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Cover Page

Federal Agency:	U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy (EERE), Bioenergy Technologies Office (BETO)	
Award Number:	DE-EE0009671	
Project Title:	Enhanced Production of Algae Lipids and Carbohydrates for Fuel and Polyurethane Precursors	
Recipient Organization:	University of California, San Diego (UC San Diego)	
Project Period:	Start: October 1, 2021	End: March 31, 2025
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Teaming Partners:	University of California, Davis (UC Davis) Algenesis Corporation (industrial partner)	

Acknowledgment and Disclaimer

Acknowledgment: *This material is based upon work supported by the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy (EERE) under the Bioenergy Technology Office (BETO) Award Number DE-EE0009671.*

Executive Summary

This Final Technical Report summarizes the goals, approach, and outcomes of the project **"Enhanced Production of Algae Lipids and Carbohydrates for Fuel and Polyurethane Precursors"**. The project addressed the challenge of improving microalgae biomass productivity and simultaneously producing valuable **polyurethane precursors (PUPs),** from both lipids and carbohydrates, that can be converted into renewable **biofuels** and **bio-based polyurethane (PU) products**. By integrating advanced genetic engineering, traditional breeding, high-throughput screening, pilot cultivation, and chemical conversion technologies, the team achieved significant advancements in algae biotechnology. Key accomplishments include:

Increased Understanding of Algal Co-Product Production: The project expanded fundamental knowledge of algae metabolic pathways by identifying and quantifying naturally occurring **polyurethane precursors (PUPs)** in both a cyanobacterium and a green algal production strain. This baseline characterization revealed abundant carbohydrates (e.g. starch, extracellular polysaccharides) and lipids (fatty acids) that can serve as chemical feedstocks for biofuels and biopolymers.

Technical Effectiveness: Through a combination of **directed evolution and breeding**, the team achieved >20% improvement in algal biomass productivity under industry-relevant conditions (high salinity and high pH) relative to the baseline, surpassing the initial target (Milestone M2.1). Concurrently, new **synthetic biology tools** (promoters, vectors, and genome sequences) were developed for two non-model production strains, enabling their genetic modification for the first time. Using these tools, improved algal strains were created with >20% higher production of target PUP molecules (Milestone M3.2). The methods investigated proved effective: the engineered algae accumulated significantly more starch or lipids, and the robust strains maintained high growth in stress conditions, demonstrating the feasibility of synergistically improving biomass and co-product yields.

Economic and Environmental Benefits: The project demonstrated an integrated process wherein algae-derived carbohydrates and lipids can be converted into polyurethane precursors and fuels. A **biorefinery approach** was validated at lab-scale: the extracted algal sugars were purified to >90% purity (Milestone M4.1) and converted to bio-based polymer building blocks, and residual algal oils were concurrently processed into renewable fuel components. This co-product strategy can improve the techno-economics of algal biofuels by generating additional value streams. A preliminary techno-economic analysis (TEA) and life-cycle assessment (LCA) indicate that producing high-value PU co-products alongside fuels could substantially reduce the minimum fuel selling price and lifecycle carbon emissions of algae biofuels, making the technology more economically viable and beneficial to the public. The **public benefits** include the prospect of sustainable drop-in fuels and **biodegradable polyurethane** products (such as foams and plastics) derived from

algae, which address both energy security and plastic pollution. By using atmospheric CO_2 and sunlight to produce fuels and materials, this project contributes to U.S. clean energy goals and circular economy objectives.

In summary, this work has delivered new commercially viable algal strains and open-source genetic toolsets that are now available to the entire algal community. It demonstrated improved biomass production well beyond expected targets, and showed, at proof-of-concept scale, the conversion of algal biomass into valuable polymers and fuels. These outcomes significantly advance the state-of-the-art in algae biotechnology and lay the groundwork for future commercialization of specific products from integrated algae biorefineries.

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Background

Algae have long been recognized as a promising resource for sustainable biofuels due to their fast growth and high lipid content (Jha et al., 2024). However, a major challenge has been the economic viability of algal biofuel production, partly because a significant fraction of the algal biomass (e.g., carbohydrates) can remain as low-value residue after lipid extraction. This project targeted that challenge by converting the residual components into **polyurethane precursors (PUPs)**, effectively utilizing the majority of the biomass and thereby increasing the overall value of the biomass, which in turn helped drive down the cost of the associated biofuel (SAF). This approach addresses a long-standing problem of "carbon waste" in algae biofuel processes by developing **high-value co-products**, the economic and sustainability profile of algae biofuels can be dramatically improved.

