.
Milestone 2.5.2: Determine optimal ratios for high salinity food waste blending and account for any microbial inhibitions occurring.	High salinity food waste (1:0) produced VFAs at 36.04 g/L with no microbial inhibition.	Continuous testing of five high salinity food waste and regular food waste ratios: 1:0, 3:1, 1:1, 1:3, 0:1 over 62 days.
Milestone 2.5.3: Clarify the role of MEC incorporation in VFA production and the effect of the MEC surface.	Application of a voltage to fermentation significantly enhanced odd carbon chain VFAs (2x increase compared to reactors at open circuit potential).	Test the effect of poised and unpoised MEC on VFA production and profile**.**

To accomplish Task 2, anaerobic and microaerobic dark fermenter (DF) reactors (35 L each) were operated for over 2 years in semi-continuous mode at various HRTs (6-12 days). The digestate (7.5 L) was collected twice a week, followed by adding fresh food waste (FW) into the DF reactors to reach the desired organic loading rate (OLR). Each reactor was adjusted to pH 7 weekly using potassium hydroxide. The microaerobic reactor was sparged with O_2 twice per week for 30 minutes (10 - 15 L O_2). The semi-continuous reactors were tested weekly for pH, total solids (TS), volatile solids (VS), VFAs, chemical oxygen demand (COD), biogas quantity, and biogas quality (CO_2, CH_4, and H_2). The reactor contents were used as inoculum for batch tests (Figures 7.1.1 and 7.1.2).

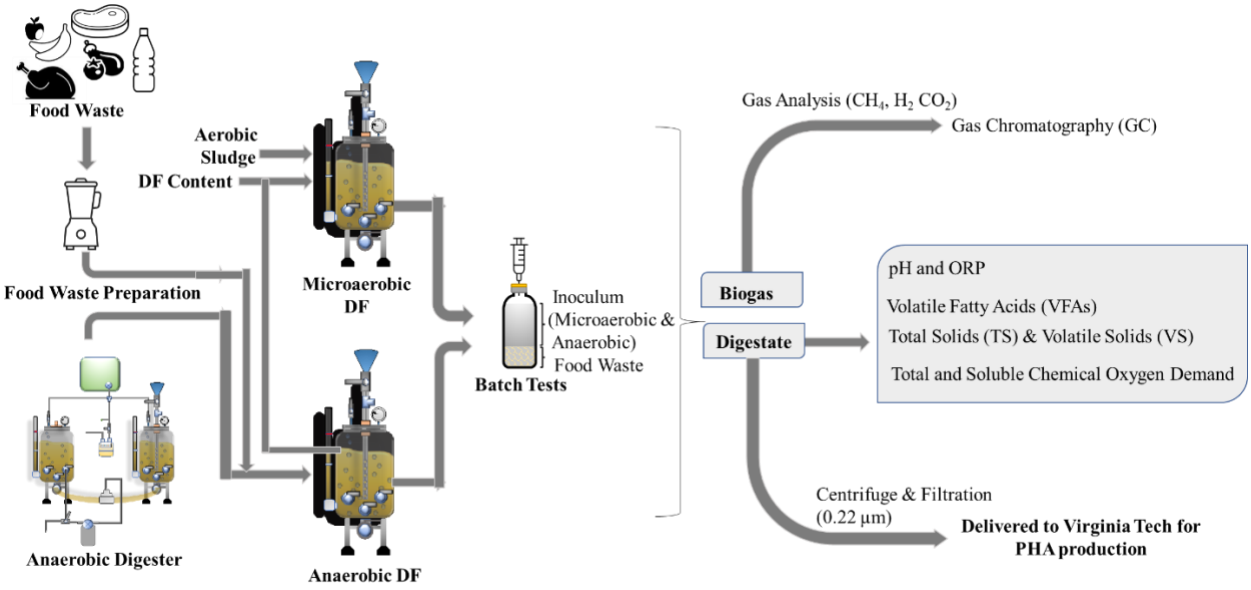


Figure 7.1.1: Anaerobic and microaerobic DF fermentation reactors were tested and used as inoculum in batch experiments to enhance VFA production in Task 2.

Figure 7.1.2: The left steel reactor is the microaerobic dark fermenter (DF), and the right steel reactor is the anaerobic DF, with the black reactors in the background being the anaerobic digestion inoculum reactors used for the initial DF reactor seeding.

Subtask 2.1: Residence Time and Temperature: Identify the effect of temperature and time on VFA concentration and methanogenesis inhibition and determine competitive drivers between VFAs and CH_4.

- **Milestone 2.1.1:** VFA increase of >35% from 1.5 g/L to 2.025 g/L to with temperature and optimal time frame established compared to our baseline.

The semi-continuous trials showed the anaerobic DF reactor (37.8 L) achieved 24.5 g/L VFAs, and microaerobic DF reached 26.4 g/L VFAs when operated at an HRT of 12 days and OLR of 2.5 g VS/L/day. These conditions facilitated acidogenesis for VFA production which inhibited CH_4 production in the fermentation gas.

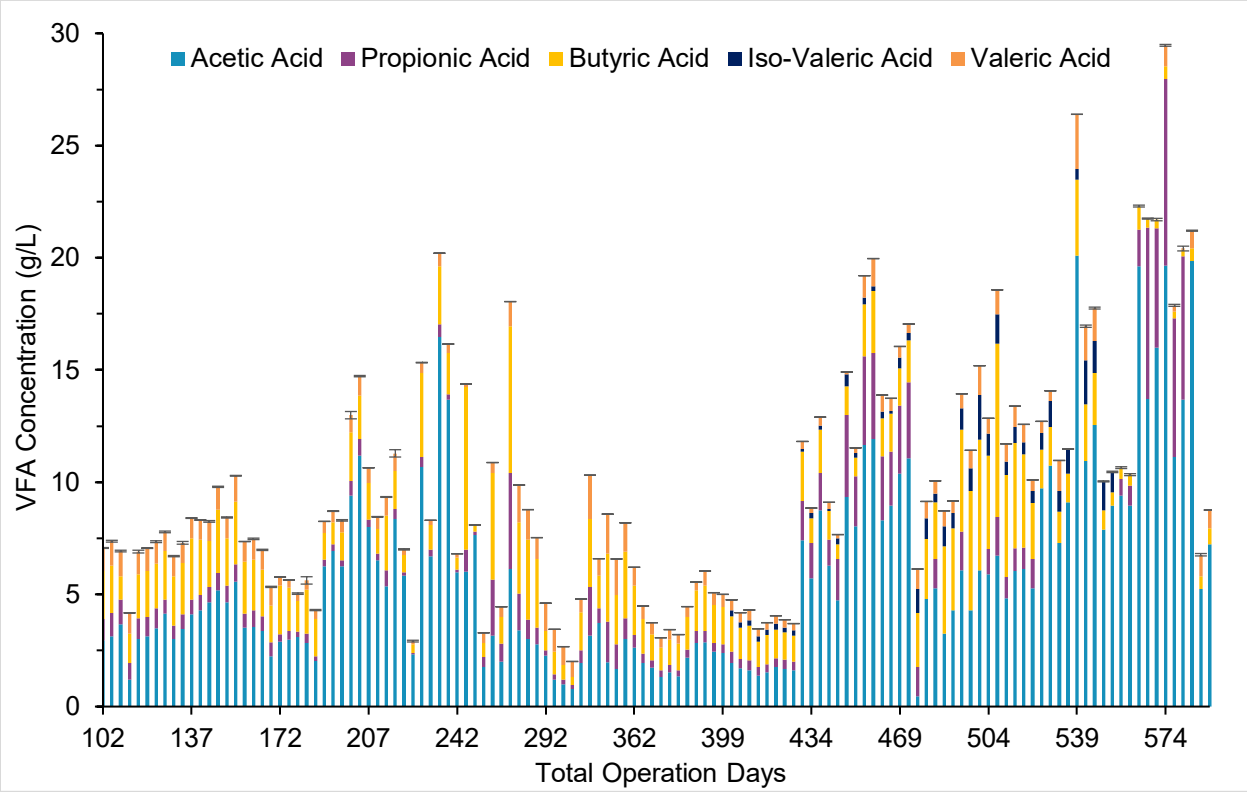


Figure 7.1.3: Anaerobic dark fermentation reactor showing individual and total VFA concentrations from Day 102 to Day 585 at a loading rate of 2.5 g VS/L-day.

The study optimized residence time and temperature in batch experiments to enhance VFA concentrations, which far exceeded the milestone target (2.025 g/L). Batch trials conducted with microaerobic DF inoculum at 55°C for 12-days, and an inoculum-substrate ratio of 1:4, and initial pH of 7 produced a maximum VFA concentration of 30.64 ± 0.48 g/L, which was more than a 1,500% increase from the baseline. Confidence in the results included: calibration and validation of GC and HPLC systems, and statistical analyses (ANOVA, Tukey-Kramer) to confirm significance.

Subtask 2.2: Thermohydrolysis Pretreatment (THP): Identify the effect of THP prior to DF on VFA production and CH_4 inhibition.

- **Milestone 2.2.1:** VFA increase >40% from 1.5 g/L to 2.1 g/L.

The project successfully met this milestone by conducting batch thermal hydrolysis pretreatment (THP) of food waste trials. Food waste was subjected to two temperatures (120,150°C) and treatment durations (15, 30 min) of THP prior to dark fermentation (Figure 7.1.4). The highest VFA concentration (21.25 g/L) was observed with THP conditions of 150°C for 30 minutes, followed by dark fermentation using microaerobic DF inoculum at an inoculum to substrate ratio (ISR) of 1:4 and pH of 6. While the VFA concentration with THP exceeded the milestone (2.1 g/L VFAs), the small increases in VFAs through implementing THP were negated by the large increase in capital costs.

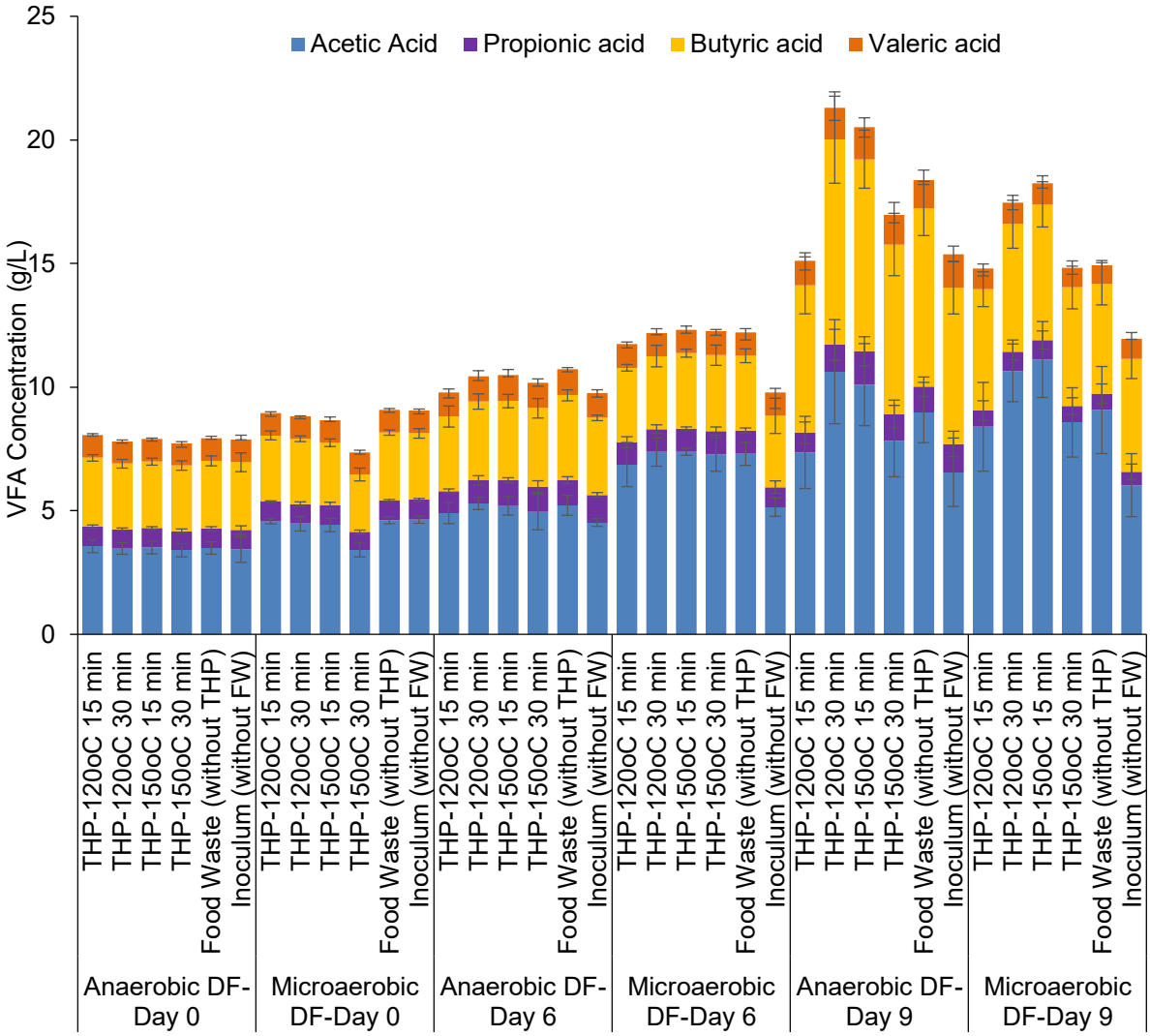


Figure 7.1.4: VFA concentrations for anaerobic and microaerobic fermentation with thermohydrolysis pretreatment (THP) applied at 120°C and 150°C for 15 or 30 minutes, treatments without THP and inoculum only with no additional food waste (FW) added.

Subtask 2.3: pH and Salinity Control: Identify the effect of pH and salinity on VFA production and CH_4 inhibition.

- **Milestone 2.3.1:** VFA increase >40% from 1.5 g/L to 2.1 g/L.

Three batch experiments at 35°C were conducted to determine the effect of pH (unadjusted pH at 5.5, pH 7, and pH 10), salinity (0, 10, 25, 50, 100, and 150 g NaCl), and ISRs (1:0 and 2:1) on VFA production in triplicate, with total VFAs and individual VFAs (acetic, propionic, butyric, and valeric acids) analyzed every three days. The anaerobic and microaerobic dark fermentation were used as inoculum and analyzed individually in separate batch reactors. Milestone 2.3.1 was achieved as the VFAs increased by >40% to > 2.1 g/L. The maximum VFAs achieved were 9.04 ±1.34 g/L with microaerobic inoculum, adjusted pH to 7, at a 6-day HRT and 1:0 ISR. The salinity experiments had increased VFAs. of 1,900% from 2.1 g/L VFA to 42.01 ±0.32 g/L using anaerobic inoculum, 100 g NaCl/L, 1:4 ISR, and a 15-day HRT.

The salinity was also tested using the high salinity food waste (HSFW) supplied by the Quasar Energy group that contained a salinity level of 113 g/L Na^+. Four ISRs (1:4, 1:8, 1:12, 1:16) and three pHs (unadjusted at 10 or adjusted to 7) were tested. The VFAs increased by 1,143% to 24.01 ±1.22 g/L using the microaerobic dark fermentation inoculum, with a 9-day HRT,1:2 ISR, and the pH adjusted to 7 (Figure 7.1.5). There were also high VFAs when the pH started at 10 for the high salinity food waste.

