Final Technical Report (FTR)

Cover Page

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1. Executive Summary:

The motivation of the project was to expand the domestic resource potential of the bioeconomy through creation of a low cost supply of algae biomass for bioproducts. The importance of the project relates to evaluating the potential to create a low cost supply of algae biomass through scale-up of a specific outdoor biofilm microalgae system identified as a rotating algae biofilm reactor [RABR] for the potential for full scale sustainable application to municipal water resource recovery facilities (WRRF) utilizing anaerobic digestion (AD). The overall goal of the project was the effective integration of municipal wastewater treatment of AD effluent in a sustainable operation with upcycling of the nutrients nitrogen and phosphorus into value bioproducts. Because of the large amount of the mass of nutrients in a WRRF, including phosphorus and nitrogen, that are present in the AD stream, the focus of the project was on utilizing that stream as a medium for algae cultivation.

This project added understanding of the positive potential to produce microalgae biomass in a biofilm construct cultivated on high nutrient concentration water and to utilize the harvested biomass for production of liquid biofuels and other bioproducts including bioplastic and biofertilizer. In addition, through this project, the discovery and understanding of biofilm microalgae enhanced precipitation of the slow release fertilizer struvite was accomplished. This project also produced an understanding of the improvement of bioplastic structure through blending with biofilm microalgae with a high-ash content. Improved understanding of the recycling potential of the bioplastic was accomplished through demonstration of the biodegradability of the bioplastic in a full-scale aerobic composting system after six months of treatment at the largest WRRF in Utah, the Central Valley Water Reclamation Facility (CVWRF) in Salt Lake City, Utah.

Concerning the technical effectiveness of the RABR system, this project demonstrated the potential for both cultivating and harvesting biofilm microalgae in the same biological reactor using mechanical harvesting of the cultivated biofilm thus eliminating the need for a separate reactor for separating the microalgae from the water used as a medium for growth. The technical effectiveness and economic feasibility of the RABR system was demonstrated through a techno-economic analysis (TEA) (Watkins, et al., 2024). The TEA demonstrated that RABR-grown algae can contain a high content of ash (30–60 wt %, dry basis), especially in AD effluent, that influences the technical and economic feasibility of bioproduct conversion processes. This project produced bioplastic as an economically viable bioproduct from multi-culture biofilm microalgae.

This project is beneficial to the public through its contribution to the bioeconomy in the form of bioproducts while simultaneous improving public health and the environment. The conversion process for bioplastic production from whole algae has the highest income:expense ratio and the most cost-competitive pricing compared with modeled processes (Watkins et al., 2024). The biodegradability of the produced bioplastic protects public health from accumulation and exposure of plastic chemicals, while the removal of nutrients from wastewater contributes to environmental protection by mitigating harmful algae blooms in receiving waters used for recreation, power, and drinking water supplies. In conclusion, this project has demonstrated upcycling of wastewater with sustainable production of bioproducts that support the bioeconomy.

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3. Background:

The word algae is most often used within the context of including both eukaryotic and prokaryotic organisms, however the term microalgae is a more accurate description that includes both types of photosynthetic microorganisms that are microscopic as individual cells or cell clusters, but can be visible in the form of biofilms and floating algae blooms on lakes and other water bodies. Macroalgae are multicellular, visible to the naked eye, and commonly known as seaweed that includes kelp, nori, and sea lettuce. The project described in this report utilized microalgae that include cyanobacteria and eukaryotic photosynthetic cells. Therefore, in this project report, the more general term algae is used and refers to microalgae and algae biofilms refers to microalgae biofilms.

The engineering of algae-based biofilm reactors for dual applications for treating municipal and industrial wastewaters and for providing feedstock for biorefinery and bioproducts has been reviewed and addressed by Christenson and Sims (2011, 2012), Kasaano and Sims (2014), Arora, et al. (2021), Goldsberry et al. (2023), Wood et al. (2022), and the Water Environmental Federation (WEF 2025). Algae biofilm systems have been demonstrated to remove total dissolved solids and suspended solids in wastewater (Wen, et al., 2023; Hodges et al., 2017). Algae biofilm reactors for water reclamation through nutrient recovery from municipal and industry wastewaters are a mode of biological treatment utilizing naturally occurring mixtures of algae types and bacteria that self-organize into a fixed-film or attached-growth contiguous structure held together by a self-produced extracellular matrix that is easily harvested by physical scraping. Phosphorus-hyperaccumulating microalgae including the green alga *Chlamydomonas pulvinate* and diatoms *Eolimna minima* and *Craticula molestiformis* have been identified in biofilm systems (Schaedig, et al., 2023). These reactors therefore combine both an upstream process (biofilm nutrient uptake) and a downstream process (biofilm separation) in one unit process/operation (WEF, 2025). In addition to applications for removing nutrients, cultivated algae biofilms have recently received increased focus for producing value bioproducts, including biofuels, biofertilizer, bioplastics, and high value chemicals, as well as serving as sources of chemicals for improved animal gut health and plant growth (Watkins, et al., 2024; Wood et al., 2015; Chang, et al., 2021; Goldsberry et al., 2023).

These reactors generally consist of a substratum or support platform for algae cultivation. The substratum is rotated or moved in a pattern to alternatively expose the growth surface to the water to contact chemical constituents and inorganic carbon in the water and to the air to contact light, as photosynthetically active radiation, and carbon dioxide. Criteria for the design, operation, and monitoring of algae-based biofilm reactors are being developed based on testing at laboratory, greenhouse, pilot scale, and full-scale utilizing a growing body of evidence generated with new tools and methods of molecular biology and of biofilm instrumentation to understand algae-bacteria interactions within high population densities and contiguous environments within biofilms (Bernstein et al. 2014, WEF, 2025). Mixed cultures of algae and bacteria generally occur within biofilms and provide intimate mass transport of oxygen from algae to bacteria and carbon dioxide from bacteria to microalgae (Bernstein et al, 2014).

Algae biofilm reactors have several configurations that have been described in the literature (WEF, 2025), with variations including revolving algae biofilm (RAB) reactors (Schaedig, et al., 2023; Zhao et al., 2018; Gross, et al., 2013), rotating algae biofilm reactors (RABR) (Christenson and Sims, 2012; Watkins, et al., 2024; Wood et al. 2015, 2022; Peterson, 2024; and Davis, 2025), and Algal Turf Scrubbers[R] (ATS) where the water to be remediated is pulsed in waves across a sloped floway over biofilm to removed nutrients (Adey, 2011). Sandia's integrative algae flow-way system allows net-CO_2 negative biofuel production from waste nutrients resulting from a treating waterway. This system remediates (or reclaims) major nutrients, metals, and organic and inorganic carbon from surface waters (Sandia, 2025). These designs illustrate different patterns of contacting biofilm, liquid, and gas phases. A summary and review of several microalgae biofilm technologies has been conducted by Osorio et al. (2021).

