Project Narrative

USDA SBIR/STTR Program Priorities

The proposed SBIR/STTR Phase I project is aligned with the following USDA Strategic Goals FY 2022-2026:

- Strategic Goal 1:
  - 1.3 Restore, Protect, and Conserve Watersheds to Ensure Clean, Abundant, and Continuous Provision of Water Resources
  - 1.4 Increase Carbon Sequestration, Reduce Greenhouse Gas Emissions, and Ce Economic Opportunities (and Develop Low-Carbon Energy Solutions)
- Strategic Goal 2:
  - 2.2 Build Resilient Food Systems, Infrastructure, and Supply Chain
  - 2.3 Foster Agricultural Innovation
- Strategic Goal 3:
  - 3.2 Expand Markets for Emerging Technologies, Sustainable Products, and Novel Products

The SBIR/STTR Phase I project is also aligned with the following USDA Science and Research Strategy 2023–2026 priorities:

- 1. Priority 1: Accelerating Innovative Technologies & Practices
  - 1.2 Technology-Enabled Decision Support System
  - 1.5 Diversified Future Systems
- 2. Priority 2: Driving Climate-Smart Solutions
  - 2.5 Bioeconomy
- 4. Priority 4: Cultivating Resilient Ecosystems
  - 4.3 Sustainable Agro- & Aquatic-Ecosystems
  - 4.2 Microbiome Research

Significance of the Problem

In the following section we summarize key challenges in traditional Wastewater Treatment Facilities (tWWT) and microalgae-bacteria (biogenic) Wastewater Treatment Facilities (bWWT)
We also delineate

- Challenges that are considered within the scope of this work
- Challenges that are proposed for future implementation

Table 1: Challenges in microalgae-bacteria wastewater treatment

Challenges	**Reference**
Challenges with traditional Wastewater Treatment Facilities (tWWT)	
high energy consumption	[26.]
stringent pollutant limits necessary before discharge of wastewater	[26.]
greenhouse gas emissions	[26.]
recyclable resource wastage	[26.]
excessive solid landfilling	[26.]
low-value biomass	[26.]
Challenges associated to bWWTs (In scope of this work)	
Any utility-connected algae systems should have their life-cycle evaluation, techno-economic analysis, and energy intensity carefully assessed before being put into utilization	[55.]
life-cycle-assessment (LCA) and techno-economic-assessment (TEA) should justify the economic feasibility and environmental impacts	[48.][49.][40.][55.]
Need further studies that compare tWWTs to bWWTs in terms of LCA and TEA	[32.][37.][36.]
System construction require a high investment	[38.][41.][42.][48.]
High operational cost of microalgae cultivation and biomass harvesting	[34.][42.][48.][51.]
The process should minimize the impact on natural water bodies	[51.]
Algae and bacteria could produce the greenhouse N_2O, which has a global warming potential 298 times higher than CO_2	[40.]
The use of open photobioreactors could increase water footprint in water-stressed areas due to the high evaporation rates	[40.][33.]
There may be risks associated with attempts to remediate water bodies using microalgae, such as the presence of toxic compounds or the occurrence of pollutants in the resulting biomass	[44.]

Lack of basic design and operation guidelines for bWWTs	[32.][34.]
Complicated operation	[32.][37.]
Need to improve process design by optimizing the operating conditions that achieve good yields and productivity	[40.][42.][36.][51.][53.][48.][51.]
Process optimization should combine algal production, pollutant removal rate, atmospheric emissions (NH3, CO2, N2O), and associated costs	[35.]
Limited large-scale studies	[28.][29.][36.][37.][40.][42.][47.][54.][49.][55.]
Limited long-term studies that account for fluctuations seasonal and wastewater composition conditions	[29.][33.][41.][47.][48.][42.]
Downstream processing alone can account for approximately 60% of the total cost of the production process	[54.]
Need high efficiency and low-cost downstream processing (harvesting, dewatering/drying, extraction, conversion)	[48.][51.][53.][54.][49.][51.]
There are many challenges associated with the separation of suspended microalgae biomass from the treated effluent after the bioremediation where the treated wastewater must be free from the microalgae biomass	[28.][32.][49.][37.]
The harvesting procedure can be somewhat inefficient and must be coupled with recirculation systems to minimize water and biomass losses, while incentivizing nutrient depletion from the culture medium	[48.][54.][51.][44.]
Advances in automation technology, remote and efficient monitoring, and troubleshooting in production systems may mitigate high operational costs	[36.]
Monitored the whole system regularly to: ● optimal performance ● changes in the environmental conditions ● detect and mitigate the formation of algal blooms and other ecological impacts	[32.][37.][51.][55.]
Infrastructure designs should consider effective integration with existing wastewater treatment infrastructure	[51.]
Open systems may require a large cultivation area	[58.]
Wastewater environments stimulate the growth of algal feeders, protozoans, and other organisms that could negatively impact a microalgae culture	[28.][32.][48.][34.]