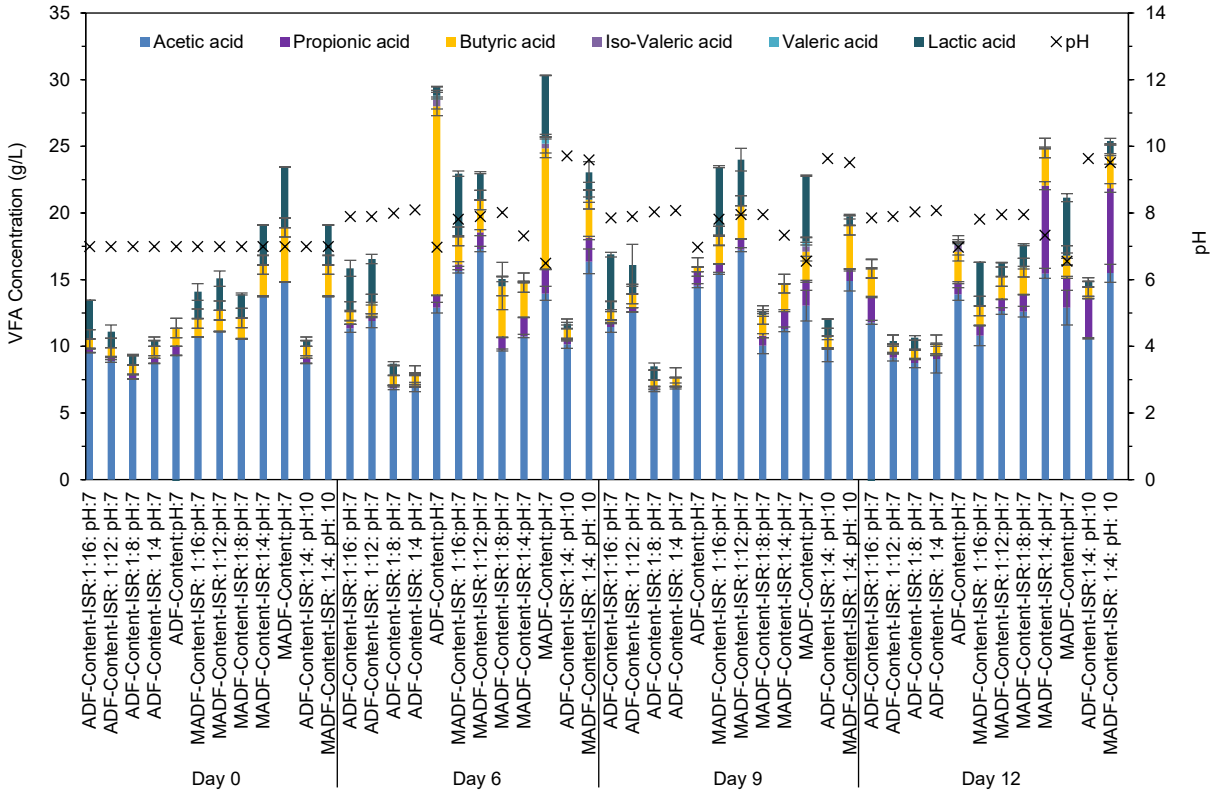


Figure 7.1.5: VFA concentrations for anaerobic dark fermentation (DF) and microaerobic DF contents at different pH control applied at various inoculum-substrate-ratio (ISR) processing high salinity food waste.

Subtask 2.4: MEC Inclusion: Identify the effect of varied voltages (0.9, 1.5, 3.5 V) on VFA production and CH_4 inhibition.

- **Milestone 2.4.1:** Show that inclusion of MEC, temperature, and thermohydrolysis into the system increases VFA content by >50% from 1.5 g/L to 2.5 g/L.
- **Milestone 2.4.2**: Further refinement of the applied voltages in the complete system will be used to increase VFAs >100% 1.5 g/L to 3.0 g/L and identify microbial communities.

To meet this milestone, we incorporated an MEC into fermentation reactor under three conditions of set voltage (1.5 V), open circuit potential (OCP) with no voltage given to the electrodes, and without electrodes as a control. The OCP reactors were operated to test the effect of high surface area in the fermentation process, which meets the reporting requirements for Milestone 2.5.3 below. We combined these data to satisfy both Milestones for conciseness and enable a side-by-side comparison of all data relevant to incorporation of MECs into the fermentation process. We deviated slightly from the milestone and did not test multiple applied voltages. This is due to some issues encountered early on in the project with determining the optimal MEC reactor design and inoculum source. After successfully solving those issues, we determined that time was better spent performing experiments with a sufficiently long duration to assess the technology rather than setting up multiple conditions with a relatively short duration. In either case, we met the milestones here by producing approximately 6.5 g/L VFAs, more than double our proposed milestone (3.0 g/L VFAs).

To assemble the MEC-fermentation reactors, carbon fiber brush anodes and stainless-steel mesh (fastened to titanium wire) cathodes were inserted into triplicate single-chamber 500 mL media bottles through a butyl rubber sheet cut to fit inside the top of a screw cap with a hole in the middle (Figure 7.1.6). Care was taken to ensure that the electrodes did not touch each other upon fixing the electrodes into the cap. The electrodes were held in place by sealing the top of the cap, rubber sheet, and electrodes with epoxy resin (3M Scotchcast resin). The resin viscosity facilitates flow into small potential gaps between electrodes and the rubber stopper. All reactors were continuously stirred at 200 rpm, incubated at 35°C, and tested in triplicate.

Figure 7.1.6. Image of MEC-fermentation reactors. Left to right: No electrode control reactor (NE), open circuit control reactor (OCP) and applied voltage reactor (MEC).

Reactors were inoculated with 400 mL (500 mL total reactor volume) of inoculum taken from the 37.8 L fermenters and using the same food waste substrate. After inoculation, the reactors were equilibrated for 17 days with no feeding to enable formation of a biofilm on the electrode surface of the MEC and OCP reactors. After 14 days of incubation, semi-continuous feeding commenced for 45 days with an OLR of 1.5 g VS/L-day and an HRT of 12 days. Samples were taken for analysis twice weekly. The pH of all MEC-fermentation reactors was adjusted to 7 at the start of each feed cycle with 1 M NaOH. Reactors were operated for 63 days total.

After initialization of semi-continuous operation after the initial enrichment period, the no electrode condition had the highest VFAs. However, within one sampling period the no electrode control had significantly less VFAs than the other two conditions (Figure 7.1.7). The MEC-fermentation reactors produced significantly more volatile fatty acids over the first 35 days of the experiment. From Day 40 to 63, the VFAs produced were not significantly different between any operating conditions. This difference is likely explained by the electrochemical data, which is discussed below. Throughout the experiment, and especially during the second half, the MEC-fermentation reactors had a significantly lower standard deviation than the other two conditions, indicating that the application of a voltage enabled a more consistent output from the reactors.

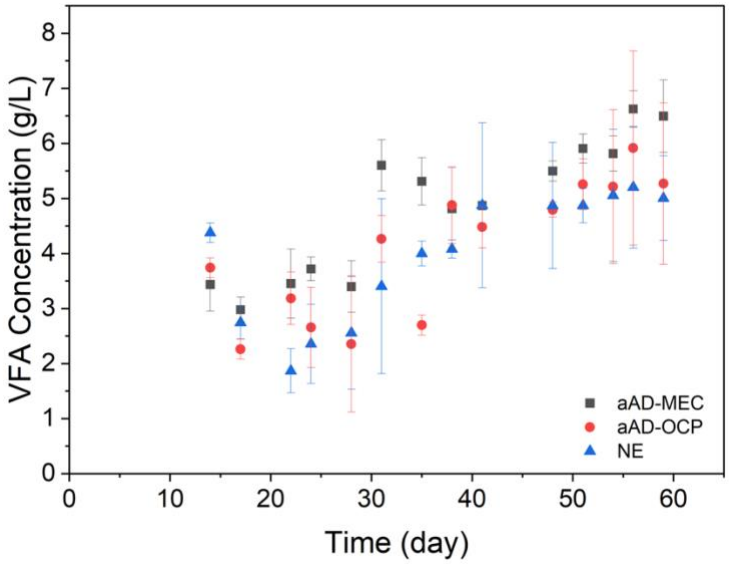


Figure 7.1.7. Volatile fatty acid (VFA) concentration after fermentation, shown as arrested anaerobic digestion (aAD), with a microbial electrolysis cell (MEC) at 1.5 V, open circuit potential (OCP), and no electrode (NE). The aAD-MEC with 1.5 V applied produced significantly more VFAs for the first 35 days.

We analyzed the odd (C3 and C5) carbon chains (propionate and valerate) and even (C2 and C4) chains (acetate and butyrate) throughout the experiment. We aggregated the data in this way, because the odd carbon chain VFAs are more desirable for downstream production of PHBV polymers. These data indicate that the amount of acetate and butyrate consistently rose from approximately 1 g/L to 4 g/L in the reactors over the duration of the experiment. For the first 35 days, the acetate and butyrate contents of the reactor were significantly higher in the MEC-fermentation reactors (shown as arrested anaerobic digestion (aAD-MEC reactors) and aAD-OCP reactors compared to the no electrode (NE) control reactors. By the end of the experiment, the aAD-OCP reactors were not significantly different in acetate and butyrate content than the NE reactors (Figure 7.1.8). The propionate and valerate content in the reactors

remained similar (~1.4 g/L) throughout the experiment for the aAD-MEC reactor (Figure 7.1.8). The aAD-OCP and NE reactors had significantly higher variability for the duration of testing. Similar to the acetate and butyrate content and the total VFA content, the propionate and valerate content was significantly greater in the aAD-MEC reactors compared to the aAD-OCP and NE control reactors in the first 35 days of the experiment. After 35 days, no significance was observed between the three conditions.

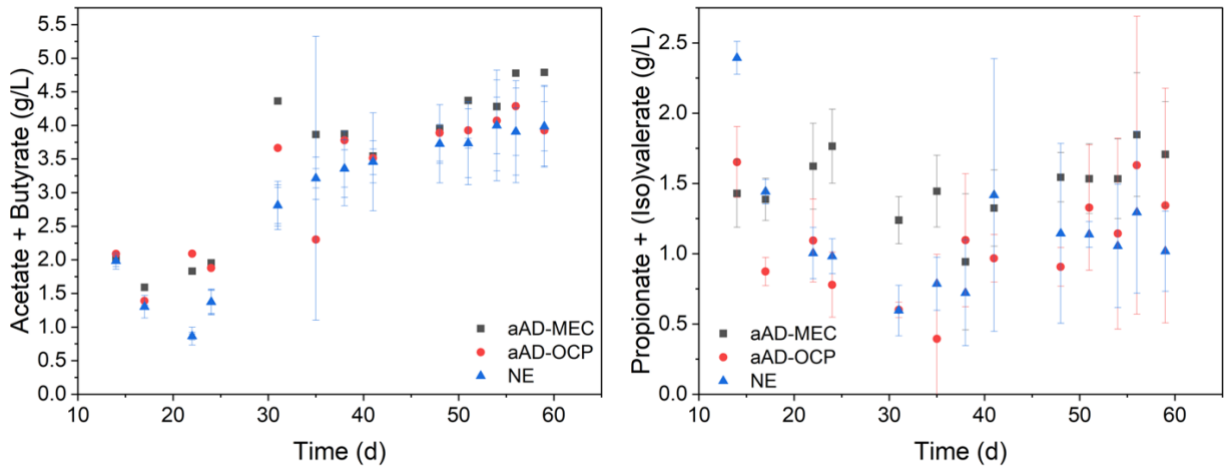


Figure 7.1.8. The combined acetate and butyrate concentrations (left) and combined propionate and valerate concentrations (right) from the reactors. The data show higher propionate and valerate concentration in the aAD-MEC reactors for the first 35 days.

The pH of the reactors was monitored over time and showed a consistently higher pH in the aAD-MEC reactor before each feed compared to the aAD-OCP and NE reactors (Figure 7.1.9). Interestingly, after 35 days, the pH values for all reactor conditions were statistically insignificant. The total and volatile solids of the aAD-reactors was similar to the other two conditions (Figure 7.1.10) for the first 35 days and significantly lower after, indicating that there was higher microbial activity in those conditions or a microbial community that had a higher metabolic potential and could degrade more substrate types than in the other two reactors.

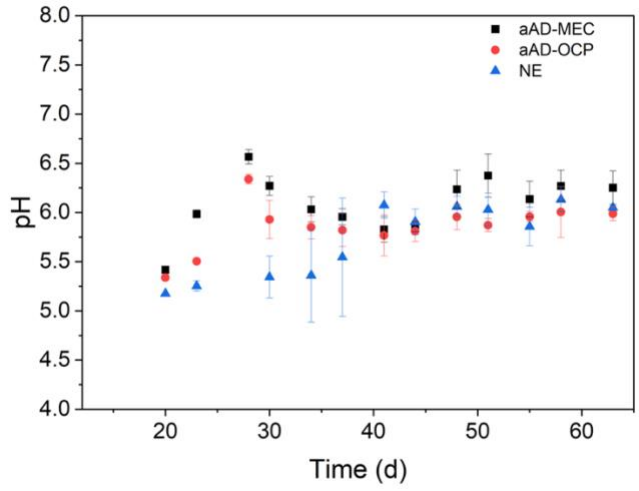


Figure 7.1.9. pH of the reactors over time showing higher pH in the aAD-MEC reactors, particularly over the first 35 days of operation, compared to the reactors without the 1.5 V applied (aAD-OCP) and no electrode (NE) included.

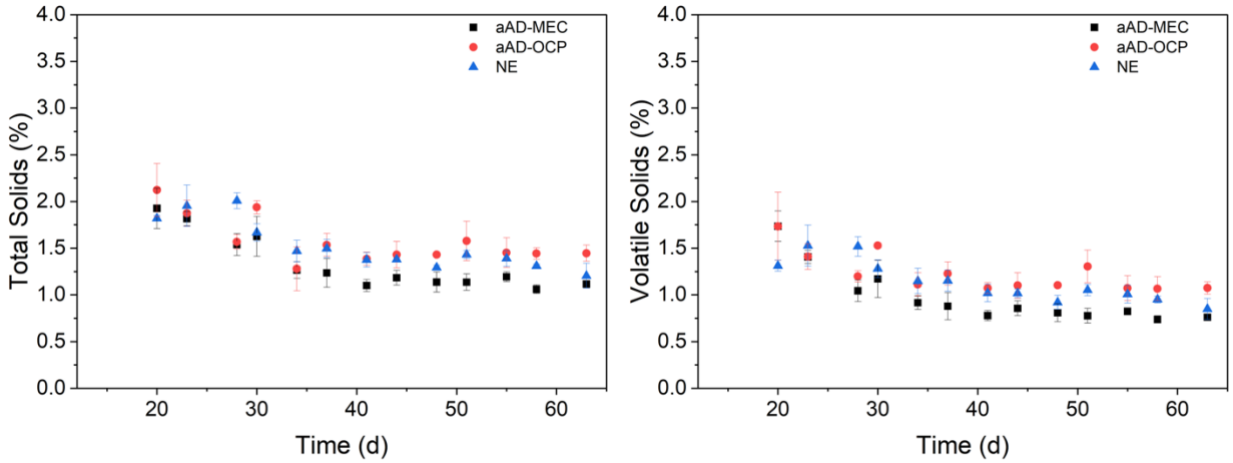


Figure 7.1.10. The total solids (left) and volatile solids (right) and in the reactor effluent.

Microbial community analysis showed the largest difference in the community structure between reactors with no electrode and with electrodes, regardless of whether there was voltage applied (Figure 7.1.11). The reactors with an applied voltage showed differences in proportions of the major organisms present, although the top two organisms were similar. As with all community analysis data, relative abundance may not be necessarily correlated with the most active organisms in a microbial community.

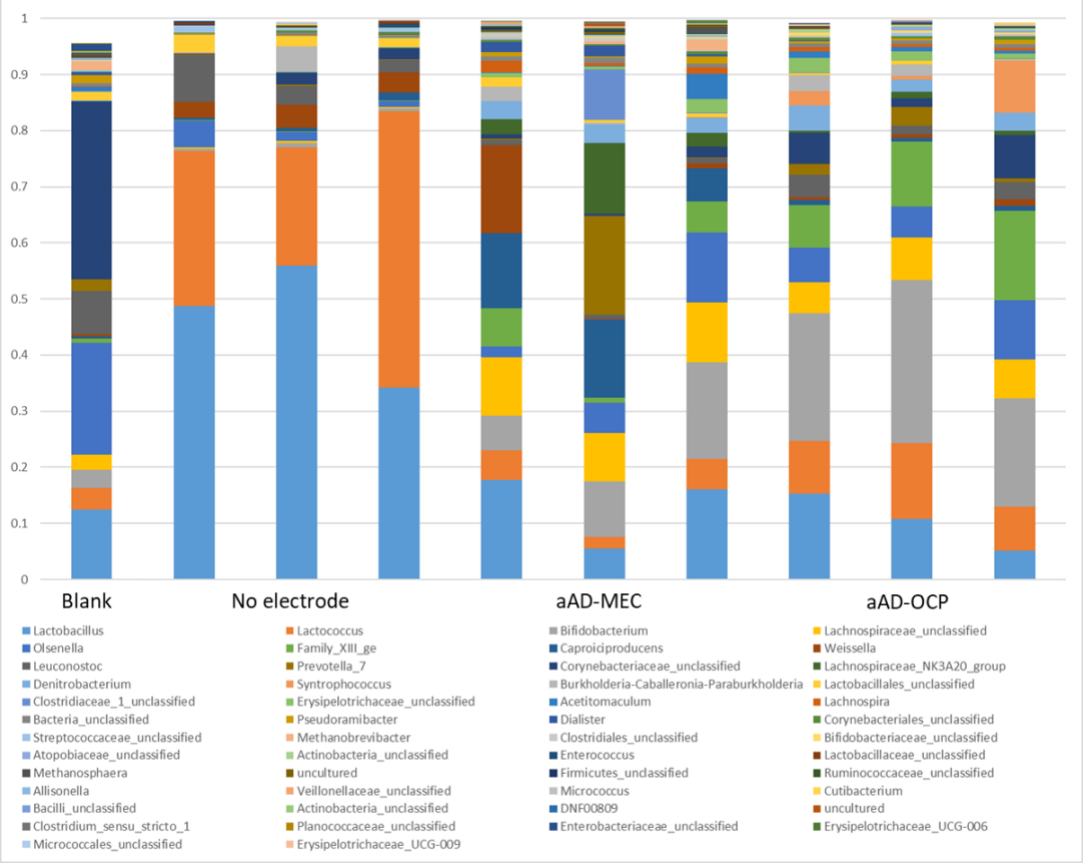


Figure 7.1.11. Microbial communities with no electrode, aAD-MEC, and aAD-OCP reactors compared to a blank (negative control).