Some advantages of algae-based biofilm systems include the following (WEF, 2025):

- Combines algae cultivation and separation in one reactor, eliminating the need for clarification, filtration, or centrifugation.
- Minimizes power requirement for solids handling through harvesting by simple scraping
- Eliminates need for oxygen addition, thereby reducing power costs,
- Reduces power costs of operation through management of duty cycle (intermittent rotation),
- Eliminates oxygen toxicity, pH increase to toxic levels, and self-shading that occurs in suspended culture photobioreactors,
- Facilitates treatment of turbid and/or colored water as biofilm is rotated above water surface,
- Avoids limitation of shallow depth of light penetration through the water.

Sandia's integrative algae flow-way system allows net-CO2 negative biofuel production from waste nutrients resulting from treating waterway. This system remediates (or reclaims) major nutrients, metals, and organic and inorganic carbon from surface waters. It consists of two separators at each end of the waterway to allow efficient flow-through and nutrient collection of water for the attached algae. The first water flow-through allows the attached algae to absorb nutrients and trace metals before flowing out to the other end. In addition, the flow-way system contains an embedded hinged container which allows automated harvest of the grown algae into a reservoir without using external power sources. Once harvested in the reservoir, the hinged container can return to its original position for further harvest of the rest of the algae. The flow-way system allows customized algae to grow alongside the device to collect fertilizer runoffs including nitrogen (N), phosphorus (P) and/or trace metal pollutants: arsenic, selenium, lead, mercury. Algae grown from the device can be further collected and processed for biomass production with an embedded hinged container within the system without the use of external power sources.

The state of the practice with other published projects is similar to this project in the use of multiculture algae types in engineered biofilm systems for CO_2 sequestration, nutrient removal from treated water, and production of liquid biofuels (WEF, 2025). However, the project reported here is different in the emphasis on treatment of anaerobic digester (AD) effluent, production of other bioproducts including bioplastic with high ash content, and algae-facilitated production of struvite fertilizer using algae biofilm technology. While Chen et al. (2012) found that non-filamentous green algae like Chlorella could tolerate elevated nutrient levels, thriving in effluent with 200 g m^{-3} of total nitrogen and 2.5 g m^{-3} of total dissolved phosphorus that allowed open suspension system to achieve an algal biomass productivity of 6.83 g m^2 d^{-1}, AD effluent contains nutrients at even higher concentrations, with total nitrogen at 500 mg/L and total phosphorus at 50 mg/L at the host site for the project reported here.. The project reported here is also different in the testing of the biodegradability of the bioplastic product. This project is also different in developing the first TEA that incorporates bioplastic production and comparison with liquid biofuels production from AD wastewater.

Another difference of this project from other published work is the evaluation of power requirements as affected by the direction of rotation of the biofilm platform through the water to be treated. Power is used to rotate the biofilm and support additional systems such as pumping media. For example, rotating the biofilm in a pond requires about 0.65 W m^{-2}, while another 0.22 W m^{-2} is needed for operations like pumping water and running blowers (Morales et al., 2020). In biofilm systems, managing HRT also involves controlling shear stress to prevent sloughing, or detachment of biofilm fragments due to liquid flow. Adjusting flow velocity in stationary systems or rotation speed in rotating biofilm systems can mitigate this issue, ensuring stability and productivity in the culture (Gross et al., 2013). For the research discovery in the project reported here, the direction of rotation of the shaft connecting the biofilm shelves was observed to affect the power demands for maintaining the same rate of rotation through the anaerobic digester effluent that served as feed to the pilot scale RABR.

This project has leveraged the published work of other projects for use in determining effect of nutrient concentration on microalgae, scale up opportunities, selection of operating parameters including harvesting frequency and rate of movement of the biofilm through nutrient enriched water, as well as applications of biomass feedstock for bioplastic production based on the work of Algix Bloom that utilized microalgae cultivated in suspension and with different nutrient sources to produce bioplastic materials.

Table 1. Biofilm reactor types, media, substrata, productivity, and information sources

Biofilm Reactor Type	Culture Medium	Substratum Material	Footprint Productivity (g/m2 - day)	Substratum Productivity (g/m2 - day)	Reference
Revolving algal biofilm (RAB) cultivation	Synthetic	Cotton duck	46.8	N/A	(Gross et al., 2015)
RAB cultivation system	Synthetic	Cotton duck	21.5 18.9 (ash-free)	5.8 5.1 (ash-free)	(Gross and Wen, 2014)
RABR with spool harvester	Municipal wastewater	Cotton rope	31	N/A	(Christenson and Sims, 2012)
Hybrid high-rate pond biofilm reactor	Municipal wastewater	Cotton interlace	N/A	9.99	(De Assis et al., 2017)
Algal turf scrubber	Eutrophic riverine	3D mesh screen	53.7	N/A	(Witarsa et al., 2020)
Algal turf scrubber	Dairy manure wastewater	Mesh nylon netting	24	N/A	(Mulbry et al., 2008)
Algadisk system	Municipal wastewater	Polyvinyl chloride	8.4	4.7	(Sebestyén et al., 2016)
Phototrophic biofilm reactor	Synthetic	Polyethylene woven geotextile	N/A	4.5	(Boelee et al., 2014)
Twin-layer biofilm photobioreactor	Synthetic	Printing paper	50[a]	12.5	(Schultze et al., 2015)
Rotating drum biofilm reactor	Synthetic	Canvas	54.5	N/A	(Shen et al., 2016)
Pilot-Scale RABR	Anaerobic Digester Effluent	Polyethylene Carpet	8.8 4.5 (ash-free)	2.8 1.4 (ash-free)	(Jeppesen, 2024)
Modified Pilot-Scale RABR	Anaerobic Digester Effluent	Polyethylene Carpet	**26.8 ± 2.7** **15.5 ± 1.3 (ash-free)**	**5.3 ± 0.5** **3.07 ± 0.2 (ash-free)**	(Haag, 2025)