Need to remove total suspended solids (TSS) (could clog or cover the surface area of the microalgae, limiting their ability to photosynthesize)	[55.][51.][37.]
pH adjustment	[33.]
adjust concentration of nitrogen and or phosphorus in wastewater	[33.]
Remove contaminants that can inhibit microalgae growth or pollutant removal capabilities ● elevated concentration of toxins such as phenolic compounds and many other organic contaminants. ● Antimicrobial agents and antioxidants inhibit the eradication process.	[51.][37.]
Challenges associated to bWWTs (Future work)	
Growth of microalgae requires a considerable time.	[55.]
Need better understanding of strain metabolic acclimatization to wastewater	[30.][33.]
Strain prospecting - adaptability to harsh conditions	[32.][30.][40.][49.][42.][48.][41.][43.][36.][53.][55.][51.][29.][36.]
Strain prospecting - increased ability degrade pollutants (including emerging pollutants)	[32.][30.][40.][49.][41.][42.][55.][29.][36.]
Strain prospecting - higher productivity in terms of growth and composition profile	[29.][30.][49.][42.][48.][36.][55.][36.]
Strain prospecting - increased CO2 fixation ability	[42.]
Genetic/metabolic engineering - improved adaptability to harsh conditions	[32.][30.][37.][40.][46.][56.]
Genetic/metabolic engineering - increased ability degrade pollutants (including emerging pollutants)	[32.][30.][40.][46.]
Genetic/metabolic engineering - tendency to form flocs to facilitate harvesting	[40.]
Need further understanding of metabolic consortia interactions and key pathways used ● better synergy to promote growth ● better pollutant removal capacity (including trace elements) ● better stability	[40.][42.][55.][33.]

• withstand harsh conditions • better understand metabolic changes under at different conditions • better understand the specific bio-flocculation requirements of polyculture systems	
Need more algae-fungi consortia studies	[40.][36.]
Contamination and presence of toxic metals in the wastewater make thie microalgal biomass unsuitable for food applications directly	[34.]
Biosafety and regulatory affairs (including GMO) • proper biosafety analysis is required to ensure the quality of products, which should comply with regulations. • It is important to determine the chemical composition and presence of toxic compounds in the microalgal biomass, especially if used as feedstock for biostimulant, biofertilizer, or animal feed production, all applications that are extremely important to feeding the starved and growing population	[40.][45.][53.][46.][47.][51.]

Background

tWWT strategies are an important part of human settlement and an ongoing challenge owing to the increasing human population [71.]. Existing challenges related to tWWT infrastructure include high financial costs, in the form of installation and operational and maintenance costs (energy consumption, machinery, labor), environmental concerns related to the excess sludge discharge, generation of large amounts of greenhouse gasses (carbon dioxide (CO_2), methane (CH_4), and N_2O), uneven removal efficiency, and land availability. Circular economy principles, which impose stringent pollutant limits that are necessary before discharge and reuse of wastewater, have also added to these pressures [101.]. Moreover, emerging pollutants, such as pharmaceutical and personal care products are contributing to the pollutant removal complexity [21.]. Therefore, the evolution of well-structured WWT technologies and practicable economic approaches is becoming an increasingly important challenge.

These struggles may be validated by the high amount of violations found in the Clean Water Act (CWA) Compliance Monitoring program by the United States Environmental Protection Agency (EPA) [70.]:

- Municipal Plants (a.k.a publicly owned treatment works (POTW))
    - 9,281 out of 15,234 have existing violations
- Biosolids Facilities
    - 1,619 out of 2,329 have existing violations

Furthermore, in 2021, a detailed US infrastructure report graded the wastewater treatment facilities in the United States with a D+ [69.].