In summary, the results here indicate that the fermentation with MEC (shown as aAD-MEC) significantly increased the VFA concentration and profile toward odd-chain carbon VFAs, which is desirable for PHBV production. Further, the application of a voltage was important to maintain performance over only having a high surface area material compared with no voltage applied (OCP). Microbial analyses were completed.

Subtask 2.5: Optimization of VFA production: Determine pre-treatment and DF conditions for VFA product based at the lab-scale to assist and troubleshoot the pilot-scale system. Complete microbial community analysis of the DF reactor conditions to understand underlying processes. Determine effect of incorporating high-salinity food waste and explore inhibition mechanisms on the mixed microbial community. Clarify the role of MEC inclusion in VFA production increases, including the surface area effect, surface type, and using MEC brush electrodes (poised and unpoised) on VFAs increases.

- **Milestone 2.5.1:** Increase C conversion by 50% from 37% to 56%, with >25% less cost (from $75/dry ton), identify microbial communities.

This milestone was achieved through microbial community analyses of the developed anaerobic and microaerobic reactor (35 L) inoculum sources, and batch dark fermentation and anaerobic digestion experiments of the un-fermented solids left behind after VFA extraction. Initial fermentation of food waste results in a VFA-rich liquid used for PHAs, but residual solids from separation processes after initial fermentation results in solids that can be re-fermented or used in anaerobic digestion system for renewable energy production. This study optimized C conversion to increase VFAs and co-production of VFAs with renewable energy from un-fermented solids with integration of the produced fermentation gas. The fermentation gas produced during dark fermentation, mainly comprised of H_2 and CO_2, was injected into the AD reactor headspace at four H_2 to CO_2 ratios (1:1, 1:2, 1:3, 1:5), and the residual solids were fermented at three ISRs (1.5:1, 2:1, 4:1) to determine bioenergy and VFA production rates.

Residual solids after dark fermentation had similar bioenergy potential to unfermented food waste (464 and 435 mL CH_4/g VS, respectively). The highest VFA concentration (28.05 g/L) was produced using residual solids after dark fermentation of food waste at nine days and a ISR of 1.5:1 (Figure 7.1.12). Adding dark fermentation gas to anaerobic digestion reactors decreased CH_4 yields by 77 - 89% (Figure 7.1.13), and therefore it is not recommended to use the fermentation gas in anaerobic digesters.

This work showed high bioenergy and VFA potential from food waste, and the residual solids after VFA separation could be digested to create bioenergy on-site or re-fermented for additional VFA production. The maximum carbon conversion efficiency increased from 47.9% to 60.6% when residual solids were processed in an additional fermentation step, which exceeded the milestone target (56%).

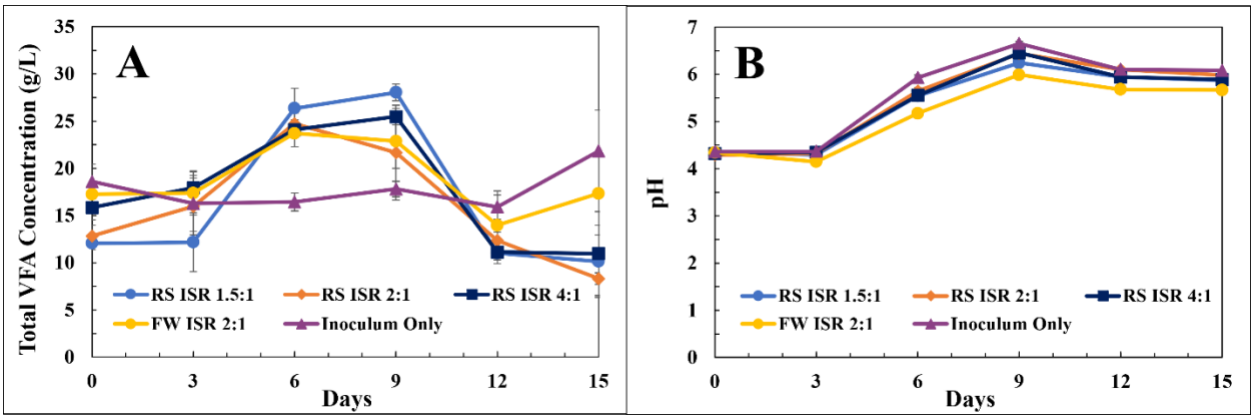


Figure 7.1.12. Total volatile fatty acids (VFA) concentration in (A), and pH in (B) over 15 days of arrested anaerobic digestion (i.e., dark fermentation). Treatments included residual solids (RS) from VFA separation, food waste (FW), inoculum to substrate ratios (ISR) for RS of 1.5:1, 2:1, 4:1, and inoculum-only, with standard error bars of triplicates.

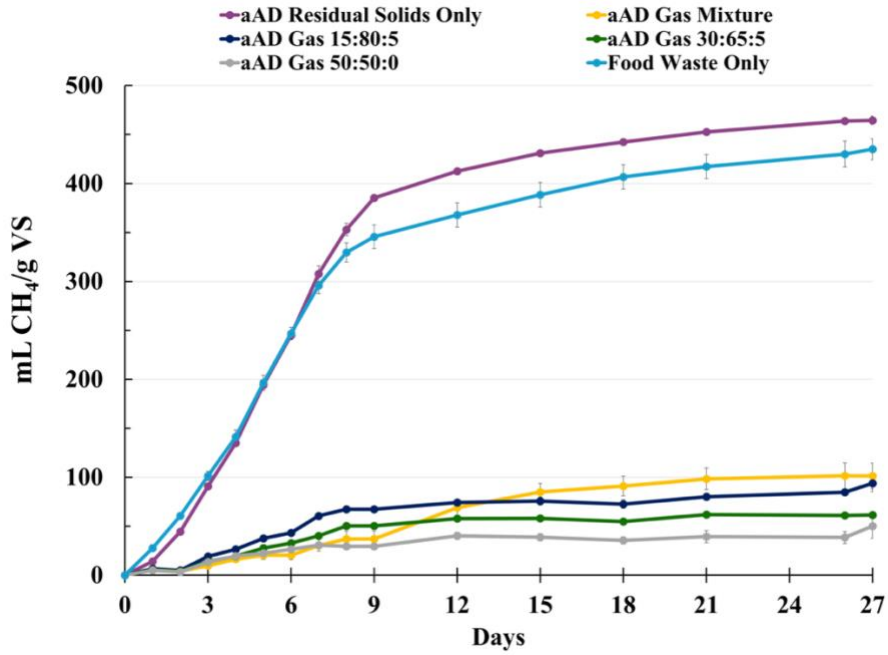


Figure 7.1.13: Methane (CH_4) production based on volatile solids (VS) of food waste and four arrested anaerobic digestion (aAD) gas injections based on ratios of H_2:CO_2:CH_4. All treatments used residual solids. Error bars based on standard error from triplicate reactors.

A microbial community analysis was conducted on the anaerobic and microaerobic fermentation inoculum reactors (35 L reactors operated for 2 years) starting from the inoculum development phase. The anaerobic fermentation inoculum was developed from anaerobic digestion inoculum with low pH shocking to reduce the methanogens present. The microaerobic fermentation inoculum was developed from a combination of anaerobic fermentation inoculum and aerobic wastewater treatment plant inoculum.

Analysis of the microbial community using 16S amplicon sequencing revealed that the proportional abundance of microbes in the anaerobic fermentation inoculum shifted from *Streptococcus* on Days 14 and 26, to *Lactobacillus* on Days 41 to 107, and then

Olsenella on Days 143- 515 (Fig. 7.1.14). The microaerobic fermentation inoculum demonstrated a similar shift from *Lactobacillus* to *Olsenella* but continued to have higher proportions of other bacteria as well such as *Prevotella* and *Family XIII* shown on Day 317 (Fig. 7.1.15). The anaerobic fermentation inoculum was dominated by *Lactobacillus* after inoculum development while the microaerobic fermentation inoculum had higher proportions of other bacteria in addition to *Lactobacillus*.

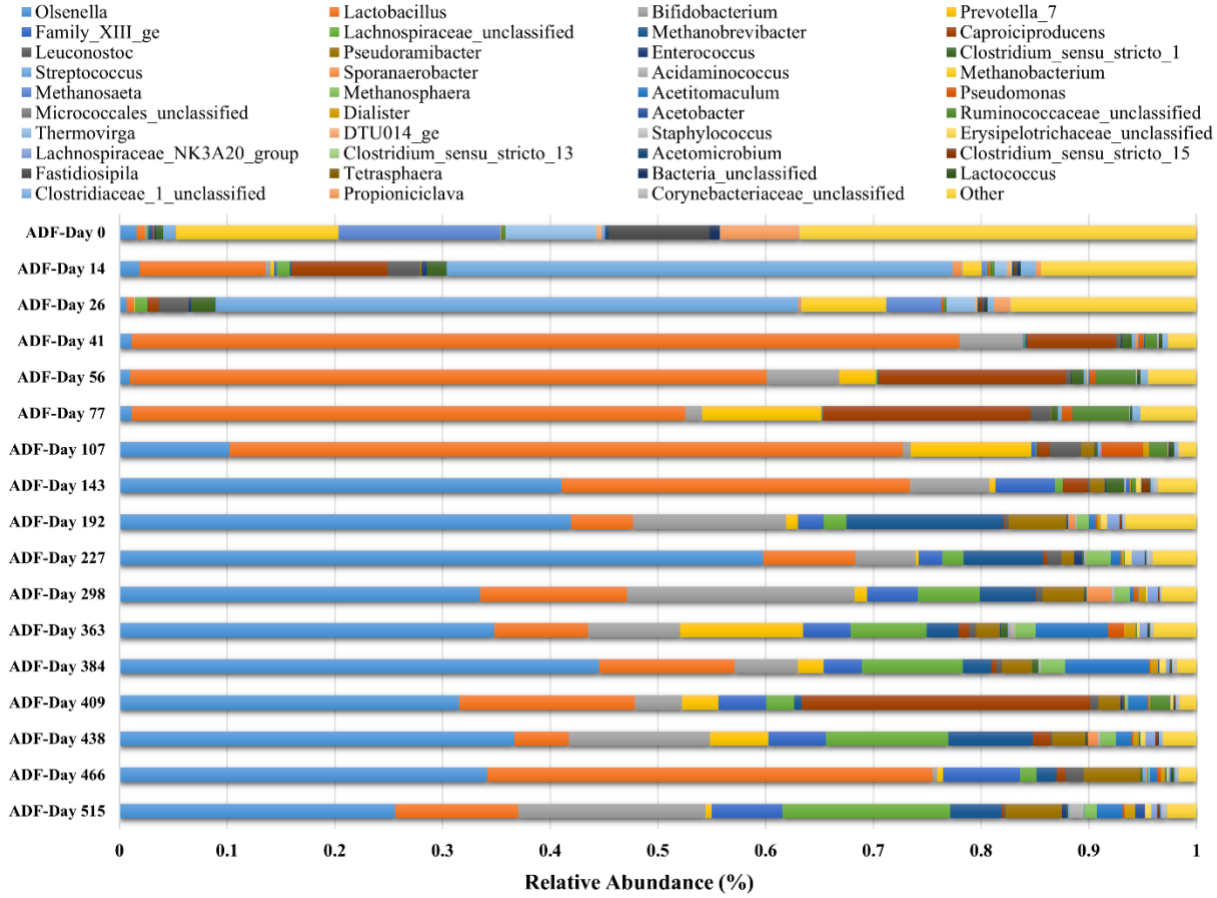


Figure 7.1.14. Community succession in anaerobic dark fermentation (ADF) inoculum samples. Relative abundance of bacteria and archaea in samples as determined by 16S rRNA gene amplicon sequencing. Only the top 39 genera are displayed, with all other identified genera grouped together into the “other” category.

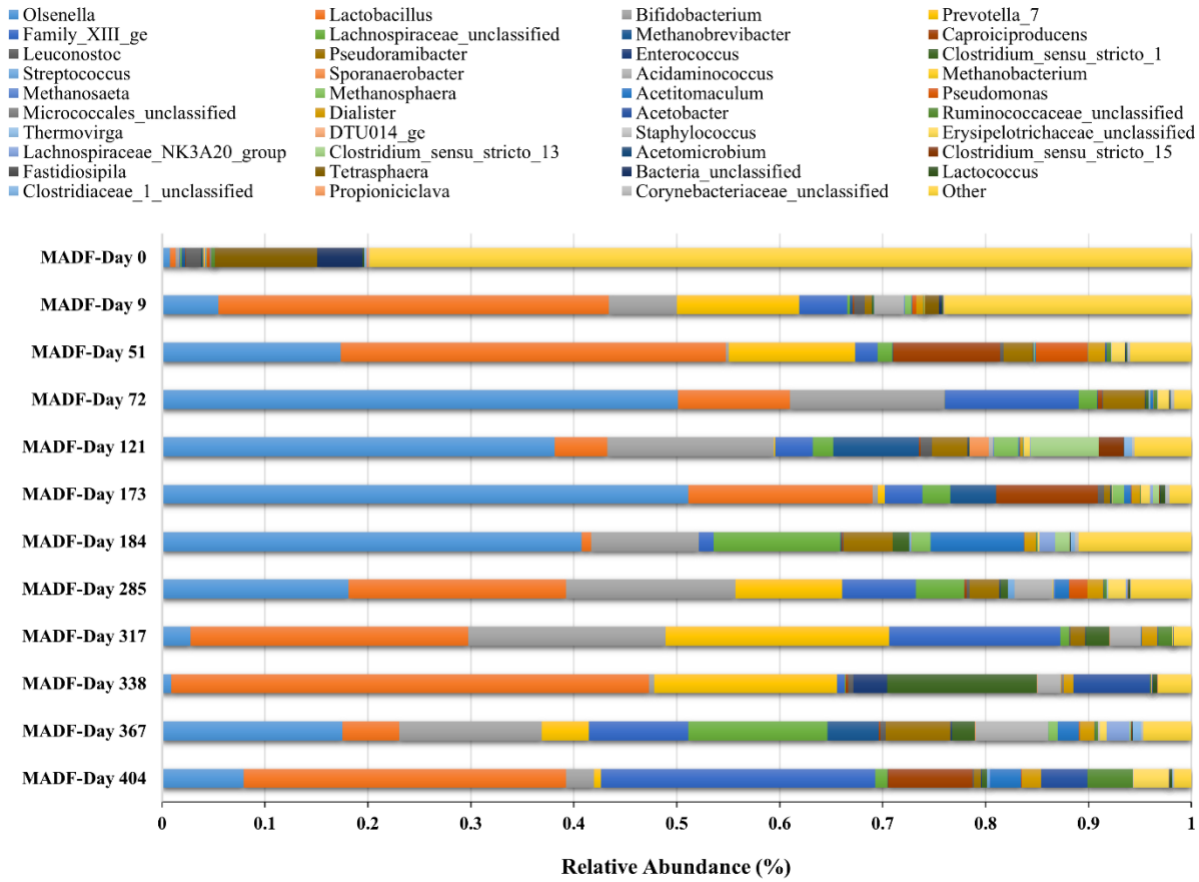


Figure 7.1.15. Community succession in microaerobic dark fermentation (MADF) inoculum samples. Relative abundance of bacteria and archaea in samples as determined by 16S rRNA gene amplicon sequencing. Only the top 39 genera are displayed, with all other identified genera grouped together into the “other” category.