4. **Project Objectives:**

This project will have impacts on achieving national goals of clean energy progression and economic benefit to the U.S. Experimental studies and economic analysis were conducted to compare different processes for bioproducts conversion of a high-ash microalgae biofilm grown using a RABR treating 0.6 million gallons per day of anaerobic digestion centrate at the CVWRF. Process and economic models were developed and compared for three conversion processes: 1) the production of bioplastics using whole biofilm algae, 2) the production of bioplastics with a lipid-extraction pretreatment, and 3) the production of biocrude via hydrothermal liquefaction. Techno-economic analysis was performed for each conversion process, including three cases for algae productivity: 231, 391, and 577 metric tons per year (dry basis). The calculated value for the minimum plastic selling price (MPSP) of bioplastics produced from biofilm algae ranged from $4,050 to $3,520 per metric ton based on baseline and final productivity cases of the RABR, respectively. The extraction of lipids in addition to bioplastic production results in an MPSP of $4,570 to $4,000 per metric ton for the same productivity cases. The relatively small production scale and complex processing for hydrothermal liquefaction results in a minimum fuel selling price of the biocrude of $5.32 per gallon of gasoline equivalent. The MPSP for the extraction of lipids in addition to bioplastic may be further improved by upgrading the extracted lipids into biofuels or high-value plastics. The conversion process for bioplastic production from whole algae has the highest income:expense ratio from 108% to 120% and the most cost-competitive pricing of the three modeled processes. The calculated value for the MPSP of bioplastics produced from algae is within the range of commercial bioplastics products. Based on the results of the TEA conducted for this project, bioplastic production from whole biofilm algae is an economically viable process that can be used to valorize biofilm algae biomass harvested from rotating algae biofilm reactors treating anaerobic digester centrate at municipal wastewater reclamation facilities.

A summary of the tasks within the Statement of Project Objectives (SOPO) is provided below. These tasks include: (1) Increase energy efficiency for Total P and Total N removal; (2) Improve Yield of polyculture biofilm microalgae feedstock in indoor controlled tests and in outdoor tests; (3) Improve cost of Total P and Total N removal; (4) Meet Total P target after remediation; (5) Produce bioplastic from biofilm microalgae; (6) Provide TEA for algae cultivation and conversion to biofuel via HTL and to and conversion to biofuels and bioplastic via lipid extraction; and (7) Provide LCA.

7.Project Results and Discussion:

Objective 1. Increase energy efficiency for total phosphorus removal and total nitrogen removal. Milestones 3.1, 8.1, and 9.1

The effect of reduction in RPM/peripheral velocity and duty cycle (25% time "on" and 75% time "off") on increasing energy efficiency through reduction in power consumption is shown in Table 2. Power consumption was reduced by 37% by reducing the rotational speed from 1.0 to 0.5 RPM at a duty cycle of 1.0, which represented reductions from 0.38 ft to 0.2 ft s^{-1}. At a duty cycle of 0.5, power consumption was reduced by 50% at a rotation speed of 1 RPM. The maximum reduction in power was 75% that was achieved by reducing the rotation from 1.0 to 0.25 RPM and the duty cycle from 1.0 to 0.5. These results were determined for the pilot RABR with six total units of surface area with $6m^2$ per unit for a total of $36m^2$ surface area for a volume of $11m^3$ with a packing factor of 3.3/m (surface area/volume). Therefore an increase in energy efficiency was possible to achieve by adjusting either the RPM and/or the duty cycle. Therefore, We were able to achieve power reductions by reductions in rpm and duty cycle that are applicable to long-term management of power requirements for the RABR.

Table 2. Reductions in power consumption with pilot RABR RPM/peripheral velocity values

RPM/ft s^{-1} peripheral velocity	1.0/0.38	0.5/0.2	0.25/0.1
Instantaneous power draw (Watts)	64	40	32
Decrease in power consumption (%) Duty Cycle 1.0	N/A	37	50
Decrease in power consumption (%) Duty Cycle 0.5	50	69	75

We also measured power requirements for RPM values greater than 1.0 PRM and duty cycle reductions from 1.0 to 0.5 to 0.25 (Table 3). Results demonstrated significant decreases in power required of up to 75% as revolutions per minute (RPM) and duty cycle were reduced from approximately 2 RPM to 1 RPM and duty cycle was reduced from 1.0 (continuous rotation) to 0.25.

Table 3. RABR power measurement in Watts for different RPM/HZ and Duty Cycle values

RPM	Motor HZ	(Watts) Duty Cycle (100%)	(Watts) Duty Cycle 50%	Reduction (%)	(Watts) Duty Cycle 25%	Reduction (%)
1.1	15	56	28	50	19	66
1.4	30	93	46	51	25	73
1.8	45	143	79	45	36	75

Objective 2. Improve Yield of polyculture biofilm microalgae feedstock (>20%)
Milestones 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 11.1,11.2, 11.3

Laboratory indoor testing of treatment for nutrient removal and biofilm biomass productivity is communicated in the publication by Watkins, et al. (2024a) at 1 L and 5 L volume sizes. Variables tested included harvesting period, hydraulic retention time, light intensity, and temperature. Two-way interactions harvesting period*light intensity (LI), harvesting period*temperature, and LI*hydraulic retention time (HRT) had significant effects on biomass productivity: at high temperature and low LI, highest biomass productivity was achieved with a 14-day harvesting period, but at medium temperature and high LI, highest biomass productivity was achieved with a 7-day harvesting period. At high HRT, highest biomass productivity occurred at low LI, but at low HRT, highest biomass productivity occurred at high LI. Phosphorus removal was strongly influenced by LI and occurred most rapidly during the first 2 days HRT, which suggests precipitation contributed significantly to phosphorus removal. Results of controlled laboratory testing were used for designing scale-up greenhouse testing at 100 L volume.

Pilot scale RABR footprint productivity was increased by 182% (almost doubled) while substratum productivity was increased by 37% comparing 2024 and 2025 (Table 4). This increase in productivity was associated with an increase in temperature of the influent AD centrate from an average of 16 ^{o}C to 27 ^{o}C. This increase in temperature was achieved by increasing the flow of heated AD centrate through the RABR to compensate for heat loss through the steel tank (above ground).