Microalgae-based wastewater refinery modules have the potential to mitigate existing tWWT burdens in terms of improving the financial burden and environmental impact.

Research and Development

As many infrastructure components are soon to reach EOL and face challenges previously described, better planning mechanisms are needed:

- We focus on taking a step back and modularizing many existing needs to characterize the problem.
- We provide a tool that attempts to capture the heterogeneity inherent to effluents, process design, valorization, environmental impact, investment cost, and operation.
- The idea is that it is as close to physical systems as possible.
- We also attempt to include important TEA and LCA studies into the design space.

Our proposal for Phase 1 is to first start with an end-to-end process flow and continually improve upon it during Phase 2.

We expect that if successful, this work may contribute toward the following future efforts:

- We expect that the initial effort can help plan future infrastructure needs as we start planning for the replacement of existing tWWT infrastructure that is soon to reach end-of-life (EOL).
- We hope this work can help regulators like CWA make better recommendations and design choices.
- We expect that our end-to-end analysis may elucidate important upgrading pathways that may otherwise remain hidden.
- Finally we aim to contribute the developed work back to the open source community, such that better designs can be further developed by the research community.
- Lead to the development of a new innovation in sensors,)
- Lead to the development of new innovation in:
  - process design,

        - automation
        - improved effluent quality
    - sensor and infrastructure technology

Technical Objectives

- Technical Objectives
    - Develop an end-to-end algae-bacteria wastewater refinery process simulator that can adapt to various optimization criteria.
    - Finetune the model by leveraging datasets generated from real-world cultivation systems.
    - Identify key factors influencing the performance of the models for a given optimization criteria.
    - Compare the model against existing wastewater, saltwater, and freshwater biogenic simulation systems in terms of operational costs, environmental impact, economic investment, and real-world adaptability.
- Technical Questions
    - Does the model accurately predict the operational costs, environmental impacts, economic investment, and real-world adaptability?
    - What are the key factors that most significantly influence the performance of the simulation model under given target optimization criteria?
    - How do the algae-bacteria wastewater refinery modules compare against existing biogenic wastewater treatment systems?
    - How do the algae-bacteria wastewater refinery modules compare against freshwater or saltwater cultivation systems?
    - Under what conditions would the economic benefits justify an investment in an algae-bacteria module?
    - Are there any bottlenecks or limitations in the current approach that could hinder applicability to real-world settings?

Work Plan

<u>Background</u>

To achieve comparability with other wastewater simulation processes, we standardize our study to the "QSDsan" framework [14.]. This framework facilitates the integration of system design, process modeling & simulation, techno-economic analysis & life cycle assessment (TEA-LCA). This framework also helps us to benchmark our solution with other wastewater and bioconversion designs that have been developed over the years.

- The QDSsan framework has a LCA and TEA module that helps facilitate this type of analysis.

<u>Scope and Limitations</u>

Covered in a previous section (**"Significance of the Problem"**)

<u>Approach</u>

The study is structured to systematically assess the design space of algae-bacteria cultivation strategies under a variety of media, cultivation modes, environmental factors, infrastructure, biomass conversion modules, and target applications.

For the overall methodology we follow 5 sections:

1. Data Gathering, Preprocessing, and Loading
2. Define of System Boundaries and Analytical Workflow
3. Calibrate and Validate the Analytical Workflow with Real-world experimental monitoring campaigns
4. Run Scenario Analysis and Gather results
5. Reporting and Dissemination

<u>Work Sections</u>

1. Data Gathering, Preprocessing, and Loading

- Overview
  - The main purpose of this task is to gather all the relevant datasets, standardize their data-structures, and load the data into a database.
- Outcome
  - This will allow the simulation models to have a consistent view of the data.
- Considerations
  - These values should be represented as a discrete point-in-time capture
- Validation
  - Report a unit test for each dataset that reflects proper data access and schema consistency at a given point in time.
- Recommended Data sources