- **Milestone 2.5.2:** Determine optimal ratios for high salinity food waste blending and account for any microbial inhibitions occurring.

This milestone was achieved through a 62-day semi-continuous fermentation experiment that determined the optimal ratio of high salinity food waste (HSFW) to food waste. Food waste and HSFW were tested at five ratios (1:0, 3:1, 1:1, 1:3, 0:1). The alkaline pH (9 – 10) conditions of the HSFW and high organic load (6.3 – 17.8 g VS/L-day) with HSFW inclusion resulted in high VFA production, despite the high salinity (214 g/L NaCl) of the HSFW substrate used. The VFAs produced from mono-aAD of HSGS (36.04 g/L VFAs) were significantly higher than dark fermentation of food waste (9.29 g/L), with the highest VFA concentration observed on Day 51 (Fig. 7.1.16). The results showed there were not any microbial inhibitions when using HSFW.

The FW-only treatment produced the most VFAs per unit of VS loaded during steady state (0.65 – 1.24 g VFA/g VS). The wide range of salinity concentrations (53.5 – 214 g/L NaCl) with FW and HSFW co-fermentation did not significantly influence VFA

production efficiency ($p > 0.05$). The inclusion of HSGS increased VFA production and mitigated acidic inhibition during fermentation. No potassium hydroxide (KOH) additions were needed to buffer acidic conditions during fermentation with HSFW inclusion >50%. KOH additions to keep a neutral pH with a 3:1 FW: HSFW was 95% less than FW-only.

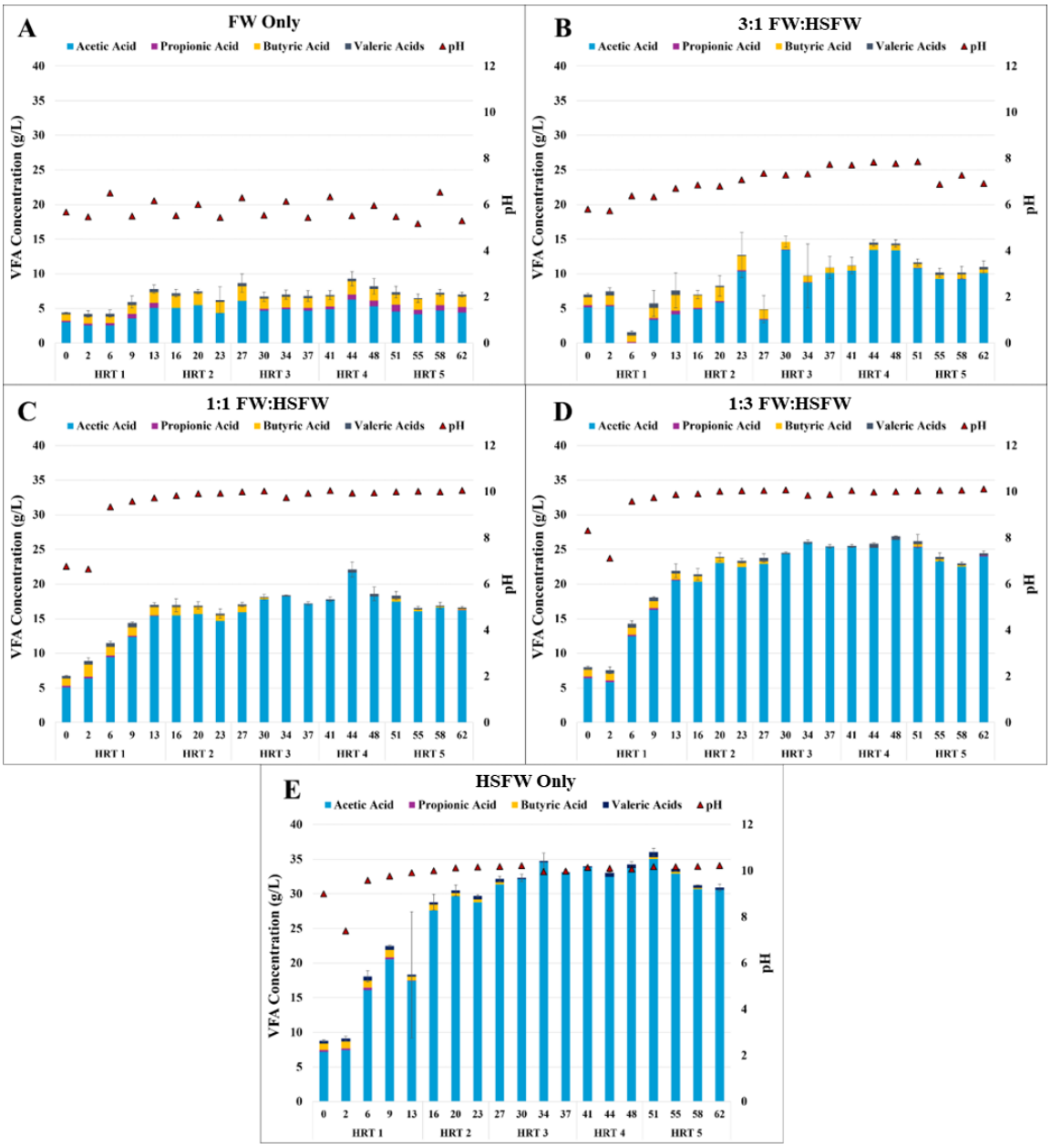


Figure 7.1.16. The composition of volatile fatty acids (VFA) is shown as bars with pH shown as triangles over five hydraulic retention times (HRT). Each HRT was 12 days. The food waste (FW)-only in 'A', high salinity food waste (HSFW)-only in 'B', and three volumetric ratios of FW to HSFW in 'C, D and E', with standard error from triplicates.

HSFW inclusion drove a shift from a community dominated by Olsenella and Methanobrevibacter to communities with higher diversity and a higher proportion of acetic acid-producing fermenters (Fig. 7.1.17). This work showed that HSFW inclusion has high VFA potential and can be used singularly or with FW to increase VFA production from wet organic waste. Milestone 2.5.2 was achieved. The HSFW could be mono-fermented with a maximum of 36.04 g/L VFAs, with no microbial inhibition.

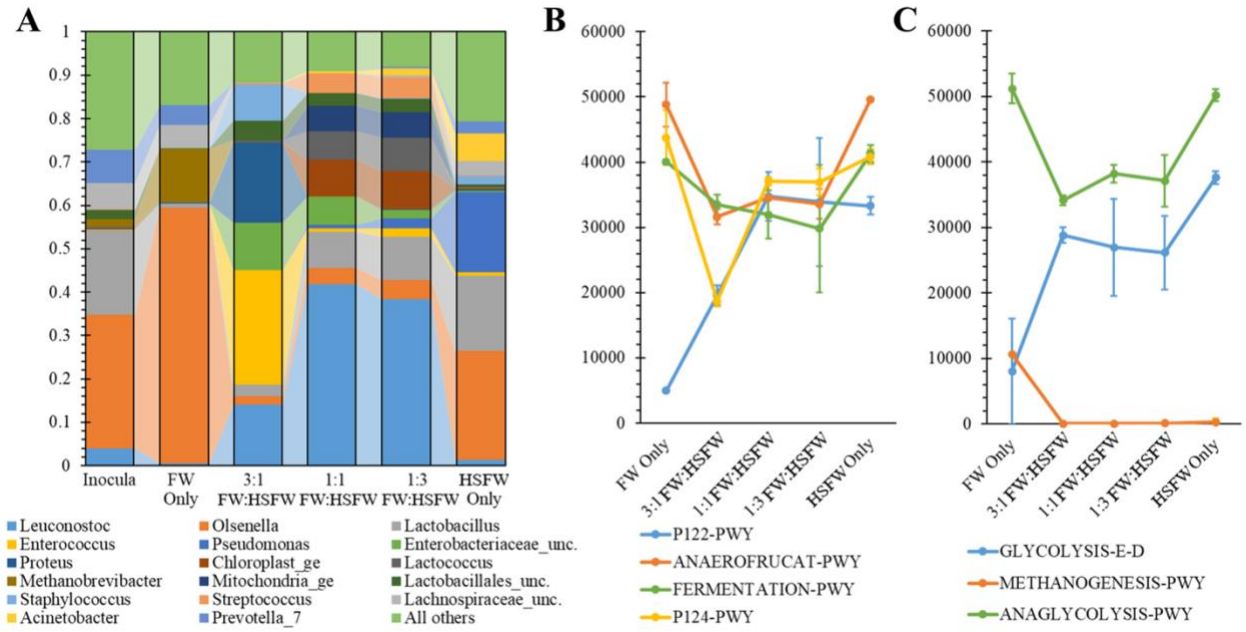


Figure 7.1.17. Microbial community assessment based on relative abundance of 16S rRNA variable region 4 amplicons associated to known genera of bacteria and archaea. The relative abundance of the 17 most abundant microbial genera averaged among the triplicate reactors for each condition shown in 'A.'. FW = food waste, HSFW = high salinity food waste, unc. = unclassified genera, ge = genus. The pathway abundance for major fermentation types predicted by PICRUSt 2.5.2 shown in 'B." MetaCyc pathway identifiers are P122-PWY = heterolactic fermentation, ANAEROFRUCAT-PWY = homolactic fermentation, FERMENTATION-PWY = mixed acid fermentation, P124-PWY = Bfidobacterium shunt fermentation. Units are community wide pathway abundance with standard deviation of triplicate reactors. The central metabolic pathway abundance predicted by PICRUSt 2.5.2 shown in 'C.' GLYCOLYSIS-E-D = Entner-Doudoroff pathway and ANAGLYCOLYSIS-PWY = Anaerobic glycolysis.

- **Milestone 2.5.3:** Clarify the role of MEC incorporation in VFA production and the effect of the MEC surface (M33).

Reporting for this milestone is incorporated into reporting for Milestone 2.4.1 so that all data from related work can be assessed side-by-side. To summarize, addition of a voltage gives a net positive effect on VFA production compared to having a high surface area material in the reactor and no applied voltage.

7.2 Lab-Scale Polyhydroxyalkanoates (PHA) Production

Task 3.0: PHA Production: Verify PHA fermentation using halophilic cultures compared to mixed cultures. Determine optimal PHA recovery process, source of salinity, and salt recovery systems.

A summary of the anticipated project outcomes (milestones), achieved results, and key methodologies under Task 3.0 is presented in Table 7.2.1, with detailed descriptions of each milestone provided in this section below.

Table 7.2.1: Summary of milestones, achieved results, and methodologies for Task 3.0.

Anticipated project outcomes	Achieved results	Methodologies
Milestone 3.1.1: Achieve the cellular PHA content > 30% wt/wt.	70% wt/wt cellular PHA content.	Flask experiment with optimized conditions.
Milestone: 3.1.2: Achieve HV content of PHA > 8% wt/wt.	12% wt/wt HV content of PHA.	MEC-aAD system.
Milestone: 3.1.3: Achieve COD removal efficiency > 60%.	70% TOC removal efficiency.	Flask and SBR.
Milestone: 3.1.4: Stabilize the halophilic fermenter for more than 60 days.	450-day stabilized halophilic fermentation.	Long-term SBR operation.
Milestone 3.2.1: Salt recovery of > 50% and purification of PHAs > 90%.	50-70% salt recovery; 96% PHA purity.	SBR at volume exchange ratios of 0.3-0.5; Water-based lysis followed by ethanol purification.
Milestone 3.3.1: Displace 10% of salt utilized by adding high strength saline wastewater, while maintaining performance metrics.	46% salt displacement.	pH 2 treatment of high salinity wastewater.
Milestone 3.3.2: Displace 50% of salt utilized by adding high strength saline wastewater derived from a food waste source.	46% salt displacement	pH 2 treatment of high salinity wastewater.
Milestone 3.4.1: Achieve a cellular PHA content >50% wt/wt while increasing carbon conversion >50% and decrease costs >25%, successful dewatering of *HM* cells to >40% dryness using coagulants, and clarification of lysis mechanism and high salinity food waste inclusion effects on PHA production, and give insights into cell lysis mechanism, and explore ways to mitigate high salinity food waste inclusion effects on PHA production.	70% wt/wt cellular PHA content; dewatering of 50% dryness; long chain fatty acids are inhibitory compounds; pH 2 treatment can mitigate inhibitory effects.	Flask experiment with optimized conditions; high-speed centrifuge followed by belt press; LC-MS; pH 2 treatment.

Subtask 3.1: Halophilic Pure Culture PHA Production.

- **Milestone 3.1.1**: Achieve the cellular PHA content > 30% wt/wt.

This milestone was achieved through flask, lab-based experiments, where optimized fermentation conditions resulted in a cellular PHA content of 70%, more than double the target value of 30%. The optimal setup involved two-fold diluted VFAs from food waste fermentation supplemented with 156 g/L NaCl, ATCC-level nutrients, and an initial pH of 7. These conditions supported strong halophilic growth and PHA accumulation. Detailed methodology, confidence-supporting quality metrics, and data visualizations can be found in our publication (see Zhang et al., 2024), with methods detailed in Section 2.4, results detailed in Section 3.1 and Figure 3.

- **Milestone: 3.1.2**: Achieve HV content of PHA > 8% wt/wt.

The HV content target was successfully met and exceeded using a MEC-integrated aAD system, which enriched C3 and C5 VFAs in food waste digestate. These VFAs served as metabolic precursors for HV synthesis, enabling *HM* to produce PHA with an HV content of 12 wt%, which is equivalent to approximately 20 mol%; a composition well suited for most bioplastic applications, including flexible packaging films. A detailed explanation of the MEC-aAD operation, confidence-supporting quality metrics, and HV enhancement results is available in our publication (see Zhang et al., 2025), with methods detailed in Section 2.3 and results detailed in Section 3.3 and Figure 3C.

- **Milestone 3.1.3**: Achieve COD removal efficiency > 60%.

High chloride concentrations (~156 g/L NaCl) in *HM* media substantially interfere with dichromate-based COD assays, leading to falsely elevated readings and risks of vial over-pressurization (Hang, 2017). To avoid these issues, organic removal was quantified using TOC analysis with a Shimadzu TOC-LCSN analyzer (Shimadzu Corporation, Tokyo, Japan) equipped with a high salt combustion tube kit, which measures organic carbon via high-temperature combustion (~680 °C) and is unaffected by chloride interference. TOC removal exceeded 70%, confirming effective substrate utilization by *HM*. These results are consistent with observations from both single-batch experiments and long-term SBR operation. Detailed methodology and supporting data are available in our publication (see Zhang et al., 2024), with methods detailed in Sections 2.5, and results detailed in Sections 3.1 and 3.2 and Figures 3A and 4B.

- **Milestone: 3.1.4:** Stabilize the halophilic fermenter for more than 60 days.

This milestone was successfully met by operating a SBR continuously for 450 days, demonstrating stable halophilic fermentation performance well beyond the 60-day requirement. Throughout this period, consistent PHA production (50–65%) and HV content (~10%) were maintained without contamination, despite variations in cycle time and volume exchange ratio, which are the two key operational parameters of the SBR. Detailed methodology and data are presented in our publication (see Zhang et al., 2024), with methods detailed in Sections 2.3 and results in Section 3.3 and Figure 5A.

Subtask 3.2: Separation for Salt Recovery.

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

Salt recovery was achieved at 50-70% at volume exchange ratios between 0.3 and 0.5 in the SBR. Specifically, a defined portion of spent media was discharged and replaced in each cycle, allowing the remaining salty supernatant to be recycled and reused in subsequent cycles. This strategy enabled 50-70% salt recovery without causing any substantial compromise in cellular PHA content. Detailed methods and results supporting these findings are available in our publication (see Zhang et al., 2024), with methods in Sections 2.3 and results in Section 3.3 and Figures 2A and 5A.