Table 4. Productivity values for polyculture biofilm microalgae feedstock

	Quarter 1: Apr – Jun 2024	Quarter 2: Jul – Sep 2024	Quarter 3: Oct – Dec 2024	Quarter 4: Jan – Mar 2025	Final Month: April 2025
Footprint Productivity (g/m^2-day)	7.8 ± 3.7 4.3 ± 2.1 (ash-free)	11.5 ± 4.1 7.2 ± 2.6 (ash-free)	8.16 ± 0.36	22.3 ± 6.64 13.2 ± 3.9 (ash-free)	29.2 ± 2.3 16.3 ± 1.3 (ash-free)
Substratum Productivity (g/m^2-day)	1.3 ± 0.6 0.7 ± 0.3 (ash-free)	1.9 ± 0.7 1.2 ± 0.4 (ash-free)	7.86 ± 0.51	4.3 ± 1.4 2.5 ± 0.8 (ash-free)	5.8 ± 0.4 3.2 ± 0.3 (ash-free)

Next generation sequencing methods were implemented to characterize the prokaryotic, eukaryotic, fungal, and phototrophic communities for a well-rounded understanding of the biofilm microecosystem in laboratory, greenhouse, and pilot scale RABRs and also the trickling filter source of inoculum of RABRs (Moravek, 2025). 16S, 18S, ITS, and 23S rDNA amplicons, respectively, were processed and taxonomically classified using Mothur and amplicon-specific reference databases. The data were then subjected to the non-parametric multivariable statistical analysis, ADONIS2, to determine relationships between communities and environmental parameters. The Kruskal-Wallis test was used to find significant genera, and LEfSe was used to find correlations between genera and significant relationships. Microscopy was used as a complimentary and secondary method of microalgal classification.

Within the algal biofilms various strains and groupings were identified (Matthews, 2025) for the pilot scale RABR. The 16S sequencing showed a variety of *Cyanobacteria* using the DADA2 analysis. Both the Mothur and the DADA3 methods showed alpha, beta, and gamma variants of *Proteobacteria* as well as high levels of *Weeksellaceae*. All of these microbes are useful for biofilm growth and nutrient removal. The 18S sequencing showed ciliate protozoa within the pilot scale RABR and high levels of *Hexapoda* or insects within the trickling filter. The 23S sequencing showed high levels of eukaryotic algae such as *Chlorella* as well as cyanobacteria such as *Leptolyngbya* and *Synechococcus*. Notably, *Chlorella* has been shown to be resilient to bacterial contamination. The ITS sequencing showed high levels of *Chlorellales* and *Chlamydomonadales*. However, the ITS analysis did not reveal the biofilm fungi. A repeat of the analysis using the UNITE database may be better able to classify the fungi.

Analysis and comparison of biofilm algae in both the pilot RABR and the trickling filter was conducted (Moravek 2025; Matthews, 2025) over a two-year period. The 16S taxonomy results indicated that the most common algae were the cyanobacteria Planktothrix and Phormidium, Chlorophyta, and Stramenopiles. The most abundant cyanobacteria in samples were Planktothrix and Phormidium. Planktothrix is currently accepted as a synonym for Oscillatoria, which is a filamentous cyanobacteria that often exhibits an oscillating motion. Other cyanobacteria present included Chroococcales and Synechococcus. Synechococcus are unicellular cyanobacteria. Phormidium and Planktothrix was found in all samples collected. The 16S analysis comparison showed that the DADA2 analysis detected cyanobacteria better than the mothur analysis across the various algal biofilms. Oddly, mothur detected a different strain of *Synechococcus* within the control samples than DADA2. It should be noted that DADA2 did have lower specificity at the family and genus taxonomic levels.

At the laboratory-scale, results indicated that temperature can be used to modify prokaryotic and eukaryotic communities, while harvesting frequency can be used to alter phototrophic communities (Moravek, 2025). At the greenhouse bench-scale, polyester substratum tended to allow for higher species richness while cotton hosted a high abundance of Chlorella. A significant find was discovering that none of the communities significantly changed with scale-up suggesting long-term stability of the microalgae-bacteria populations.

Objective 3. Improve cost of total phosphorus and nitrogen removal with decrease in cost $\geq$ 20% (Milestones 4.2, 8.1, 9.1, 10.1)

Decreases in the cost of power consumption of up to 75% were achieved by managing the RABR peripheral velocity/RPM is shown in Table 5. The Rocky Mountain Power cost for the Central Valley Water Reclamation Facility is \$0.09/kWh. Reducing the peripheral velocity from 0.38 to 0.2 ft s^{-1} decreased the power cost by 39%, and a further reduction to 0.1 ft s^{-1} decreased power cost by 50%. These cost decreases were achieved with a duty cycle of 1.0 where the RABR motor was engaged continuously (24 hours per day).

When duty cycle (percentage of time power used to rotate the substratum) was reduced to 0.5, decreases in cost of 50%, 69%, and 75% are achievable with peripheral velocities of 0.38 ft s^{-1}, 0.2 ft s^{-1}, 0.1 ft s^{-1}, respectively. These results were generated through operating the pilot RABR at the CVWRF for Duty Cycle of 1.0 and estimated for Duty Cycle of 0.5. WesTech-Inc. will install controls on the pilot RABR so that the duty cycle can be programmed to be reduced at night.

Table 5. RABR power cost (\$/day) for different RPM/HZ and Duty Cycle values

RPM	Motor HZ	(\$/day) Duty Cycle 100%	(\$/day) Duty Cycle 50%	Reduction (%)	(\$/day) Duty Cycle 25%	Reduction (%)
1.1	15	0.12	0.06	50	0.04	66
1.4	30	0.20	0.10	50	0.05	75
1.8	45	0.31	0.17	45	0.08	74

Example calculation: 56 watts x 24 hr/day = 1,344 watt-hrs/1000watts/kW = 1.344 kW x 0.09\$/kWh = \$0.120/day

Cost reduction was also impacted through testing different substrata at different base costs (\$/m^2) for algae biofilm yield that was evaluated as \$/ gm biofilm for the different substrata tested. Polyester carpet substratum cost based on algae yield was \$0.02/gm biofilm compared with cotton (baseline) substratum at \$5.91/gm biofilm.

Objective 4. Meet total phosphorus target concentration of 0.3 mg/L.
Milestone 12.1. Go-No/Go RABR TP$\geq$70% removal and CVWRF TP$\leq$0.3 mg/L.