    - Wastewater Composition
        - [101.], [2.], [3.].
    - Weather Data
        - [7.], [8.].
    - Multi-year Algae-Bacteria Campaigns
        - wastewater algae-bacteria monitoring campaigns
            - [9.], [10.], [11.].
        - algae cultivation monitoring campaigns
            - [12.], [13.], [65.], [66.].
- Tasks
    - Gather the following data points from the recommended literature specified in this section
        - Time
        - Culture Media
            - Wastewater
                - Municipal sewage water
                    - Secondarily Treated Sewage
                - Aquaculture wastewater
                    - Fish and Shrimp farms
                - Agro-industrial wastewater
                    - Swine, piggery, cattle, and poultry industries
            - Saltwater
            - Freshwater
        - Culture Media Parameters
            - pH value
            - Volume
            - temperature
            - water composition
        - Gasses
        - Weather Patterns
        - Coarse Grained Algae-Bacteria Microorganisms
    - Preprocess the datasets

            - Handle missing values
    - Register the data schemas into a "Feast" [4.] metastore.
        - Follow "Feast" documentation for best practices to populate a featurestore
    - Load the dataset into a "DuckDB" [5.] database.
        - Follow "DuckDB" documentation for best practices to populate a featurestore
    - Validate that you can query each database through the "Ibis" [6.] dataframe framework.
        - Follow "Ibis" documentation for best practices to populate a featurestore.

2. System Boundaries and Analytical Workflow System Design
    - Overview
        - The main purpose of this task is to define and standardize all the process units (traditional and microalgae-based WWT) that are within the system boundaries of this study.
        - This task should also group the process units into dependent, independant, and control variables.
        - Furthermore, each process unit should be laid-out as part of an end-to-end system flow.
    - Outcome
        - The process units are grouped into dependent, independant, and control variables within the system boundaries.
        - The process units allow for cultivation sensor data input
    - Validation
        - Each process unit is properly interconnected and validated by reporting the results of integration tests.
    - Tasks
        - Cultivation
            - Influent (INDEPENDENT VARIABLE)
                - Water composition
                    - Municipal sewage water
                        - Secondarily Treated Sewage
                    - Aquaculture wastewater
                        - Fish and Shrimp farms

            - Agro-industrial wastewater
                - Swine, piggery, cattle, and poultry industries
    - Saltwater composition
    - Freshwater composition
- Reactor Type (INDEPENDENT VARIABLE)
    - Outdoor
        - Cylindrical PBR
    - Semi-Outdoor
        - Cylindrical PBR
        - Tubular PBR
        - Membrane PBR
- pH Model (CONTROL VARIABLE)
    - NH4+, NH3, CO2, HCO3-, CO32-, H+, OH-
- Wastewater Pretreatment prior to microalgae cultivation
    - Secondary Treatment scenario
        - Primary treatment (solids and fats removal)
        - Dilution
    - Tertiary Treatment scenario
        - Anaerobic Pond
            - Sedimentation
            - Sedimentation and Filter
        - Dilution
- Cultivation Mode (INDEPENDENT VARIABLE)
    - Batch
        - 12-25 days per growth/harvest cycles
    - (Semi)-Continuous
        - harvest every 2-7 days.
- Biomass Model based on the ALBA model (Casagli et al., 2021) [10.].
    - Biomass composition (CONTROL VARIABLE)
        - Algal biomass composition (C100, H183, O48, N11, P)
        - Bacterial biomass composition (C60, H87, O23, N12, P)

            - Growth dynamics and constraints (INDEPENDENT VARIABLE)
                - physical growth parameters (CONTROL VARIABLE)
                    - Light
                    - Temperature
                    - Gas-liquid transfer
                    - Hydraulics
                        - Evaporation
                        - Precipitation
                - chemical growth parameters (CONTROL VARIABLE)
                    - pH growth dependence
                - Growth kinetic type (CONTROL VARIABLE)
                    - multiplicative/minimum
    - Harvesting (INDEPENDENT VARIABLE)
        - Sedimentation
        - Flocculation
        - Membrane Filtration
    - Biomass Recovery (INDEPENDENT VARIABLE)
        - (Continuous) Belt-filtration
        - (Continuous) Centrifugation
    - Drying (INDEPENDENT VARIABLE)
        - Sun
        - Belt-drying
        - Oven
    - Applications of converted Biomass (INDEPENDENT VARIABLE)
        - Biofertilizer
            - None
        - Feed
            - protein Extraction
    - Storage (CONTROL VARIABLE)
        - Transportation