State of the Art: Previous research has shown that many microalgae species accumulate substantial carbohydrates (such as starch or extracellular polysaccharides), in addition to lipids, under various conditions. For instance, certain green algae can store >50% of their biomass as starch under stress (Xu et al., 2022), and others excrete polysaccharides into the culture medium (Cristofoli et al., 2023; Qiu et al., 2020). These algal carbohydrates have only recently been explored as renewable chemical feedstocks (de Carvalho Silvello et al., 2022). One especially promising route is converting algal sugars to **furandicarboxylic acid (FDCA)**, a renewable monomer that can substitute for petroleum-derived aromatic diacids in polymers such as terephthalic acid. To date, only one study has linked algal polysaccharides to furan chemicals, highlighting the novelty of this project's approach in developing algae-to-FDCA pathways (*Sustainable PoLymers from Algae Sugars and Hydrocarbons | FP7 | CORDIS | European Commission*, n.d.; van Putten et al., 2013).

On the lipid side, algal oils are typically similar to vegetable oils and can be converted to biodiesel or hydrocarbon fuels. Recent advances also demonstrate that **monounsaturated fatty acids** from algae can be chemically transformed into diacids suitable for polyurethane production (Burkart & HAI, 2021). These findings from the literature set the stage for this project, indicating that both algal lipids and carbohydrates can yield valuable chemical building blocks (diacids, polyols) for polymers like polyurethanes. By leveraging such advances, this project differentiates itself by **combining biological and chemical innovations**: improving algal production of those precursors and integrating their conversion into **drop-in replacements for petrochemical polymer components**.

In addition to focusing on co-products, the project builds on a decade of algal biotechnology progress at the California Center for Algae Biotechnology (Cal-CAB). The team has developed robust **genetic engineering tools for algae and cyanobacteria** in prior projects, including broad-host-range vectors and synthetic promoters for these organisms. Those tools historically were limited to model strains; here they are extended to **commercial production strains** that industry uses, which

is a significant step toward real-world application (addressing the BETO **Algae Productivity Exceeding Expectations (APEX)** program challenge of translating lab breakthroughs to commercial strains). Moreover, the project's collaboration with industry (Algenesis and BASF) ensures alignment with current state-of-the-art industrial practices in algae cultivation and polymer chemistry.

In summary, this project's background is rooted in two key concepts from recent advancements: (1) **Algal biomass fractionation for co-products** – harnessing all major biomass fractions (lipids and carbohydrates) to improve biofuel economics, and (2) **Enabling technologies** – new algal genetic tools, strain improvement methods, and chemical conversion techniques that together make the above concept feasible. By building upon and fully leveraging these developments, the project aimed to accelerate progress beyond the state-of-the-art, demonstrating a viable pathway to produce both renewable fuels and polyurethane materials from algae in a single integrated process.

Project Objectives

The overarching goal of this project was to develop an **integrated algae biorefinery** process that produces **renewable fuel** and **polyurethane (PU) co-products** cost-effectively, thereby supporting U.S. clean energy and manufacturing goals. Specifically, the project sought to **improve algal biomass productivity** to unprecedented levels while simultaneously increasing the accumulation of lipids and carbohydrates that can serve as polyurethane precursors. By doing so, the project addresses national clean energy objectives in two ways: (1) advancing sustainable biofuel production (displacing petroleum, reducing carbon emissions) and (2) creating bio-based and biodegradable plastics to replace petrochemical plastics. Success in this project would demonstrate a new model of algae utilization with enhanced economic viability and environmental benefits, directly contributing to BETO's mission of developing **innovative biotechnologies** for fuels and products.

Specific Objectives and Targets:

- **O1. High-Productivity Strains:** Develop algae strains (one green microalga and one cyanobacterium) capable of at least a **20% increase in biomass productivity** in high-salinity, high-alkalinity growth conditions compared to baseline (Milestone M2.1). This target supports the APEX program goal of greatly exceeding conventional algae growth rates, which is crucial for lowering biofuel production costs.
- **O2. Enhanced PUP Production:** Increase the production of at least one polyurethane precursor (PUP) molecule (such as a specific lipid or carbohydrate) by >20% in the engineered strains relative to the unmodified baseline strains (Milestone M3.2). The intended outcome was to demonstrate

significant metabolic enhancements leading to higher yields of target feedstocks for PU monomers.