The Pilot RABR at the host site (Central Valley Water Reclamation Facility [CVWRF]) was physically moved during the month of March (2024) to another location at the site due to construction activities related to upgrading of the CFWRF. The Pilot RABR was moved to co-locate it with the Annamox Process, which was initiated in the summer of 2023, that resulted in an increase of the influent water temperature to near 80 F that is required for the operation of the Annamox process versus the average temperature of 25 F at the original site. The new location also allowed the elimination of solids clarifiers as a pre-treatment process for the Pilot RABR. The influent to the Pilot RABR at the new location has the same chemical water quality with regard to concentrations of nutrients (phosphorus and nitrogen) as the influent to the Pilot RABR at the previous site because it is the same effluent from the anaerobic digestion process. Re-location of the pilot RABR at the host site resulted in increased nutrient removal by the RABR biofilm. Maximum TP removal was measured at 76% for the period from April 1 through June 30 with minimum TP removal at 42%.

CVWRF has implemented upgrades for nutrient removal independent of the RABR testing that will achieve an effluent quality of 0.3 mg/L TP that meets the goal of this objective. This goal is being achieved through the installation of MagPrex for phosphorus removal and Anammox (AnitaMox) for nitrogen. The RABR contribution will be in the form of biomass production that can serve as feedstock for bioproducts, including bioplastic, biofertilizer, and biofuels. A TEA was conducted specifically for bioplastic and biofuels from biofilm microalgae produced in this project (Watkins, et al. 2024).

Objective 5. Produce bioplastic from biofilm microalgae (Milestones 5.1, 5.2, 13.1).

Information developed for achieving this objective is presented in detail in the thesis of Clayton Lords (2025). Dry algae biomass (10 Kg) harvested from the CVWRF was shipped to Algix in Meridian, Mississippi for bioplastic production. Algix provided five different polymers for analysis: (1) bioplastic composite containing 30% algae biomass from the CVWRF RABR; (2) bioplastic composite containing 30% algae biomass from the CVWRF RABR with an additional biodegradation-enhancing resin; (3) bioplastic composite containing 30% algae biomass sourced from Algix; (4) biodegradable polyester containing no algae biomass as the positive control; and (5) polyethylene negative control. All samples were provided as dog-bones, disks, and thin films; the dog-bones and disks were of identical thickness and density.

Biodegradation testing of the bioplastic parts and controls produced by Algix occurred within the aerobic full scale composting system at the CVWRF (host site). Temperature was maintained between 60 and 70 °C for both compost periods. Triplicate compost vessels for each plastic type were incubated in the compost system. For this testing, thin film samples were used instead of dog bone samples. The samples were each encased in separate compost vessels with compost inoculum. Five compost vessels were built using perforated stainless steel spherical containers, each with an approximate volume of 8 L. Each vessel was filled with compost inoculum and 5 g thin film sample. The containers were then buried approximately 1 m deep into the compost pile for 42 days. The samples were removed from the pile for measurement and examination, and then replaced into the stainless-steel containers and incubated in the compost pile for another 42 days at a depth of 1 m. At the end of each biodegradation period, the degradation of the materials was measured quantitatively by screening the contents of each vessel through a ½-in. and ¼-in. stainless steel sieve to recover the remnants of the plastic. The mass of the remaining plastic was measured.

According to Section 6.2 of ASTM D6400, if no more than 10 % of the original dry mass remains after sieving on a 2 mm sieve, then a plastic is considered to demonstrate satisfactory disintegration. After the first incubation period of six weeks at 60-70 ^{o}C, biodegradation for all samples was measured between 20 and 54% except for the polyethylene control with no loss of mass. After 12 weeks of aerobic composting treatment at 60 – 70 ^{o}C, no measurable mass was retained on the 1/4-in. sieves except for the negative control of polyethylene. Therefore, the bioplastic materials derived from the biofilm algae were demonstrated to be biodegraded by aerobic composting with 12 weeks of treatment at 60-70 ^{o}C.

Objective 6. Provide TEA for algae cultivation and conversion to biofuel via HTL and to bioplastic and conversion to biofuels and bioplastic via lipid extraction (Milestone 4.1, 6.1, 6.2, 14.1, 14.2).

The following information is taken from the abstract and content from the refereed publication that is a product of this award with Jacob Watkins. et al., (2024) titled “ Techno-economic analysis of bioplastic and biofuel production from a high-ash microalgae biofilm cultivated in effluent from a municipal anaerobic digester.” A Techno-economic analysis (TEA) was conducted to compare different processes for bioproducts conversion of a high-ash microalgae biofilm grown using a RABR treating 0.6

million gallons per day of anaerobic digestion centrate at the CVWRF. Process and economic models were developed and compared for three conversion processes: 1) the production of bioplastics using whole biofilm algae, 2) the production of bioplastics with a lipid-extraction pretreatment, and 3) the production of biocrude via hydrothermal liquefaction. Techno-economic analysis was performed for each conversion process, including three cases for algae productivity: 231, 391, and 577 metric tons per year (dry basis). The calculated value for the minimum plastic selling price (MPSP) of bioplastics produced from biofilm algae ranged from $4,050 to $3,520 per metric ton based on baseline and final productivity cases of the RABR, respectively. The extraction of lipids in addition to bioplastic production results in an MPSP of $4,570 to $4,000 per metric ton for the same productivity cases. The relatively small production scale and complex processing for hydrothermal liquefaction results in a minimum fuel selling price of the biocrude of $5.32 per gallon of gasoline equivalent. The MPSP for the extraction of lipids in addition to bioplastic may be further improved by upgrading the extracted lipids into biofuels or high-value plastics. The conversion process for bioplastic production from whole algae has the highest income:expense ratio from 108% to 120% and the most cost-competitive pricing of the three modeled processes. The calculated value for the MPSP of bioplastics produced from algae is within the range of commercial bioplastics products. Based on the results of the TEA conducted for this project, bioplastic production from whole biofilm algae is an economically viable process that can be used to valorize biofilm algae biomass harvested from rotating algae biofilm reactors treating anaerobic digester centrate at municipal wastewater reclamation facilities.

Microalgae assisted struvite formation was demonstrated in the Pilot scale RABR that resulted in the precipitation of nitrogen and phosphorus within the struvite (Goldsberry, et al., 2023; Goldsberry, 2023). As the microalgae increase the pH with the uptake of CO_2 within the biofilm, struvite precipitates within the biofilm matrix. Also, evaporation of water in the low humidity outdoor environment results in residual salts exceeding the solubility product for struvite and the corrugated structure of the substratum provides sties for crystallization. The addition of ash in the form of struvite to the biofilm adds value to bioplastic materials by providing nutrients and also structure in the form of filler for the bioplastic matrix (Watkins, et al., 2024).