For PHA recovery and purification, over 90% PHA recovery was successfully achieved by soaking *HM* cells in water with mixing for 2 hours (Table 7.2.2). However, the crude PHA powder obtained (Figure 7.2.1A) exhibited a relatively low purity of 84 ±

2% (Table 7.2.2), likely due to impurities such as carotenoids, a well-known *HM* cell byproduct that imparts a reddish color (Giani et al., 2021), as seen in Figure 7.2.1A. To achieve higher purity aligning with industry product standards, an ethanol washing step was introduced after PHA recovery, effectively improving the PHA purity to 96 ± 2% (Table 7.2.2). PHA recovery and purity were calculated according to Equations (1) and (2). PHA recovery using NaClO was assumed as baseline values to represent 100% recovery, as it is a widely adopted standard method known to achieve near-complete cell lysis and maximal PHA extraction (Cui et al., 2017). All results were based on triplicate biological replicates to ensure data reliability and confidence.

$$\text{PHA recovery } (\%) = \frac{\text{mass of PHA recovered from cells using fresh water}}{\text{mass of PHA recovered from cells using NaClO}} \times 100 \quad (1)$$

$$\text{PHA purity } (\%) = \frac{\text{mass of PHA monomers contained in freeze dried PHA powder}}{\text{mass of freeze dried PHA powder}} \times 100 \quad (2)$$

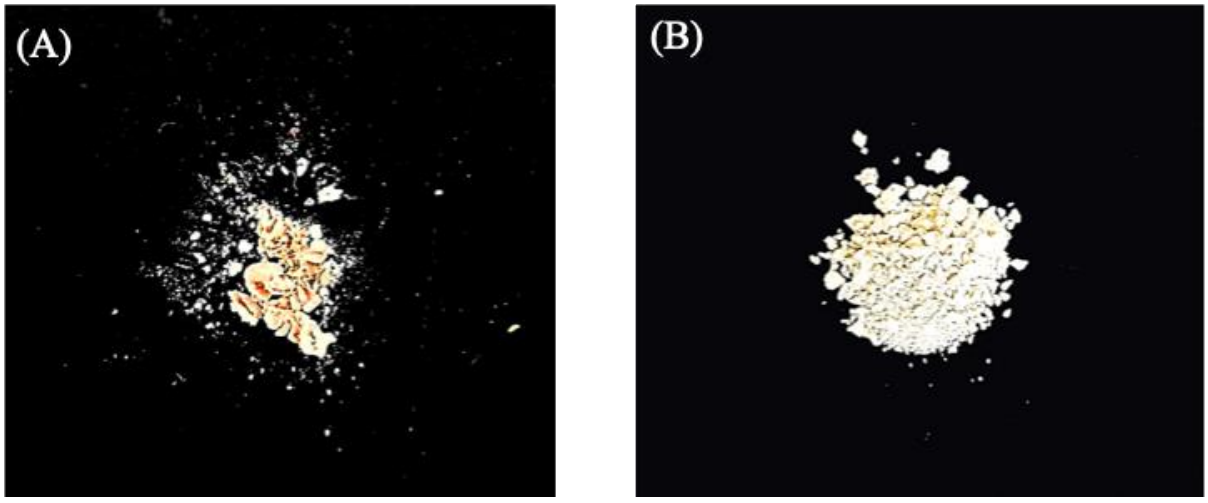


Figure 7.2.1: Crude IN (A) and purified in (B) polyhydroxyalkanoate (PHA) powder.

Table 7.2.2: PHA recovery and purity using water-based methods.

Recovery method	Recovery (%)	Purity (%)
Water (2 hour)	93 ± 3	84 ± 2
Water (2 hour) followed by ethanol purification		96 ± 2

Subtask 3.3: Blending high salinity wastewater to enhance the economic and environmental viability of the halophilic PHA production.

- **Milestone 3.3.1:** Displace 10% of salt utilized by adding high strength saline wastewater, while maintaining performance metrics.
- **Milestone 3.3.2:** Displace 50% of salt utilized by adding high strength saline wastewater derived from a food waste source.

Experimental results showed that even a 10% blending of high salinity wastewater inhibited *HM* growth to the extent that no PHA production was observed, let alone at 50% substitution. This indicates strong inhibitory effects associated with high salinity wastewater, likely due to the presence of toxic compounds. In response to these

findings, Milestone 3.4.1 was established to investigate high salinity food waste inclusion effects on PHA production and explore ways to mitigate high salinity food waste inclusion effects on PHA production. By applying this mitigation approach, up to 46% of the salt could be successfully displaced with high salinity wastewater, as detailed under Milestone 3.4.1 below.

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/wt with >8% HV content, and >90% PHA recovery compared to baseline values.

We achieved PHA production at 70% wt/wt with 12% wt/wt HV content, and 93% PHA recovery compared to baseline values. We also achieved carbon conversion efficiency of minimum of 47.9% and maximum of 60.6%. This meets minimum of 25% increase over baseline of 37% carbon conversion efficiency. This also corresponds to 50% decrease in cost of disposal, meeting the go/no-go target of >10%.

Subtask 3.4: Optimization of PHA production: Determine optimal conditions to integrate PHA fermentation, with co-product formation from dark fermentation; integrate dewatering using coagulants to guide pilot-scale operation; give insights into the lysis mechanism during fresh water PHA extraction, and explore ways to mitigate the inhibitory effect of high salinity food waste inclusion on bacterial survival and PHA production.

- **Milestone 3.4.1:** Achieve a cellular PHA content > 50% wt/wt while increasing carbon conversion >50% and decrease costs >25%, successful dewatering of *HM* cells to >40% dryness using coagulants, and clarification of lysis mechanism and high salinity food waste inclusion effects on PHA production, and give insights into HM cell lysis mechanism, and explore ways to mitigate high salinity food waste inclusion effects on PHA production (M33).

Cellular PHA content reached 70 wt% as described in Milestone 3.1.1. Information related to carbon conversion efficiency (>50%) and cost reduction (>25%) is available in the TEA and LCA sections (Section 7.3 of this Final Report).

Dewatering of *HM* cells to >40% dryness was successfully achieved, with the dryness measured at 50 ± 4%. Flocculants were tested but showed no measurable effect on dewatering performance; thus, we decided not to use flocculants because the high NaCl concentration in the halophilic culture medium significantly reduces flocculant effectiveness (Zhao & Corredig, 2016). Instead, we evaluated the performance of a high-speed centrifuge operated at 16,000 × g for 30 minutes, yielding a cell pellet in Figure 7.2.2A with 39 ± 2% TS. To further enhance dryness, the pellet was transferred into a centrifuge filtration tube in Figure 7.2.2B simulating a belt press, fitted with a 0.45 µm membrane or belt-press mesh. After an additional 10-minute spin at the same speed, the final pellet in Figure 7.2.2C achieved 50 ± 4% TS. All results were based on triplicate experiments to ensure reproducibility.

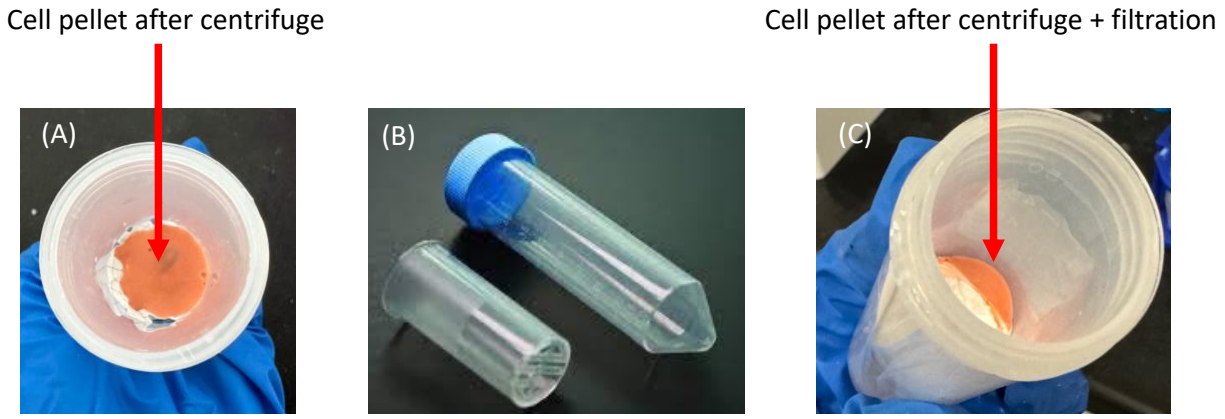


Figure 7.2.2: (A) Dewatered cell after centrifuge; (B) filtration tube; and (C) dewatered cell after both centrifuge and filtration.

A comprehensive literature review revealed that a protein layer, known as phasin, cover PHA granules in *HM* (Cai et al., 2012) provides insight into the lysis mechanism of *HM*. This structural barrier may contribute to the need for extended osmotic lysis conditions, such as 2 hours of water soaking with agitation, for effective PHA release, compared with much faster lysis of red blood cells, which typically lyse within 10 minutes in hypotonic environments (Grimberg et al., 2012).

To mitigate the inhibitory effects of high salinity wastewater on *HM*, overliming and pH 2 treatments were evaluated. Only pH 2 treatment was effective, leading to the formation of a dark, slimy precipitate (Figure 7.2.3). The supernatant, after precipitate removal, enabled *HM* to grow and produce PHA in 3× diluted high salinity wastewater. While, no growth or PHA production was observed in untreated or overliming groups (Figure 7.2.4). LC-MS analysis of the precipitate (Figure 7.2.5) identified long-chain fatty acids (LCFAs) as the primary inhibitory compounds, known to disrupt microbial physiology (Mitchell & Ellermann, 2022). These results confirmed LCFAs as the major inhibitors in high salinity wastewater. The pH 2 treatment enabled successful PHA production at 3× diluted high salinity wastewater, corresponding to 46% salt replacement.

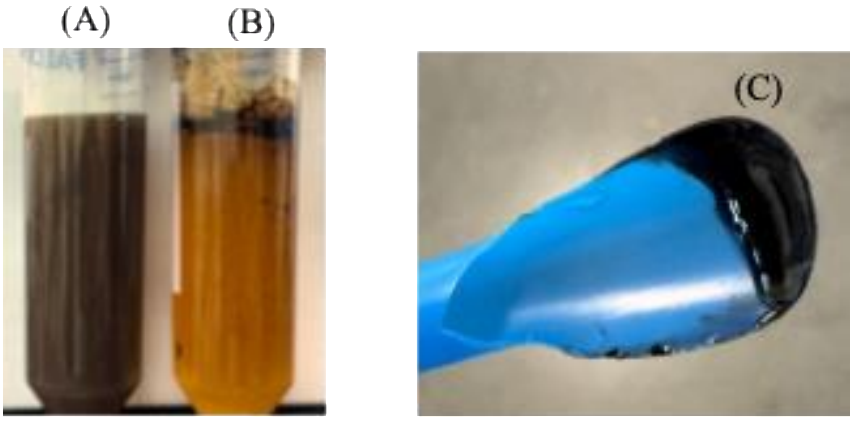


Figure 7.2.3: Visual appearance of high salinity wastewater (A) before and (B) after pH 2 treatment, and (C) dark, slimy precipitate formed following pH 2 treatment.

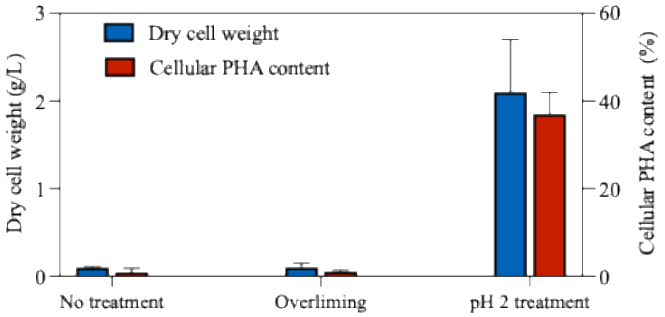


Figure 7.2.4: Cell growth and cellular polyhydroxyalkanoate (PHA) content of *HM* cultivated in 3× diluted high salinity wastewater without other treatment, with overliming treatment, and with a pH 2 treatment.

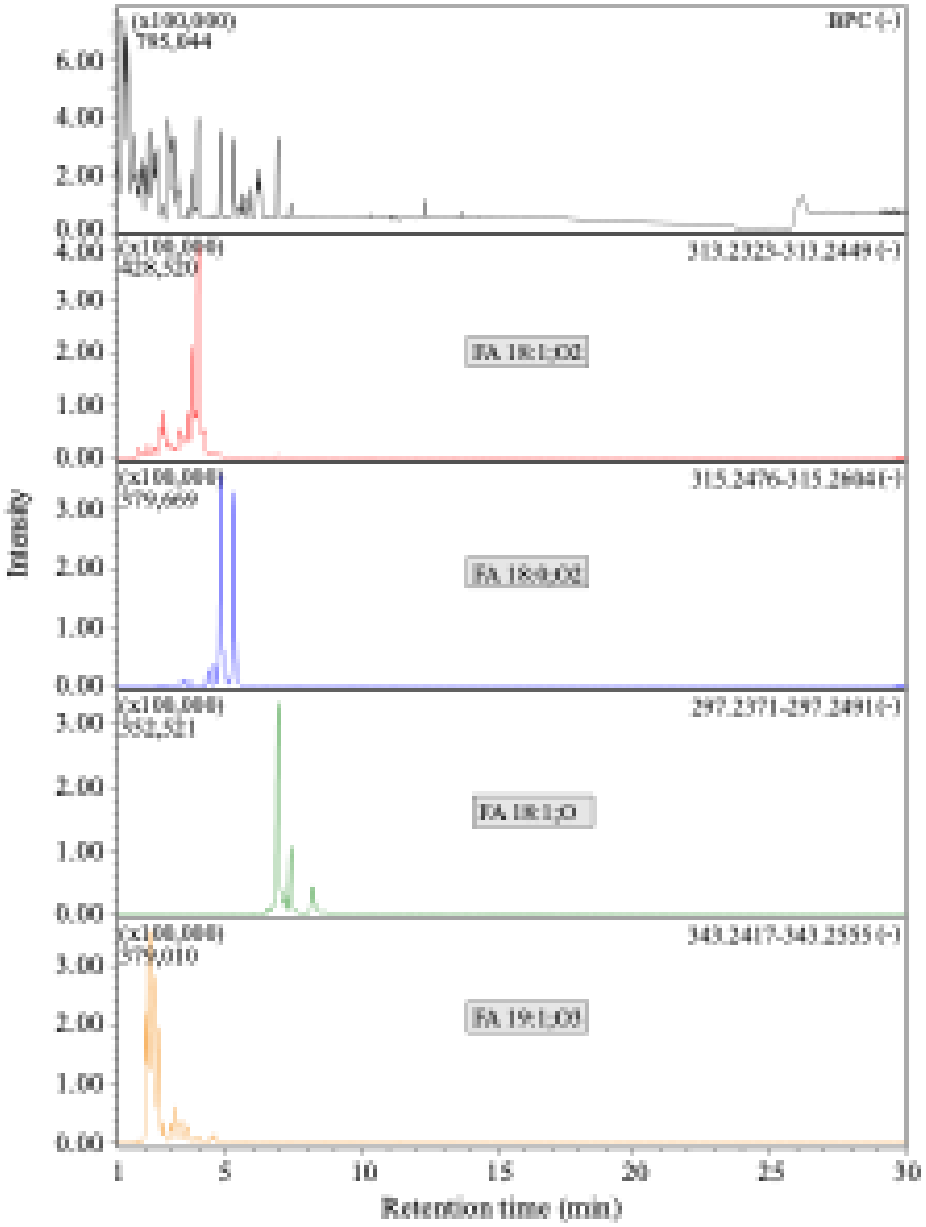


Figure 7.2.5: Liquid chromatography-mass spectrometry (LC-MS) chromatogram of the dark precipitate formed after pH 2 treatment.

7.3 Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA)

Task 4.0: Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA): TEA and LCA analyses will be conducted for Tasks 2 and 3 to determine optimal PHA culture conditions and necessary VFA enhancements.

In this project, a techno-economic analysis and life cycle analysis model was developed in excel and several TEA and LCA scenarios were run to find optimal costs, carbon emissions and carbon conversion efficiencies. With that, the cost of disposals was also calculated to see the improvements in comparison to the current and existing process that deals with food waste management. Over the course of the project, several different scenarios consisting of pilot scale scenarios with high salinity food waste were considered as the feed and conducted the analysis.

Subtask 4.1: TEA/LCA for VFA and PHA Generation: The model outputs will be used to determine optimal conditions moving forward in Tasks 2 and 3 and compared to the baseline model (Task 1). We will begin developing ratios for combined PHA-MEC-AD scenarios (further developed in Task 4.2).

- **Milestone 4.1.1**: Conduct TEA/LCA to compare VFA and PHA production to baseline conditions with targets of increasing carbon conversion efficiency >25% and reducing disposal cost by 10%.

As an intermediate task, we met the milestone to achieve carbon conversion efficiency of 25% and reduction of disposal cost by 10%. To achieve this, the following process flow diagram was considered with three scenarios: i) PHA production (all in black), ii) PHA production with recycle of residue from DF back to the DF to maximize VFA production and iii) PHA production that produces biogas from the residue obtained from DF.