                - Degradation Losses

3. Analytical Workflow Calibration and Validation

- Overview
    - The main purpose of this task is to ensure that the predictive capabilities of the analytical workflow are accurate before running more complex analytical workflows.
- Outcome
    - The Analytical Workflow System Design should be properly calibrated to reflect realistic industrial-scale and multi-year Algae-Bacteria-based WWT process designs.
    - The workflow should provide a baseline for LCA and TEA design optimization.
- Validation
    - Report a multi-year F1 accuracy of the calibrated system.
- Basic Workflow
    - 1. Initial Conditions
        - Overview
            - Describe a set of parameters that will inflow to the system at each timestep t, where t can be expressed in minutes, hours, days, weeks, months
        - Data points
            - Water influent composition
            - Gas influents composition
            - Culture Media Parameters
            - Weather Patterns
            - Initial Algae-Bacteria Biomass
    - 2. Finite State Prediction
        - Overview
            - The main objective of this finite-state-prediction model is that given a set of states/data-points, the values of next timestep t can be successfully predicted with a high degree of accuracy.
    - 3. Prediction Target

            - In general, considering that we want to understand the TEA and LCA aspects of a process design, the following prediction target will be the most important for this study:
                - Biomass concentration
                    - algae
                    - bacteria
                - (optional) Biomass Composition
                    - protein
                    - lipids
                    - carbohydrates
                - Nutrients
                    - total nitrogen (TN)
                    - total phosphorus (TP)
                    - chemical oxygen demand (COD)
                    - dissolved oxygen (DO)
                    - total organic carbon (TOC)
                    - (optional) Metals

4. Scenario Analysis

- Overview
    - The main purpose of this section is to run a variety of end-to-end design combinations based on the calibrated model.
- Outcome
    - The Scenario analysis should provide detailed and comprehensive information that leads to answering the Technical Objectives/Questions.
- Validation
    - Should be able to answer our initial questions:
        - What are the key factors that most significantly influence the performance of the simulation model under given target optimization criteria?
        - How do the algae-bacteria wastewater refinery modules compare against existing biogenic wastewater treatment systems?

            - How do the algae-bacteria wastewater refinery modules compare against freshwater or saltwater cultivation systems?
            - Under what conditions would the economic benefits justify an investment in an algae-bacteria module?
        - Ensure that the generated data is plottable.
- Workflow
    - 1. Set up the analytical workflow
    - 2. Run Scenario Analysis and Gather Summary Statistics
    - 3. Generate Reports for Optimization Targets

5. Reporting and Dissemination

- Overview
    - The main purpose of this task is to compile the results int a document and slide-deck that can serve as an advanced manuscript for online publishing, presentations, and/or a Phase 2 proposal.
- Outcome
    - This section should result in a quality manuscript that details the experimental workflow and results obtained in the study.
    - Similarly, a slide-deck should be developed for academic or related dissemination purposes.
    - Lastly, if the results were favorable, these documents should serve as a baseline to develop a Phase 2 application.
- Recommended Workflow
    - Compile Results & Generate Tables and Figures
    - Develop an outline for the Document and Deck
    - Write Document and Deck Draft
    - Review/Edit Document and Deck Draft
    - Complete Document and Deck
    - Publish Results

<u>Software tools needed</u>

- Software
    - "Python" [68.]. (latest allowable version).

    - "DuckDB" [5.]. database (latest allowable version).
    - "Feast" (Python library) [4.]. metastore (latest allowable version).
    - "ibis" (Python library) [6.]. dataframe framework (latest allowable version).
    - "qsdsan" (Python library) [69.]. Quantitative Sustainable Design for sanitation and resource recovery systems (latest allowable version).
    - "optuna" (Python library) [15.]. A hyperparameter optimization framework (latest allowable version).

Roles, Location, and Timeline

<u>Roles</u>

- Project Manager
- Data Engineer
- Software/QSDsan Engineer
- Data Scientist
- Consultants/Consultation-Topics
    - Environmental Engineer
    - Wastewater Process
    - Biogenic Wastewater Treatment
    - Wastewater Infrastructure
    - LCA for the Harvesting Unit

<u>Location</u>

For Phase 1, all the work is to be performed remotely within the United States.