Objective 7. Provide LCA (Milestone 14.3)

The LCA for this project was prepared by PNNL under the direction of Mr. Jacob Watkins and Dr. Francesca Pierobon with testing data and information generated and provided by Utah State University and the project collaborators specifically for the pilot RABR at CVWRF with a volume of 11,400 L.

This life cycle analysis (LCA) aims\ed to quantify the environmental impact of a microalgae-polylactic acid (PLA) bioplastic blend produced using microalgae cultivated at a Central Valley Water Reclamation Facility (CVWRF) in Salt Lake City, Utah. The full cradle-to-gate production process is considered, including the cultivation and drying of the algae biomass used as feedstock and the conversion of the cultivated microalgae into a usable bioplastic resin.

In the modeled process, microalgae is cultivated using a flat-panel rotating algae biofilm reactor (RABR) (Goldsberry, 2023, Jeppesen, 2024, Watkins, et al., 2024b). Microalgae cultivation using the flat-panel RABR requires three major inputs, namely (1) electricity for rotation, (2) nutrients for growth, and (3) polycarbonate and polyester materials to facilitate microalgae biofilm formation, growth, and harvesting. Following microalgae cultivation and harvesting, the microalgae biomass is vacuum dried, milled, and compounded with polylactic acid (PLA) (Watkins, et al. 2024).

The life cycle inventory (LCI) for the algae cultivation system was based on experimental inputs from an 11,400-L pilot RABR at CVWRF (Goldsberry, 2023, Jeppesen, 2024, Watkins, et al., 2024b). LCI data was provided for two alternative scenarios, based on testing data for low (12 hours) vs. high (48 hours) hydraulic retention time and high (1.0) vs. low (0.5) duty cycles for rotation. In both HRT scenarios, the overall nutrient removal rate was sufficiently low (<15 mg/L or 4% N removal, <3mg/L or 20% P removal) to assume that operation of the RABR will have no impact on other N and P removal processes at CVWRF. Thus, the impacts of N and P recovery were excluded from the LCA. Similarly, the RABR is assumed to be constructed onsite on pre-developed land at CVWRF, requiring no changes in land use and no associated environmental impacts. The LCI for the algae conversion system is based on inputs from Algix LLC, using both industrial data and testing data produced using the algae cultivated at CVWRF. Detailed inventory data for the algae-to-bioplastic conversion system and for the two algae cultivation scenarios is presented in Table 6.

Table 6. Life cycle inventory data for the algae cultivation and conversion processes

Parameter	Scenario / Quantity				Units	Reference
	S1	S2	S3	S4		
Algae cultivation						
Electricity use	10.5	6.74	6.74	6.74	kWh/kg algae	[2]
Polycarbonate use	1.64	0.57	0.06	0.00	kg/kg algae	-
Polyethylene terephthalate use	0.65	0.23	0.11	0.11	kg/kg algae	-
Algae carbon content	0.26	0.26	0.26	0.26	kg/kg algae	[3]
Algae conversion to bioplastic						
Electricity use	2.38	2.38	2.38	2.38	kWh/kg product	[4]
Natural gas use	2.04	2.04	2.04	2.04	MJ/kg product	[4]
Polylactic acid use	0.55	0.55	0.55	0.55	kg/kg product	[4]
Algae biomass use	0.45	0.45	0.45	0.45	kg/kg product	[4]
Cooling tower residue production	0.83	0.83	0.83	0.83	kg/kg product	[4]

The model used in this LCA was developed in OpenLCA version 2.3 (https://openlca.org), following ISO 14040-14044 standards (Environmental Management – Life Cycle assessment - principles and framework, 2006; Environmental Management – Life Cycle assessment – requirements and gujdelines, 2006). The Ecoinvent database version 3.10 (Wernet, et al., 2016) was used to model the impact for each energy and material input flow. Life cycle environmental impacts were assessed using the Tool for Reduction and Assessment of Chemicals and Other Environmental Impacts (TRACI 2.1 (Bare, 2011). Energy inputs are modelled as described previously (Pierobon, et al. 2019), using the Western Electricity Coordinating Council (WECC) grid. As per the IPCC Fifth Assessment Report, environmental impacts are reported in terms of 100-year global warming potential impacts (IPCC, 2014).

The GHG emission allocations across the different process components for all three scenarios are illustrated in Figure 1. The current environmental impact of producing a microalgae-PLA blend using microalgae cultivated on a flat-panel RABR is 4.15 kg CO_2-eq/kg product (S2), which includes a 1.52 kg CO_2-eq/kg product credit for biogenic carbon storage. This net value is lower than the global average for polycarbonate production (6.75 kg CO_2-eq/kg product), but higher than that for polylactic acid production (1.11 kg CO_2-eq/kg product) and low-density polyethylene production (3.25 kg CO_2-eq/kg product). In a scenario with the respective lifespans of the polycarbonate and polyethylene materials increased to 10 and 2 years (S3), the environmental impact of the microalgae-PLA blend could be reduced to 2.53 kg CO_2-eq/kg product. By replacing the polycarbonate materials with permanent stainless-steel materials (S4), this could be further reduced to 2.36 kg CO_2-eq/kg product. In this scenario, the net GHG contribution from algae cultivation is 1.72 kg CO_2-eq / kg algae, which is within the range reported for other microalgae cultivation systems (1.6 to 28 kg CO_2-eq / kg algae, dry basis) (Duran Quintero et al, 2021). Increased production of microalgae biomass would increase the amount of nitrogen and phosphorus removed from wastewater, which could further reduce GHG emissions by reducing the energy requirements for wastewater reclamation at CVWRF.