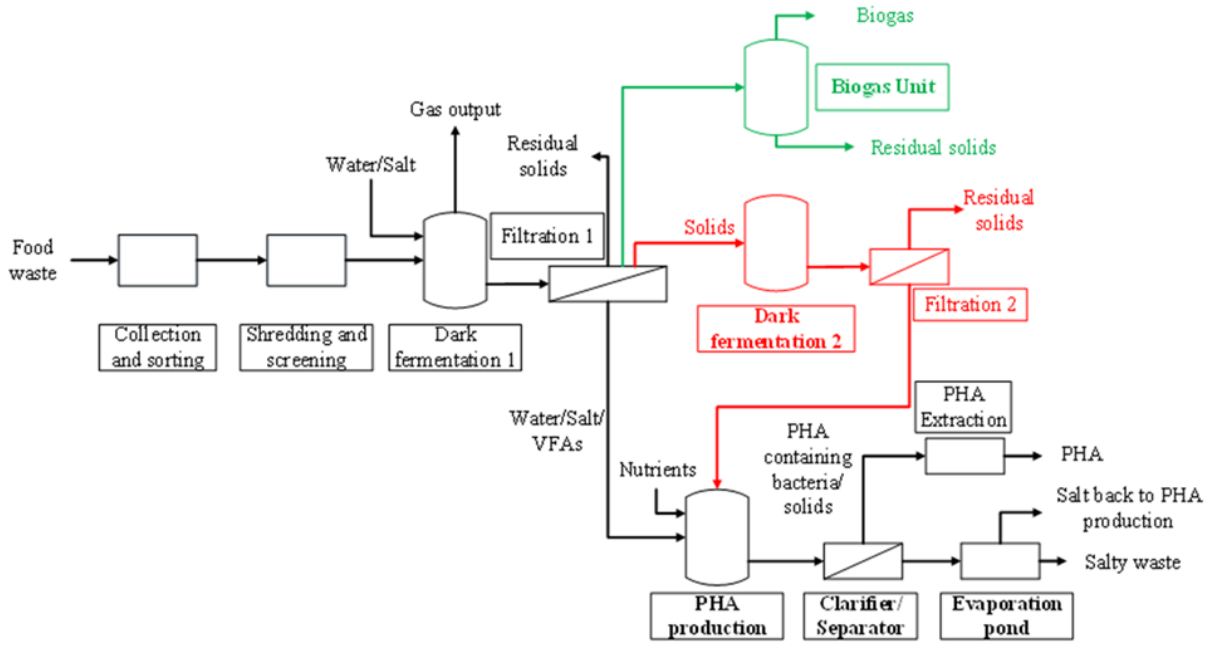


Figure 7.3.1: Techno-economic analysis and life cycle assessment flowsheet representing dark fermentation and PHA production

For the carbon conversion efficiency and disposal cost reduction, we assumed a standard 10,000 kg/h food waste processing capacity. A net carbon conversion efficiency of 47.9% was observed on the mass basis of food waste with no further modification to the production system. However, as shown in the process flow diagram, a modification of the dark fermenter was proposed where the und-fermented solids were taken back to the dark fermentation after the filtration for VFAs, which increased the overall carbon conversion efficiency to 47.9% to 60.6%. This re-fermentation also significantly reduced the cost of production from $2.73/kg PHA to $2.35/kg PHA. Similarly, re-fermentation of solids reduced emission from 5.23 to 4.50 kg CO_{2e}/kg PHA. An alternative scenario was also studied with no solids recycled back for re-fermentation but the solids were used to produce biogas. That scenario, however, only reduced the cost of production of PHA from 2.73 to 2.70 $/kg PHA, and carbon efficiency remained the same at 47.9%. Furthermore, the equivalent emissions reduced to 5.22 from 5.23 kgCO_{2e}/kg PHA product (Table 7.3.1).

Table 7.3.1: Summary of cost disposal cost reduction at different selling price of PHA.

Scenario	DF1	DF2	Biogas	$/kg PHA	kg CO_2/kg PHA	Carbon conversion efficiency (%)
1	Yes	No	No	2.73	5.23	47.9
2	Yes	Yes	No	2.35	4.50	60.6
3	Yes	No	Yes	2.70	5.22	47.9

Elaborating on the cost of Scenario 2 that recycles residues back to the dark fermenter, the capital cost is US$ 7,690K, and the total yearly operating cost is US$ 20,870K, including the amortized capital cost for 20 years of plant life. The interest rate is assumed to be 6% annually. Waste food is assumed to be $1/metric ton (Table 7.3.2). The scenario where unfermented biomass is recycled back to the reactor with a total residence time of 19 days can further reduce the breakeven cost of production to $2.35/kg PHA.

Table 7.3.2: Cost analysis of Scenario 2 system processing 10,000 kg/h food waste.

Cost type	Cost ($K)	Units
Total project capital cost	7,690	US$
Total operating cost (including amortized capital cost)	20,870	US$/year
Total food waste cost	80,000	US$/year
Total solvent cost	15,001	US$/year
Total energy cost	554	US$/year
Total breakeven production cost	**2.35**	**US$/kg PHA**

A sensitivity analysis based on the production size was performed. We varied the production size from 10 kg/h waste food to 10,000 kg/h waste food. The cost of production for a small 10 kg/h system is high to $36.35/kg PHA. However, when the processing size increases to 10,000 kg/h, the cost of production reduces to $2.35/kg PHA. It can be further reduced to $2.31/kg PHA if the processing size increases to 100,000 kg/h.

Similarly, life cycle impacts also reduce as the sizes go up, but not in the same fashion as the cost of production. For a system with 10 kg/h, 5.06 kg CO_{2e} /kg PHA; for a system with 10,000 kg/h size, 4.50 kg CO_{2e} /kg PHA, as shown in Figure 7.3.2.

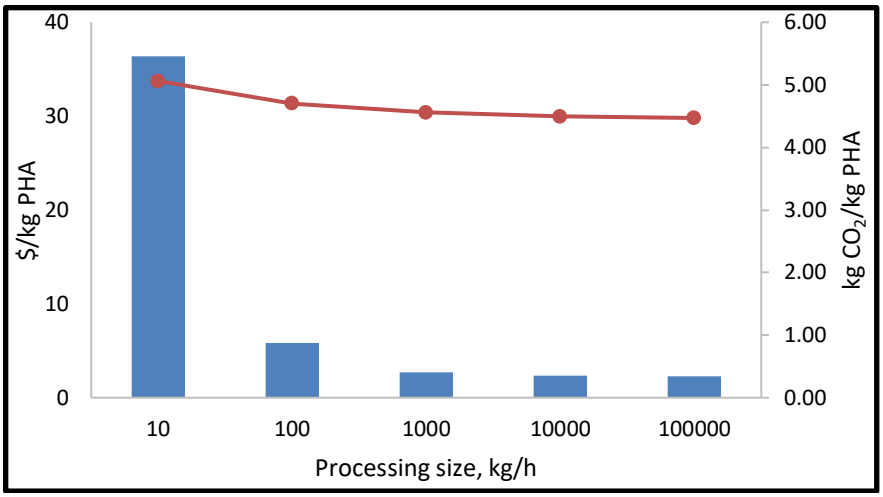


Figure 7.3.2. Sensitivity analysis based on processing size.

Subtask 4.2: TEA and LCA for PHA enhancement: A complete process model will be developed to determine optimal product ratios (PHA, H_2, CH_4) based on various input scenarios, including feedstock source characterization, use of high salinity food waste, feedstock disposal fee, co-product market prices, and current/future tariffs and policy changes. The models will be refined based on outputs from Tasks 2.6, 3.4, and 5.1.

- **Milestone 4.2.1:** Conduct cost/benefit analysis of using high-salinity food waste. Techno-economic scenario will take into consideration higher tipping fees available for this waste and its impact on salt demand for PHA fermentation, PHA yield/quality, and salt solution disposal costs.
- **Milestone 4.2.2:** Build an integrated model and conduct TEA/LCA analyses on the operation of pilot-scale reactor and design scenarios to increase economic and carbon conversion viability.

In this subtask, the model was further developed to incorporate high salinity food waste, and several pilot plant scenarios were conducted based on data obtained from pilot plant studies. Both of the milestones were met by including the tipping fees into the calculations and by recycling salt back to the process. The integrated model was also developed that performed full scale economic analysis and carbon conversion viability.

The direct production of PHA from glycerol waste is illustrated in Figure 7.3.3. Most of the inputs from the cost analysis are used for the LCA using GREET.

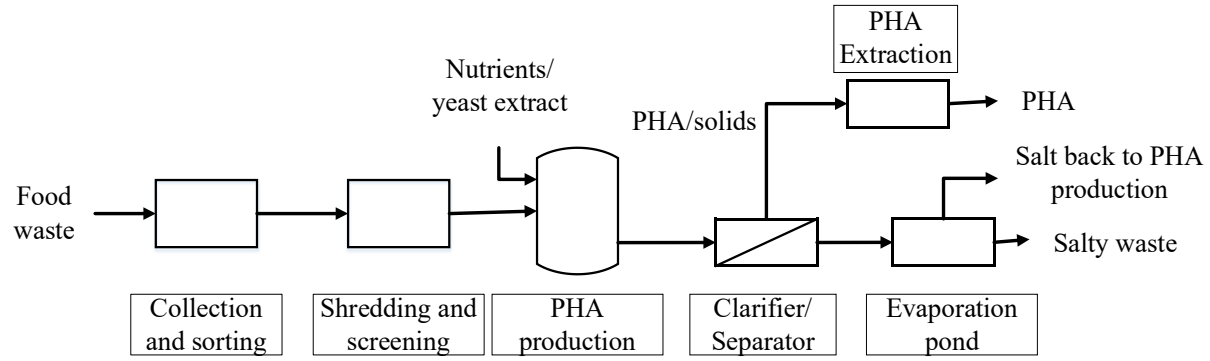


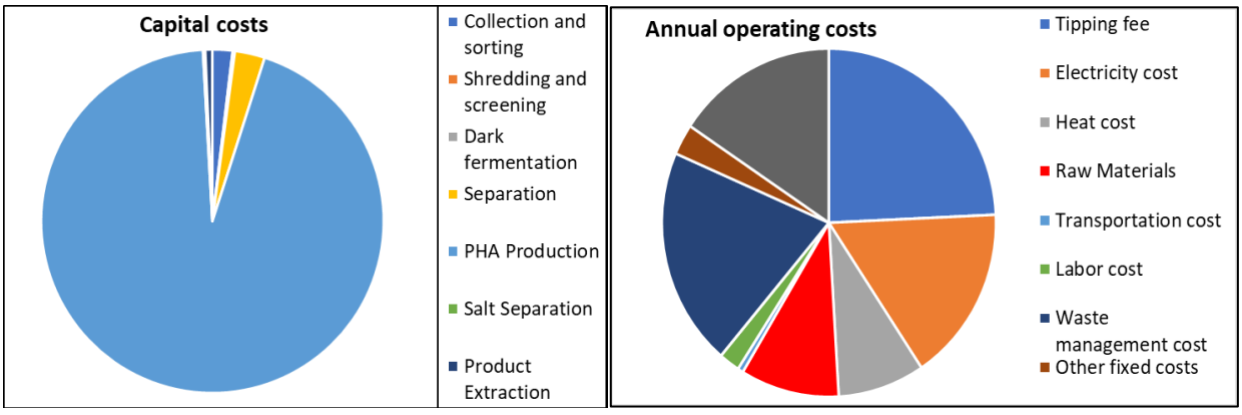
Figure 7.3.3: TEA and LCA flowsheet representing pilot-scale production where high salinity food waste or food-based glycerol waste is used to produce PHA directly.

A standard case of 10,000 kg/h system was assumed with all the parameters remaining the same. High salinity glycerol waste was assumed as feed, which was directly fed into PHA reactor in one pilot-scale study iteration. The overall mass and energy balance of the system was achieved, as shown in Table 7.3.3.

Table 7.3.3: Overall mass and energy balance of the system.

Unit name	Energy use (MJ/y)	Net input (kg/h)	Net output (kg/h)	Waste output (kg/h)	PHA production (kg/h)
Collection and sorting	0	10,000	0	0	0
Shredding and screening	276,114	0	0	0	0
Dark Fermentation	0	0	0	0	0
Separation 1	0	0	0	0	0
PHA production	492,721,112	1,456,432	0	0	5,654
Salt Separation	7,080,968	0	732,850	732,850	0
Production extraction	860,713	0	5,648	112,776	0

With this mass and energy balance, the cost, carbon conversion and carbon emissions were calculated. The overall cost of PHA production was calculated as $ 0.26/kg PHA and the total associated carbon emissions was calculated as 0.266 kg CO_{2e}/kg PHA and the overall carbon conversion efficiency was 56.4%. This meets all the milestones set for the project. The capital cost of $24,174K and annual operating cost of $7,636K was calculated for the standard-sized system with 10,000 kg/h capacity. Figure 7.3.4 shows the breakdown of annual operating cost and capital cost for the standard-sized system.



(a) (b)

Figure 7.3.4: Breakdown of (a) capital costs and (b) annual operating costs for the standard-sized system

For the same standard sized system, equivalent carbon emission was also calculated. The annual equivalent CO_2 emission of 12,000,347 kg_eCO_2 was calculated, and it is equivalent to CO2 emissions of 0.266 $kgCO_{2e}$/kg PHA. Figure 7.3.5 is the breakdown of the carbon emissions for the entire plant.

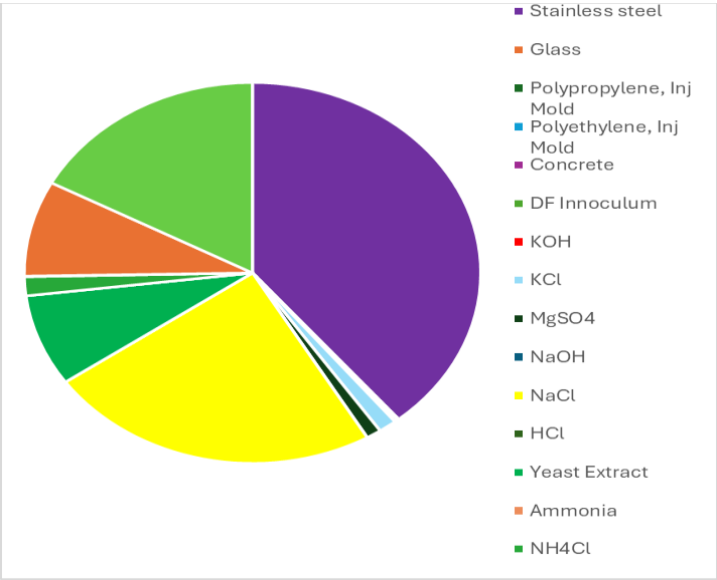


Figure 7.3.5: Breakdown of carbon emissions for the standard sized system.

A sensitivity analysis was conducted based on the processing size. The size ranged from 10 kg/h to 100,000 kg/h. The smallest size, 10 kg/h processing size, yielded a cost of production of $24.9/kg PHA and the equivalent CO_2 emissions of 0.824 kg CO_{2e}/kg PHA. The largest size, 100,000 kg/h processing size, yielded a cost of production of $0.23/kg PHA and the equivalent CO_2 emissions of 0.254 kg COe_2/kg PHA. In terms of reduction in disposal cost, the standard sized system incurred a net disposal cost of $33.9/ton waste which is 54.8% reduction from the regular $75/ton food waste disposal cost. This also meets our project milestone. Figure7.3.6 plot summarizes the cost of production and equivalent emissions based on production size.

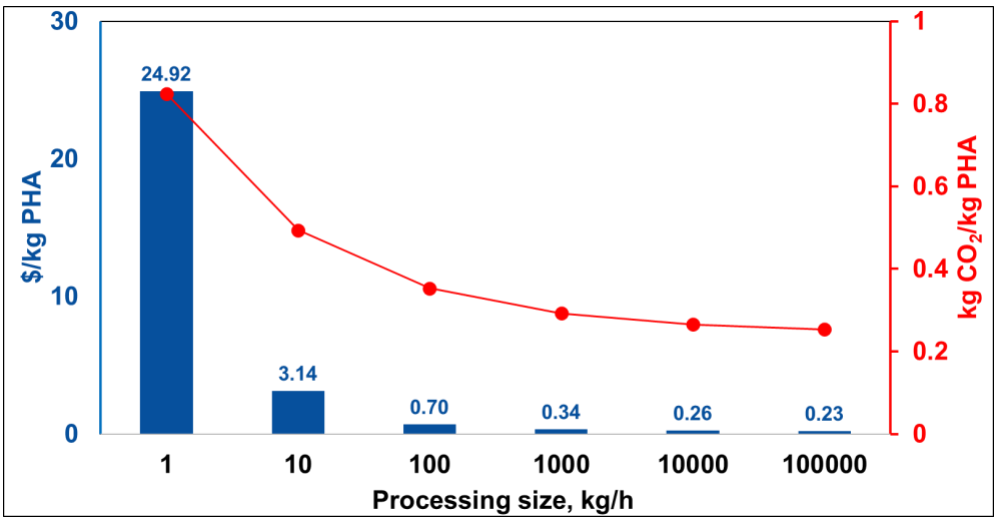


Figure 7.3.6: Sensitivity analysis based on production size

7.4 Pilot-Scale Evaluation with Lab-Scale Contributions

Task 5.0: Pilot-scale Reactor: Design and operate a pilot-scale reactor (50 L) under continuous feed for production of PHAs and test over a period of six months in >100 hour runs and evaluate for: 1) treatment efficiency, 2) PHA production, 3) energy efficiency, 4) separation and dewatering efficiency, 5) LCA and TEA, with analysis of the effect of both fossil fuel-based and carbon-free energy sources used to supply energy to a theoretical scaled-up system.