<u>Timeline</u>

Note: For planning purposes, the timeline represents a sequential dependency graph that reflects a worst-case scenario. In practice, not all tasks will be sequentially dependent.

1.Planning Phase:

(March-15 to April-15): Prep and Planning

- Compile the team and agree on implementation timelines, roles, backups, etc.
- Ready the Work Plan

(April-15 to April-30): Prep and Planning

- Team gathering, Communicate work plan, timelines, requirements, reserve any off dates, etc.

(May-1 to May-30):

- Team preparedness buffer time

(May 30) All workplan prerequisites shold be met

2.Work Plan Execution Phase

(June 1 2025) Early start

- Data (Data Engineering team in consultation with an Environmental Engineering expert)
    - Data Gathering
    - Data Preprocessing
    - Data Loading & validation
    - Buffer

  Ends Jul-30-2025
- Workflow Design (Software/QSDsan Engineering team in consultation with a biogenic and traditional WWT infrastructure expert)
    - Cultivation Module
    - Harvesting and Biomass Recovery
    - Drying, Target Application Conversion, and Storage
    - Buffer

  Ends Sep-22-2025
- Analytical Workflow Design (Data Science team and Software/QSDsan Engineering team in consultation with an LCA expert)
    - Initial Conditions Setup
    - Finite State Prediction
    - Prediction Target
    - Buffer

  Ends Nov-22-2025
- Scenario Analysis (Data Science team in consultation with an Environmental Engineer)
    - Analytical Workflow Definition
    - Run scenario Analysis
    - Generate Reports
    - Buffer

  Ends Jan-7-2026

- Reporting, Dissemination, and Phase 2 Prep (Project Manager)
    - Compile Results & Generate Tables and Figures
    - Develop an outline for the Deck and Draft
    - Write Document and Deck Draft
    - Review/Edit Document and Deck Draft
    - Complete Document and Deck
    - Publish Results
    - Buffer

  Ends Feb-28-2026

(Feb 28 2026) Phase 1 Project End

Market Opporutnity

Within the United States alone, there are over 16,000 publicly owned WWT facilities that collectively treat over 62.5 billion gallons of wastewater per day [69.]. Of these facilities, ~44% use activated sludge, ~33% are traditional wastewater ponds, and ~15% are biofilm systems [72.].
In 2019, though, the annual water infrastructure capital investment gap is $81 billion and the total gap investment gap could grow to more than $434 billion by 2029.[69.]. Estimates indicate that utilities spent over $3 billion in 2019 with cost that is projected to grow by an average of 5% annually [69.].

Our initial target market and benefit to the customer would be the following:

- First target: POTWs and Biosolids facilities that are currently failing to pass the CWA Compliance Monitoring program
    - critical need to improve pollutant removal conditions
- Second target: treatment plants nearing their EOL lifespans
    - better planning
- Third target: wastewater that are under heavy financial pressures
    - alleviate financial burden to society by maximizing valorization
- Fourth target: wastewater that are not producing activated sludge
    - maximize the valorization of biosolids

A driving key driving factor for the development of the field may be specified in the Effluent Guidelines Program Plan 15 developed by EPA [74.] which specifies industry-specific wastewater Effluent Limitations Guidelines and Standards (ELGs) based on the performance of demonstrated wastewater treatment technologies.

We expect that potential valuable market opportunities may come in developing potential product and services offerings the form of:

- Physical Modules development
    - sales
- Automated Operation
    - sensors sales
    - software licensing
- Services
    - managed services

A secondary related potential market opportunity may come in the form of developing a brand of bio-based products from the resulting wastewater biomass, such as, biogas, household items, fertilizers, feed, industrial commodity chemicals, and higher value products like enzymes and pigments.

Each year, nearly 1000 km3 of wastewater is generated around the world with about 300 km3 discharged as municipal wastewater and over 600 km3 as industrial wastewater [75.], where over 80% of the world's wastewater is released back into the environment without treatment [76.]. Wastewater contains various elements (e.g., nitrogen, phosphorus, organic compounds, and heavy metals) that can cause water eutrophication if released into natural water bodies without treatment.

We hope that our project may provide key insights toward the following categories of the The United Nations 2030 Agenda for Sustainable Development [77.]:

- Better Water utilization
- Climate Action
- Responsible Production
- Industry Innovation and Infrastructure
- Food