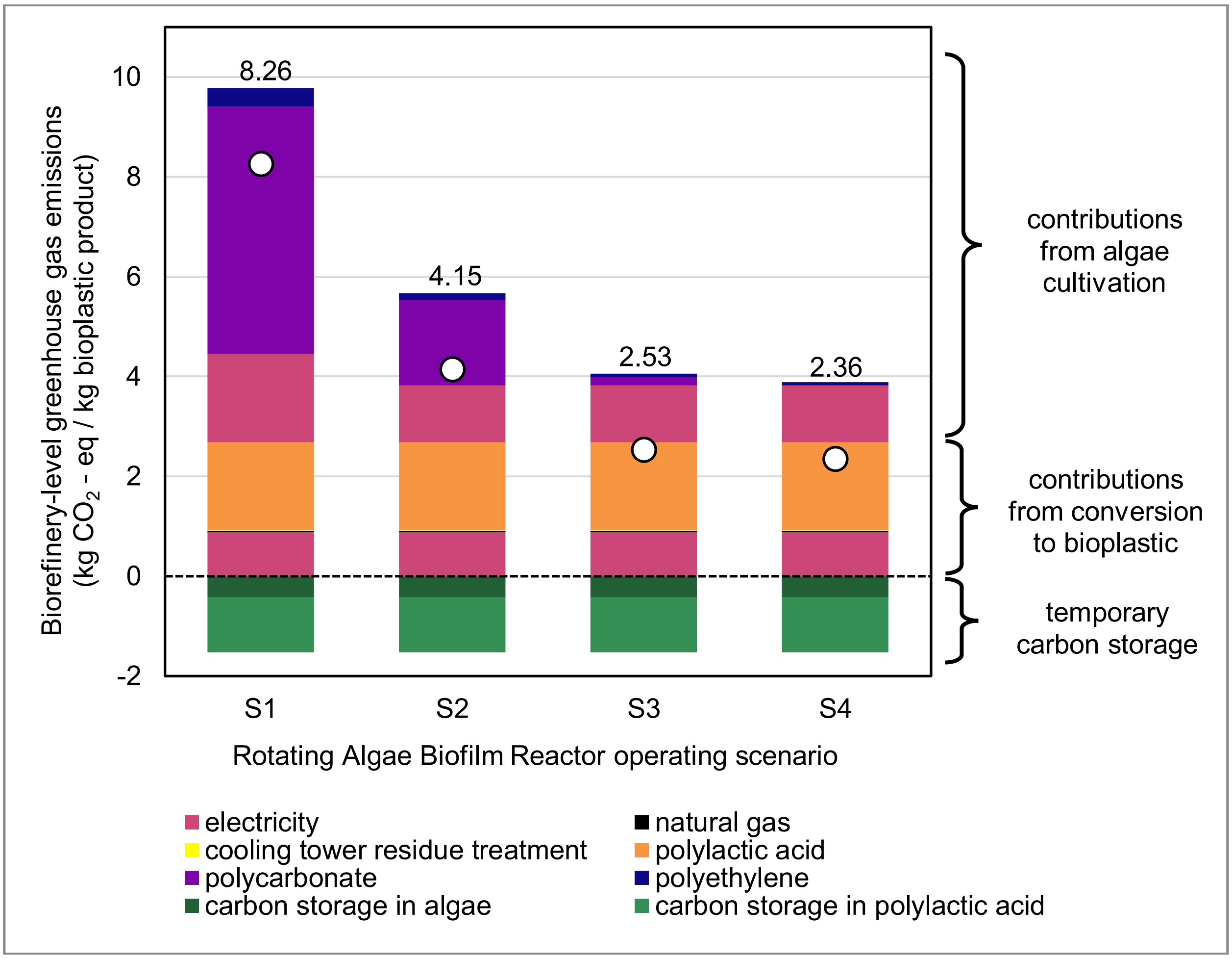

Figure 1. GHG emission allocations for a biorefinery that produces bioplastics through co-blending of polylactic acid and thermo-mechanically plasticized microalgae cultivated using a rotating algae biofilm reactor (RABR) treating municipal wastewater. (S1) baseline operation; (S2) S1 with reduced hydraulic retention time, increased duty cycle, and increased biomass productivity; (S3) S2 with reduced replacement frequency for the plastic RABR components; and (S4), S3 with polycarbonate panels replaced with lightweight stainless-steel panels.

8. Significant Accomplishments and Conclusions:

This project demonstrated a positive TEA for the production of bioplastic from biofilm microalgae feedstock with the rotating algae biofilm reactor (RABR) (Watkins, et al. 2024). The calculated value for the minimum plastic selling price (MPSP) of bioplastics produced from algae ranges from $4,050 to $3,520 per metric ton based on baseline and final productivity cases of the RABR, for 231, 391, and 577 metric tons per year (dry basis), respectively. Of the three conversion processes modeled in this TEA, including bioplastic, bioplastic and lipids, and bioplastic production from whole algae, bioplastic production has the highest carbon and energy efficiency and has the highest income:expense ratio (120 %). Based on the results of this TEA, bioplastic production from whole algae is an economically viable process that can be used to valorize algae biomass harvested from RABRs treating anaerobic digester centrate at municipal wastewater reclamation facilities.

The production of thermoplastic blends that incorporate microalgae is particularly appealing because this process can be applied to wastewater-grown microalgae feedstocks with relatively high ash content. In this process, the ash acts as a “filler” and contributes to the nitrogen, phosphorus, and mineral content available in the final biodegradable bioplastic. Fillers are already necessary in bioplastic production to reduce shrinkage during the setting process, to improve the tensile strength and hardness of bioplastics, and to reduce the cost of bioplastic resin per kg product. Although the viability of microalgae conversion into bioplastic has been demonstrated in several TEA studies with processes that fractionate biomass prior to plasticization, the direct production of these blends using whole algae has not yet been analyzed by any published TEA until this project generated results that were published (Watkins, et al., 2024).

This project also discovered the bio-physiological production of struvite through the photosynthesis process within he RABR biofilm (Goldsberry, et al. 2023). While struvite precipitation and removal from wastewater is conventionally accomplished through physicochemical methods, this research is the first report of struvite enhanced formation through algae biofilm based photosynthesis. Photosynthesis is utilized in RABR operation to enhance struvite formation by increasing the pH value within the biofilm through the uptake of carbon dioxide from solution resulting in struvite precipitation within the biofilm. Measurements of pH trended higher with depth through the biofilm when exposed to light confirming the function of photosynthesis in increasing pH and, as a consequence, struvite formation. In addition, reducing RPM allowed more time for water to evaporate through exposure of the biofilm to the atmosphere and provided a management option to exceed the struvite solubility product thereby enhancing struvite precipitation. Struvite precipitation was predicted based on chemical analysis and MINTEQ modeling of AD effluent and was confirmed using Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy, and quantified by determining ash content. Increase in pH within the biofilm was also confirmed as photosynthetic photon flux density increased. As struvite crystallizes, the crystals provide nucleation site for addition struvite precipitation. The bio-physiological production of struvite provides a management option for enriching microalgae biofilms with the slow-release fertilizer that increases the microalgae-struvite biofilm function as a biofertilizer (Goldsberry et al. 2023). The harvested microalgae struvite biofilm was compared with a commercial struvite product using yield of dwarf wheat as a metric. Algae biofilm fertilizer performed significantly better than the commercial struvite with $P = .002$ (Watson and Sims, 2025). The bio-physiological production of struvite provides a management option for enriching microalgae biofilms with the slow-release fertilizer that increases the microalgae-struvite biofilm function as a biofertilizer (Goldsberry et al. 2023).