Table 7.4.1: Result summary of Task 5.

Milestone Description	Achieved results	Methodologies
Milestone 5.1.1: Set up and operate pilot scale process capable of PHA production.	Pilot system established with 100 L DF reactor, 50 L PHBV fermenter, industrial-scale disc centrifuge and homogenizer; operated 10 fermentation runs for PHBV production.	Modular system with DF, MEC, PHBV fermentation, disc centrifuge and homogenizer.
Milestone 5.1.2: Increase carbon conversion >25% in pilot-scale system and decrease costs >15% in the pilot system at Month 26.	Carbon conversion averaged 56% and peaked at 74%. Cost reduction exceeded 25% through supernatant recycling, industrial-grade salts.	PHBV fermentation was conducted in 50 L reactor, with recycled supernatant from previous runs.
Milestone 5.1.3: Increase carbon conversion >50% (from 36% to 56% C conversion) and decrease costs >25% in the pilot system at Month 33.	Carbon conversion averaged 56% and peaked at 74%. Cost reduction exceeded 25% through supernatant recycling, industrial-grade salts.	PHBV fermentation was conducted in 50 L reactor, with recycled supernatant from previous runs.

Task 5.1 Pilot-scale Reactor: Conduct >10 iterations of co-product formation ratios using triplicate runs of >100 hours. Test for product formation (PHAs). nutrients (N, P), PHA type (PHB, PHBV), gas production and composition (CO_2, H_2, and CH_4), separation and dewatering efficiency. Inputs of carbon-free energy for MEC electricity will be tested to determine effect on LCA and TEA outputs.

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

A modular 4-stage pilot-scale system was established for PHA production from general food waste (Figure 7.4.1), consisting of the following units explored in the following sections:
7.4a: 100 L dark fermentation reactor for VFA production using food waste.
7.4b: 50 L glass bioreactor for PHBV fermentation.
7.4c: Disc centrifuge for solid–liquid separation.
7.4d: High-pressure homogenizer for PHBV extraction.

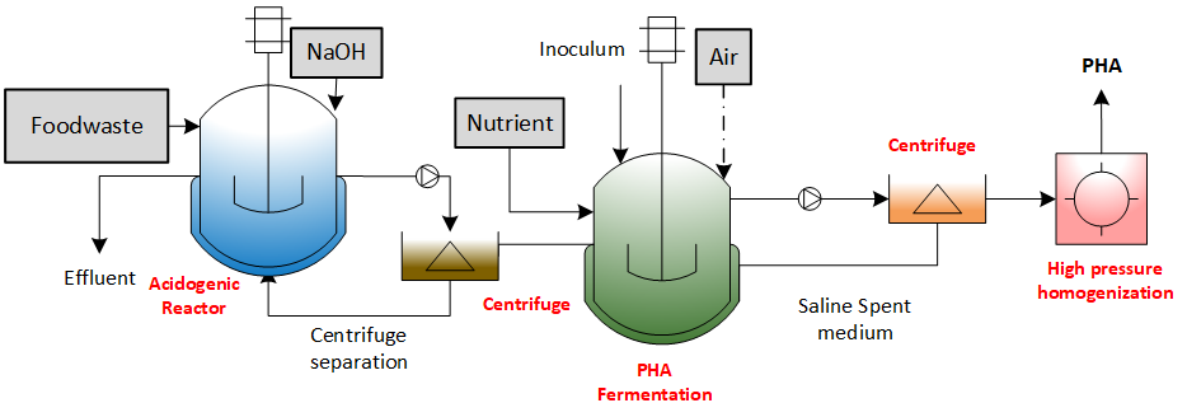


Figure 7.4.1: The 4-stage process pilot-scale system.

In addition, a short-pass process was also evaluated for PHBV production from high-salinity waste and fruit processing waste. This configuration includes only 3 stages- PHBV fermentation, centrifuge and homogenization as illustrated in Figure 7.4.2.

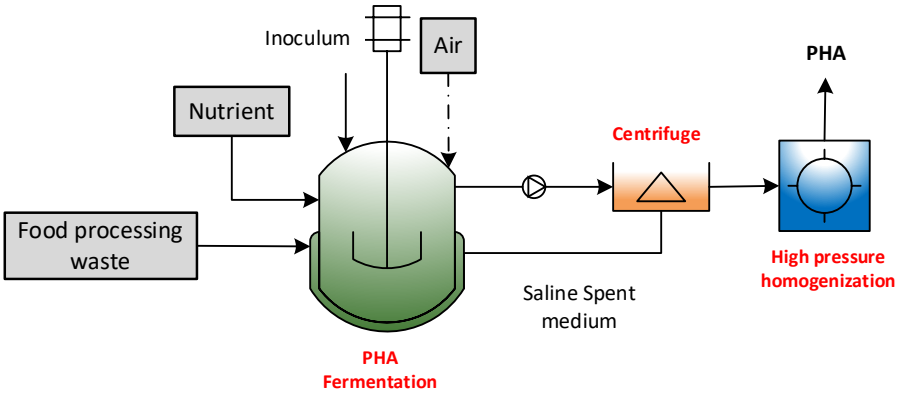


Figure 7.4.2: The short-pass process pilot-scale system.

7.4a: 100 L dark fermentation reactor for VFA production

The project successfully met the milestone by operating a 100 L reactor with an 80 L working volume using food waste as feedstock (Figure 7.4.3). Key operational parameters are as follows: the reactor is discharged and fed with 26.7 L every three days, resulting in a solid retention time (SRT) of 9 days. The organic loading rate (OLR) is 2.50 g VS/L/day. The pH is adjusted to 5.5 with NaOH every day, and the reactor is maintained under mesophilic conditions (35°C) with mixing at 150 rpm.

Figure 7.4.3: Pilot scale 100 L reactor for dark fermentation.

As shown in Figure 7.4.4, when regular food waste was used, the average VFA concentration reached 6.67 g/L with a maximum VFA concertation of 9.26 g/L, which is comparable to the 9 g/L obtained in the 35 L reactor (Figure 7.1.3). The overall VFA concentration in the 100 L reactor (Figure 7.4.4) was slightly lower than that of the 35 L reactor. In the 35 L reactor, the maximum VFA concentration reached 36 g/L when using high-salinity food waste (HSFW), surpassing the project goal of 30 g/L VFA concentration. However, HSFW was not tested in the 100 L reactor due to the concern about potential corrosion damage. Instead, the HSFW was directly fermented to PHBV in the 50 L glass reactor (see Section 7.4b).

The average volatile solids reduction (VSR) was 47.32%. The average biogas composition was approximately 12% CH_4 and 88% CO_2, indicating limited methanogenic activity under the operating conditions.

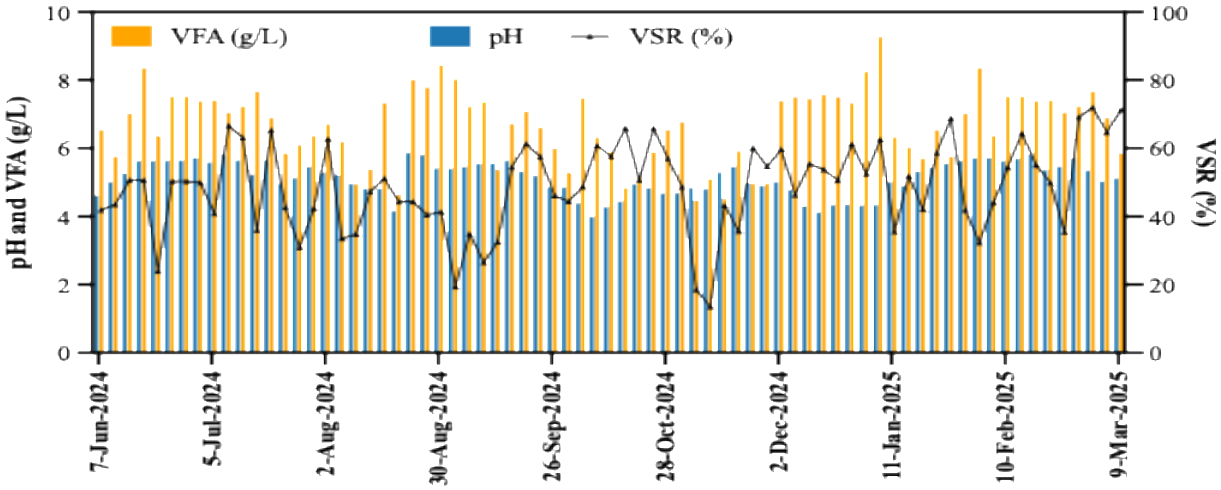


Figure 7.4.4: Volatile fatty acid (VFA) concentration, pH, and volatile solids reduction (VSR) for 100 L DF.

An MEC system was successfully integrated into a 100 L dark fermentation reactor to enhance the production of C3 and C5 VFAs in the digestate. This integration was informed by lab-scale MEC tests (Milestone 3.1.2), which showed that higher levels of C3 and C5 VFA were obtained with MEC which are beneficial for increasing the HV content during PHBV fermentation. A higher HV fraction improves the flexibility of PHBV. Initially, the MEC integration resulted in a short circuit due to contact between the electrodes and the metal reactor wall. This issue was resolved by installing a 3D-printed plastic cage with large openings to hold the electrodes. Additionally, a 100 L glass reactor was constructed to prevent conductivity-related issues.

Three stages were evaluated to validate the impact of MEC on VFA production observed at the lab scale: no electrode (NE), open circuit potential (OCP), and 1.5 V MEC. The NE stage served as a baseline to assess VFA generation without electrochemical influence. The OCP stage was used to determine whether the presence of electrodes without potential would affect VFA profiles. The 1.5 V stage involved the application of an external voltage to drive electrochemical activity. As shown in Table 7.4.2, the concentrations of C3 and C5 VFAs increased in the OCP stage compared to NE. Specifically, C3 rose from 0.04 g/L (NE) to 0.31 g/L (OCP), and C5 increased from 0.06 g/L to 0.16 g/L. At 1.5 V, C3 slightly decreased to 0.22 g/L, while C5 further increased to 0.19 g/L.

Table 7.4.2: VFA composition in NE, OCP, and 1.5 V stages.

Stage	NE (no electrode)	OCP (open circuit potential)	1.5 V (MEC applied voltage)
VFA Conc. (g/L)	6.05 ± 0.95	6.76 ± 0.44	5.66 ± 0.64
C2 (g/L)	2.94 ± 0.73	2.72 ± 0.31	1.41 ± 0.30
C3 (g/L)	0.04 ± 0.01	0.31 ± 0.05	0.22 ± 0.04
C4 (g/L)	0.78 ± 0.44	1.41 ± 0.05	1.52 ± 0.01
C5 (g/L)	0.06 ± 0.02	0.16 ± 0.11	0.19 ± 0.01
C6 (g/L)	1.94 ± 0.84	1.81 ± 0.20	1.71 ± 0.15
C7 (g/L)	0.23 ± 0.10	0.18 ± 0.08	0.52 ± 0.17

7.4b: Pilot-Scale PHBV Fermentation

A total of 10 iterations of pilot-scale PHBV fermentation were completed in 50 L glass reactor (Figure 7.4.5), totaling 2,376 hours, successfully achieving Milestone 5.1.1. Detailed information is summarized in Table 7.4.3. Feedstocks tested included high-salinity waste glycerol, fruit processing waste, and digestate from the 100 L dark fermentation. During the runs, salts, macro- and micro-nutrients were substituted with low-cost, industrial-grade products. Each cultivation lasted between 9 and 12 days.

Figure 7.4.5: Pilot scale 50 L glass reactor for PHBV fermentation.

Table 7.4.3: Summary of the 10 runs for PHA fermentation using the 50 L glass reactor.

Runs	Process	Feedstock	Variable	Duration
1	short-pass	High salinity waste	5% inoculum, lab-grade salts	9 days, 216 hours
2	short-pass	High salinity waste	10% inoculum, industrial salts	9 days, 216 hours
3	short-pass	High salinity waste	10% inoculum, industrial salts	9 days, 216 hours
4	short-pass	High salinity waste	10% inoculum, industrial salts)	10 days, 240 hours
5	short-pass	High salinity waste	Recycle spent media for 1st time	10 days, 240 hours
6	short-pass	Fruit processing waste	10% inoculum, industrial salts	12 days, 288 hours
7	short-pass	High salinity waste	Recycle spent media for 2nd time	10 days, 240 hours
8	4-stage	Fermented food waste	pH controlled at 7	10 days, 240 hours
9	short-pass	High salinity waste	Recycle spent media for 3rd time	10 days, 240 hours
10	4-stage	Fermented food waste	initial pH at 7; without pH controlled	10 days, 240 hours

The 50 L PHBV fermentation reactor was aerated at 110 L/min and agitated at 150 rpm. Recycling the fermentation broth supernatant saved 6240 g NaCl, as the salt in the previous run was reused. Performance metrics, including total organic carbon (TOC) removal efficiency, PHBV content, and production rate using different feedstocks are presented in Table 7.4.4. The best results were obtained in the runs with high salinity waste, with a TOC removal of 49.3% and PHBV production rate of 55.8%. The HB and HV content of the PHA cell was 47.3% and 8.5%, respectively. These results are relatively lower than those obtained in lab-scale tests, which showed 70% TOC removal with 58% HB and 12% HV contents, likely due to reduced oxygen transfer efficiency in the 50 L reactor compared to laboratory flasks. Regardless, the PHBV production remained high compared with values reported in literatures (Zhang et al., 2025) and met the specifications in the milestone.

Table 7.4.4: Summarized result for PHBV fermentation.

Parameters	High salinity waste	Fruit processing waste	Food waste digestate
TOC removal (%)	49.3	29.8	87.0
HB content (%, wt/wt)	47.3	23.4	40.1
HV content (%, wt/wt)	8.5	3.0	12.3
PHBV production rate (%, wt/wt)	55.8	26.4	52.4

7.4c: Disc Centrifuge for Solid–Liquid Separation

The Alfa Laval Clara 20 clarifier (Low Flow Disk Centrifuge) with a capacity of 500L/h (Figure 7.4.6a) was employed to separate *HM* cells from the fermentation broth obtained (Section 7.4b). Each batch involved feeding approximately 40 L of PHBV fermentation broth into the clarifier. Two cycles were performed consecutively for each batch, with each cycle lasting 30 minutes, ensuring efficient phase separation. Phase separation yielded approximately 18% (v/v) solid fraction and 82% (v/v) liquid supernatant (Figure 7.4.4). This process enabled efficient recovery of PHBV-rich biomass and yielded a reusable saline supernatant for subsequent fermentation. The solid fraction was further processed via homogenization for pilot-scale PHBV recovery as described in the following section (Section 7.4d).

(a) (b)

Figure 7.4.6: (a) Pilot scale disc centrifuge and (b) high salinity supernatant (liquid out) and residue cell (solid out).

7.4d: High-Pressure Homogenizer for PHBV Extraction

HM cells were effectively disintegrated using a high-pressure homogenizer (PandaPLUS 2000, GEA, Frankfurt, Germany) with a processing capacity of 9 L/h (Figure 7.4.7), eliminating the need for harsh chemical treatments. The solid fraction obtained from the disk centrifuge (Section 7.4c) was passed through the homogenizer at a pressure of 1000 bar to achieve cell wall disruption. This mechanical method enabled PHBV recovery rates exceeding 90%, while simplifying downstream processing and significantly reducing both processing time and chemical usage.