In order to increase biomass productivity, a consistent and stable increase in temperature was required that could not be met due to the heat transport and escape from the heated influent through the above ground steel tank RABR into the atmosphere, even after wrapping the 11,000 L tank with insulation. In order to achieve a stable tank water temperature above the average of 10°C, the influent flow rate was increased to the maximum flow that decreased the HRT from 40 hours to 11 hours. This change in flowrate resulted in increasing the water in the tank from an average of 10 °C to a consistent temperature of 30 °C. This increase in temperature resulted in a doubling of the biofilm productivity, but at the cost of reducing the nutrient uptake by 50 – 75%.

9.Path Forward:

While there are no immediate plans for future development or commercialization, we have identified the next R&D steps that might be taken to advance the technology. The first step is to design, test, and implement an automatic harvesting system for the microalgae biofilm. During this project, biofilm was manually harvested with garden hoe tools that required about two hours to harvest 72 square meters of RABR surface area. Harvesting guidelines include not applying aggressive pressure in order to sustain a small film of microalgae for regrowth but enough pressure to remove a majority of biofilm. Collection of the biofilm into a container or trough prevents the biofilm from move to the water in the tank that would require separation from the liquid phase. Therefore, developing a successful automatic harvesting system is needed for effective scale-up operation of the RABR.

10. Products:

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Analyzing Microalgae-bacteria Population Changes in Response to Environmental Parameters for Improved Wastewater Treatment. 2025. Amanda M. Moravek, M.S., All graduate theses and dissertations. https://doi.org/10.26076/7338-051a

11. **Project Team and Roles**: List all participants along with their individual roles and/or intellectual contribution (e.g., DOE personnel, students, collaborating org

Dr. Ronald C. Sims, Utah State University, PI, lead the development of the proposal and the execution of the project including technical, schedule, and budget aspects. Also initiated research into struvite formation mechanisms as a bio-physiological process and engineering management of struvite formation and testing as a biofertilizer. Served as the major advisor for project graduate students including Jacob Watkins, Peter Jeppesen, Clayton Lords, Parker Goldsberry, and Davis Haag.

Dr. Charles Miller, Utah State University, Co-PI, lead the research at USU addressing the microscopic and genetic characterization of microalgae biofilms at laboratory, greenhouse, and field scales.

Dr. Philip Heck, Plant Manager of the Central Valley Water Reclamation Facility and host site for the project, provided engineering information on the operation of the CVWRF, identified the location of the RABR to follow the MagPrex phosphorus removal process and be parallel to the AnitaMox ammonia removal process, provided input to the PNNL team for the TEA, and provided support personnel to assist with maintenance and repair of the RABR when mechanical problems occurred.

Mr. Navneet Prasad, Electrical Engineer, Central Valley Water Reclamation Facility, lead the design and implementation of the automatic electrical control system for the clarifiers placed before the field scale RABR to prevent suspended solids from entering the RABR tank.

Mr. James Judd, WesTech-Inc, provided the design guide on the upgrade of the field scale RABR from six units to 12 units thereby doubling the surface area for microalgae biofilm growth from 35 m^2 to 72m^2 over 36 individual shelves. He also lead the “winterization” of the RABR through wrap-around insulation of the steel tank and polycarbonate transparent roof to prevent snow and winter wind from contacting the biofilm.

Mr. Ashton Zeller, Algix Bloom, lead the industry conversion of the biofilm algae into bioplastic parts that were tested for biodegradability at the full scale aerobic composting system at the host site (CVWRF).

Mr. Jacob Watkins, Utah State University graduate student (M.S.) and PNNL employee, lead the laboratory analyses of microalgae biofilm biochemical composition and lead the organization and writing of the TEEA publication based on this project.

Dr. Yunhua Zhu, PNNL, initiated the conceptualization and TEA modeling that include three conversion pathways including: (1) production of bioplastics, (2) production of bioplastics with lipid-extraction pretreatment, and (3) production of biocrude via hydrothermal liquefaction.

Dr. Peter Valdez, PNNL, participated in the development of the TEA including methodology, data curation, and formal analysis.

Clayton Lords, Utah State University graduate student (M.S.), lead the collection of biomass for conversion to bioplastic at Algix Bloom and the laboratory and field-scale biodegradability testing of the bioplastic parts produced by Algix Bloom.

Dr. Pavlo Bohutskyi, PNNL, lead the genetic characterization of the microalgae harvested from rotating algae biofilm reactors (RABRs) at laboratory, greenhouse, and field scales using Mothur and Dada 2 search engines.

Peter Jeppesen, Utah State University graduate student (M.S.), lead the field scale RABR operation and performance functions and monitoring the RABR for nutrient removal, biomass production, and power requirement.

Parker Goldsberry, Utah State University graduate student (M.S.), lead the laboratory and field scale investigation of struvite formation as a bio-physiological process of biofilm formation and cultivation.

Davis Haag, Utah State University graduate student (M.S.), lead the field scale RABR operation and performance function following the upgrade from $36m^2$ to $72m^2$ substratum surface area and installation of an improved power meter to monitor power requirements for rotating the RABR shaft.

Amanda Moravek, Utah State University graduate student (M.S.), lead the genetic analysis and characterization of the RABR biofilms at laboratory, greenhouse, and field scale RABRs using Mothur and Dada 2 for the last first years of the project under the supervision of Drs. Charles Miller and Pavlo Bohutskyi.

Eric Matthews, Utah State University graduate student (M.S.), lead the genetic analysis and characterization of the RABR biofilms at laboratory, greenhouse, and field scale RABRs using Mothur and Dada 2 for the last two years of the project under the supervision of Drs. Charles Miller and Pavlo Bohutskyi.

Dr. Lukas Buecherl, Utah State University, assisted with RABR biofilm genetic data analysis and interpretation, and as graduate committee chair for Mr. Eric Matthews.

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