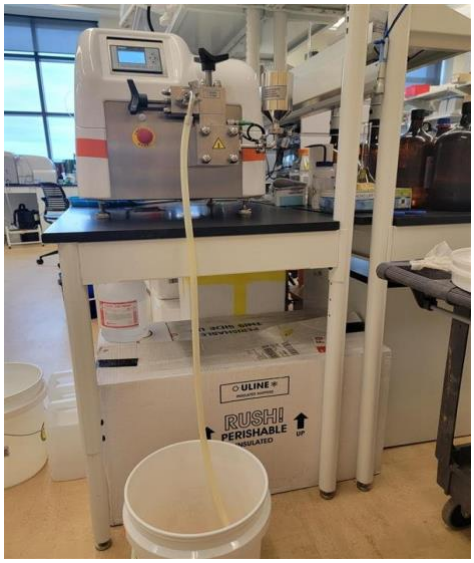


Figure 7.7.7: PandaPLUS 2000 homogenizer used for PHBV extraction.

- **Milestone 5.1.2:** Increase carbon conversion >25% in pilot-scale system and decrease costs >15% in the pilot system. (M26)
- **Milestone 5.1.3:** Increase carbon conversion >50% (from 36% to 56% C conversion) and decrease costs >25% in the pilot system. (M33)

The pilot-scale PHBV production as detailed in Milestone 5.1.1 demonstrated desired performance that met and exceeded the goals of Milestones 5.1.2 and 5.1.3. Across multiple 50 L batch fermentations using high-salinity waste, the average carbon conversion rate reached 56%, with a peak of 74% observed in run 5. This corresponded to an average PHBV yield of 0.38 g/g high salinity waste, with a highest PHBV of 0.50 g/g. These results substantially surpassed the target of 50% carbon conversion.

Cost reduction targets were also achieved, as detailed in Subtask 4.1. By recycling high-salinity supernatant across multiple runs, the process eliminated the need for approximately 6240g of NaCl per batch. Combined with the full replacement of lab-grade nutrients with industrial-grade alternatives, and recycled use of the salty supernatant, the system achieved an >25% reduction in production costs. Moreover, residual *HM* cells from disc centrifugation enabled consistent regrowth without reinoculation, reducing input requirements and potentially lowering process costs.

Task 6.0: Final Verification (M33-M36)
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.

Developed a fully integrated model and performed comprehensive TEA and LCA analyses. Best-case scenario yielded PHA production of $2.37/kg and 4.53 kg CO_{2e}/kg PHA with 60.6% carbon conversion efficiency. High salinity waste yielded PHA production of $0.26/kg and 0.266 kg CO_{2e}/kg PHA with 56.4% carbon conversion efficiency. This also yielded $33.9/ton disposal cost for high salinity food waste and is equivalent to 54.8% reduction in the disposal cost, meeting all the milestones set for the project.

8. Significant Accomplishments and Conclusions:

Task 1 was a successful initial verification. Task 2 achieved high VFA output with no inhibition from the mono-fermentation of high salinity food waste. The high salinity food waste produced the highest VFAs (36.04 g/L) with no negative effect from increasing salinity (53.5 – 214 g/L NaCl), showing high VFAs with higher salinity levels than previous literature. Regular food waste had higher efficiency when normalized by organic loading but produced less concentrated VFAs (9.29 g/L). High salinity food waste inclusion increased microbial community diversity and reduced relative abundance of methanogenic archaea, resulting in a two-fold effect of increasing the abundance of acetic acid producing bacteria (i.e., Leuconostoc, Bfidobacterium, and Pseudomonas) and reducing the relative abundance of acetate consumers (i.e., Methanobrevibacter). Results showed that alkaline, high salinity food waste increases VFA production and can be mixed with other food waste. For the first time, MEC fermentation was shown to enhance C3 and C5 VFAs for higher HV content in PHBV.

Task 3 advanced the industrialization of food waste-derived PHA production using halophilic fermentation. We established the longest continuous-flow halophilic PHA fermentation to date, a 450-day contamination-free run using a SBR, which validated process stability and supports industrial scalability. We identified LCFAs as key inhibitors in high salinity wastewater and mitigated their effects through pH 2 treatment, which enabled 46% salt substitution to reduce cost and addressed a major cost barrier to halophilic PHA commercialization. The inhibitors present in high-salinity food waste may still limit the maximum PHA titer achievable. Therefore, future efforts should focus on further mitigation strategies, such as additional pretreatment or co-fermentation with lower-salinity food waste, to improve process robustness and PHA titer.

Task 4 designed and demonstrated an integrated technoeconomic and life cycle analysis model with a sensitivity analysis that helped to identify the most cost intensive unit (dark fermentation) and other processes that could be optimized to lower production costs of production. It was identified that a processing plant needs to be at least 500 kg/h to be breakeven in the US. The PHA production cost from regular food waste was \$2.37/kg and equivalent emissions of 4.53 kg CO_{2e}/kg PHA with a 60.6% carbon conversion efficiency. The cost of PHA production from the high salinity food waste was \$0.26/kg and emissions of 0.266 kg CO_{2e}/kg PHA with 56.4% carbon conversion efficiency and without the need to use any prior dark fermentation process and recycling 50% of the salt back to the solution, which reduced the cost of disposal by 54.8%.

Task 5 successfully demonstrated the scalability and stability of a pilot-scale bioprocess integrating DF and PHBV fermentation. The pilot system achieved up to 74% carbon conversion efficiency and consistent PHBV yields of 0.38–0.50 g/g high salinity waste, validating utilization of this complex substrate. The MEC was integrated into the DF system to enhance C3 and C5 VFA composition and downstream PHBV quality. The pilot unit was operated for over 2376 hours using different 10 fermentation trials, demonstrating long-term resilience. Additional engineering strategies, such as using a 3D-printed plastic cage to prevent MEC short-circuiting, were employed. Recycled supernatant retained sufficient salinity for reuse, reducing salt input and cost. However, the industrial disc centrifuge failed to fully separate *HM* cells, although residual biomass supported regrowth without reseeding. These results support further process scale-up and long-term operation strategies in saline, non-sterile environments.

9. Path Forward:

To advance this technology toward commercialization, the project team has outlined a multi-phase path that includes product validation, feasibility assessment, and pilot-to-industrial scale-up. In the near term, PHBV polymer samples produced in this project will be evaluated by polymer companies for application-specific performance evaluation. This collaboration will provide critical feedback on material properties and inform necessary adjustments in downstream processing or formulation to meet specifications of commercial grade PHBV products.

Parallel to product validation, a feasibility study for commercial scale-up will be conducted. This will include an assessment of feedstock availability and composition, analysis of co-product streams and waste handling strategies, and identification of suitable geographic locations, preferably co-located with existing anaerobic digestion infrastructure. Process design and engineering will advance through the development of detailed process flow diagrams (PFDs) and piping and instrumentation diagrams (P&IDs), along with the specification of core equipment, including fermenters, centrifuges, and PHA separation units. Environmental permitting and regulatory compliance will be addressed through emissions quantification and risk assessments aligned with local, state, and federal regulations.

Key technical barriers remain. These include scaling up MEC systems, particularly with regard to corrosion and cost issues associated with stainless steel components, and ensuring consistent PHA product quality when feedstock composition varies by location. Additional challenges include transforming existing high-salinity waste streams under existing anaerobic digestion contracts toward PHA production and addressing logistical hurdles in sourcing food waste, especially from residential versus industrial sources. The variability in food waste composition may affect consistency of PHBV polymer, which will require process adaptation or quality control strategies.

Over the next 5-10 years, the vision includes the construction of a demonstration facility, or potentially direct transition to full-scale industrial production, depending on funding availability and technical readiness. Site-specific assessments will be performed to match feedstock supply with process capacity and evaluate community acceptance. The team plans to engage stakeholders, including municipalities and waste management authorities, to secure siting approval and feedstock commitments. The effort will also include community impact analysis and environmental permitting to ensure a responsible and inclusive scale-up process. These community impact and municipalities analyses could have been part of the food waste analysis in DOE's WASTE 'Waste Analysis and Strategies for Transportation End-use' program, as both University of Maryland and Quasar PIs from this report were part of announced WASTE projects, but the project funding has for these announced recipients has not been awarded to date.

To support these PHA production efforts, private-sector investment will be sought to cover capital and operational costs, while additional federal grants will be pursued for innovation, emissions reduction, and sustainability goals. Engineering design support will continue through collaboration with Quasar Energy Group and other partners. Regulatory guidance and market development assistance, including customer engagement and end-user partnerships, will be essential to accelerate commercialization and will continue to take this novel technology straight to market.

10. Products:

10.1 Publications:

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Olonimoyo, E.A., Amradi, N.K., Lansing, L., Asa-Awuku, A.A., Duncan, C.M., 2025. An improved underivatized, cost-effective, validated method for six short-chain fatty organic acids by high-performance liquid chromatography. J. Chromatography https://doi.org/10.1016/j.jcoa.2024.100193. OSTI ID: 2479653.

Zhang, X.Y., Wang, J.F., Zhang, Y.X., Qing, W.H., Lansing, S., Shi, J., Zhang, W., Wang, Z.W., 2024. Anhydrous volatile fatty acid extraction through omniphobic membranes by hydrophobic deep eutectic solvents: mechanistic understanding and future perspective. Water Res. https://doi.org/10.1016/j.watres.2024.121654. OSTI ID: 2540062.

Zhang, X.Y., An, Z.H., Wang, J.F., Lansing S., Amradi, N.K., Haque, M.S., Wang, Z.W., 2025. Long-term effects of cycle time and volume exchange ratio on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) production from food waste digestate by *Haloferax mediterranei* cultivated in sequencing batch reactors for 450 days. Bioresour. Technol., 416, 131771 https://doi.org/10.1016/j.biortech.2024.131771. OSTI ID: 2540060.

Zhang, X.Y., Amradi, N.K., Moore, M., Hassanein, A., Mickol, R.L., McCoy, E.L., Eddie, B.J., Shepard, J.S., Wang, J., Lansing, S., Yates, M.D., Kim Y.T., Wang Z.W., 2025. Enhancement of 3-hydroxyvalerate fraction in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) produced by *Haloferax mediterranei* fed with food waste pretreated via arrested anaerobic digestion integrated with microbial electrolysis cells. Bioresour. Technol., 430, 132536. https://doi.org/10.1016/j.biortech.2025.132536. OSTI ID: 2562203.

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10.2 Thesis/Dissertations Submitted:

McCoy, E., 2024. Generating bioenergy and high-value products from high salinity food waste. Master of Science Thesis. University of Maryland, College Park. https://doi.org/10.13016/ghea-vndl. OSTI ID: 2571058.

10.3 Conference Papers:

Zhang X.Y., An Z., Wang J., Lansing S., Amradi N.K., Haque M.S., Wang Z.W., 2025. Long-term effects of cycle time and volume exchange ratio on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) production from food waste digestate by *Haloferax mediterranei* cultivated in sequencing batch reactors. WEF Joint Conference: Residuals & Biosolids and Innovations in Treatment Technology. May

6 – 9, Baltimore, MD. https://doi.org/10.2175/193864718825159768. OSTI ID: 2571067.
Zhang X.Y., Amradi N., Hassanein A., Lansing S., Wang Z.W., 2023. Salty food waste conversion to bioplastics. WEFTEC 2023, Water Environment Federation, Sep.30 – Oct. 4, Chicago, IL. https://doi.org/10.2175/193864718825159148. OSTI ID: 2571066.

10.4 Scientific Presentations:

Amradi, N.K., Hassanein, A., Lansing, S., 2022. Dark Fermentation for Volatile Fatty Acids to Produce Bioplastics from Food Waste. American Ecological Engineering Society Annual Symposium, Baltimore, Maryland. June 20-23, 2022. OSTI ID: 2571158.
Amradi, N.K., Hassanein, A., Lansing, S., 2022. 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. OSTI ID: 2571159
Amradi, N.K., Hassanein, A., Lansing, S., 2022. Biological Conversion of Food Waste to Bioenergy and Bioplastics. Postdoc Research Symposium, University of Maryland, September 23, 2022. OSTI ID: 2571160.
Amradi, N.K., Hassanein, A., McCoy, E., Lansing, S., Wang, Z., Yates, M.D., 2023. Bioprocessing of Waste for Bioenergy and Biomaterials for Sustainable Bio-Economy in Biorefinery Framework. Symposium on Biomaterials, Fuels and Chemicals. Portland, OR. April 30 - May 3, 2023. OSTI ID: 2571161.
Amradi, N.K., Hassanein, A., McCoy, E. Rose, A., Lansing, S., 2023. Food Waste Biological Conversion into Bioenergy and Bioplastics. American Ecological Engineering Society Conference, Tampa, FL. June 6-9, 2023. OSTI ID: 2571163.
Lansing, S., Hassanein, A., Naresh K. Amradi, N., Wang, Z., Zhang, X., Wahlen, B., Adhikari, B., Yates, M.D., Ge, X., Li, Y., 2022. Valorizing Food Waste through Dark Fermentation to Bioplastic Production. Symposium on Biomaterials, Fuels, and Chemicals. New Orleans, LA. May 2 – 4, 2022. OSTI ID: 2571059
Lansing, S., 2023. Food Waste and Restoring our Circular Economy. American Society of Agricultural and Biological Engineers (ASABE). Omaha, NE, July 9, 2023. OSTI ID: 2571170.
Lansing, S., 2023. Innovations in anaerobic digestion and fermentation for a circular economy (Plenary). BioResTec. Lake Garda, Italy. May 16, 2023. OSTI ID: 2571171.
Lansing, S., 2024. Food waste and restoring our circular bioeconomy (Keynote). American Society of Ag. & Bio. Engineers Biocircular Economy. Chicago, IL. March 28-29, 2024. OSTI ID: 2571172.
McCoy, E., Lansing, S., Hassanein, A., 2023. Increasing Energy Production of Food Waste to Bioenergy and Bioplastics. An AGNR Cornerstone Event: Advancing Agricultural Production Systems, College Park, MD. October 24, 2023. OSTI ID: 2571173.
Wang M.X., Zhao F.J.Z., Zhang X.Y., Huang H.B., Young K., Wang Z.W., 2024, Potential of *Haloferax mediterranei* in conversion of food waste to Poly(3-

hydroxybutyrate-co-3-hydroxyvalerate)-based bioplastics. WaterJAM 2024, Virginia Beach, VA. September 9-12, 2024. OSTI ID: 2571063.

Wang M.X., Zhang X.Y., Zhao F.J.Z, Huang H.B., Kim Y., Wang Z.W., 2025. Pilot-scale conversion of food. waste to Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-based bioplastics by using *Haloferax mediterranei*. AEESP Research and Education Conference 2025, May 20–22, Durham, NC, USA. OSTI ID: 2571064.

Wang M.X., Zhang X.Y., Zhao F.J.Z, Huang H.B., Kim Y., Wang Z.W., 2025. Pilot-scale conversion of food waste to Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-based bioplastics by using *Haloferax mediterranei*. WEF Residuals & Biosolids Conference 2025, May 6–9, Baltimore, MD, USA. OSTI ID: 2571065.

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Zhang X.Y., Amradi N., Hassanein A., McCoy E.L., Lansing S., Yates M.D., Wang Z.W., 2024. Enhancement of 3-Hydroxyvalerate fraction in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) produced by *Haloferax mediterranei* fed with food waste digestate as a substrate. WaterJAM 2024, Virginia Beach, VA. September 9-12, 2024. OSTI ID: 2540132.

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10.5 Media:

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11. Project Team and Roles:

Dr. Stephanie Lansing at University of Maryland (UMD) was the Primary Investigator. She led the overall project and supervised a research scientist (Dr. Amro Hassanein), post-doc (Dr. Naresh Kumar Amradi) and M.S. student (Emily McCoy). The UMD team conducted all Task 2 milestones, except those involving the MEC. The MEC-based milestones and microbial assessments in Task 2 were completed by Dr. Matthew Yates at the Naval Research Laboratory (NRL). He was assisted at NRL by Dr. Brian Eddie, Dr. Rebecca Mickol, and Mr. Marty Moore. Dr. Zhiwu Wang at Virginia Tech (VT) led Task 3 and collaborated on Task 5. He advised one PhD student, Xueyao Zhang, who completed all Task 3 milestones, and another PhD student, Mingxi Wang, who completed all Task 5 milestones. Dr. Brad Wahlen at Idaho National Laboratory (INL) led and was assisted by Dr. Birendra Adhikari on completing all Task 4 milestones. Dr. Yebo Li, Dr. Xumeng Ge, and Mr. James Elias at Quasar Energy Group led Task 5 and assisted on Tasks 2, 3, and 4, including market analyses in Task 4 based on their industrial experience.

12. References

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