- **O3. Genetic Tool Translation:** Translate the complete suite of modern **genetic engineering tools** (vectors, promoters, gene editing/transformation methods) to the selected commercial algae strains (Milestone M3.1: at least one functional genetic vector for each strain). This includes constructing and validating new vectors and regulatory elements that work in these production strains, thereby empowering both this project and the broader algae R&D community to modify and improve industrial strains (an APEX "challenge" deliverable).
- **O4. Co-Product Extraction and Purification:** Develop processes to **isolate and purify PUPs from algal biomass** at laboratory scale. A success criterion was to purify at least one target compound (e.g., sugar or diacid) to >90% purity from algae (Milestone M4.1). Efficient separation of these co-products is essential for downstream conversion to polymers.
- **O5. UCSD-PEAK Process for Biomass Enhancement:** Apply the **PEAK (Productivity Enhanced Algae Toolkits)** process (a UCSD-developed cultivation strategy) to further improve biomass and PUP yields. The goal was to demonstrate >50% increase in biomass and/or PUP productivity after multiple PEAK cycles (Milestone M5.1). This objective aimed to push algae performance beyond genetic modifications alone by using process engineering (e.g.,breeding program, environmental conditioning).
- **O6. Pilot-Scale Demonstration:** Evaluate at least one improved algal strain at **pilot scale (outdoor greenhouse)** to confirm that lab gains translate to scaled cultivation (Milestone M5.2). This involved cultivating the enhanced strains in ~100–1000 L outdoor raceways or photobioreactors and measuring growth and product yields, thus bridging the gap to commercial deployment.
- **O7. Chemical Conversion to Polyurethane Monomers and Fuels:** Demonstrate the conversion of algal-derived lipids and carbohydrates into **polyurethane monomers** (polyols/diisocyanates or analogous compounds) and into **fuel precursors**. Two milestones guided this objective: M6.1, the successful synthesis of a polyurethane material at gram-scale from algae-derived inputs, and M6.2, the conversion of algal PUPs to PU precursors *and* residual lipids to fuel components in an integrated manner. Achieving these would prove the technical viability of producing both fuels and plastics from algae.
- **O8. Polyurethane Product Prototype:** Using the monomers produced in O7, fabricate a **PU product** (such as a foam or plastic prototype) containing >50% bio-based content from algae (Milestone M7.1). This objective connects the project to real-world applications by demonstrating that algae-based monomers can perform in actual polymer formulations (e.g., foams for footwear or insulation with majority renewable content).
- **O9. Techno-Economic and Life-Cycle Analysis:** Perform a comprehensive **techno-economic analysis (TEA)** and **life-cycle assessment (LCA)** for the integrated process (Milestone M8.1). The TEA will quantify the production cost (e.g., minimum fuel selling price, MFSP) under scenarios with co-product

revenue, and the LCA will quantify environmental impacts (e.g., greenhouse gas emissions per MJ of fuel, water/energy usage) for the algae fuel + PU production system. The objective is to verify that co-producing PUPs can materially improve economics (lower $/gal fuel) and reduce carbon footprint relative to an algae-to-fuel only scenario. Insights from this analysis guide future research and highlight remaining barriers to commercialization.

To ensure these objectives were met, the project was structured according to a **Statement of Project Objectives (SOPO)** with defined tasks, milestones, and two critical **Go/No-Go decision points**: one at the end of initial validation (Budget Period 1) and another mid-project (after demonstrating improved biomass and PUP production, Budget Period 2). These decision points (GN.1 and GN.2) were intended to rigorously evaluate whether the project was on track to achieve its transformative goals. In summary, the project objectives combined ambitious technical targets (exceeding prior algae productivity records and creating novel bioproducts) with practical milestones that ensure each advancement contributes to the ultimate goal of an economically viable algae-based biorefinery.

Project Results and Discussion

Project work was carried out in a series of tasks aligned with the SOPO. In this section, we provide a detailed account of the results for each task, comparing the anticipated outcomes (milestones and deliverables) with the actual achievements. Overall, the project met or exceeded the majority of its technical milestones, as discussed below. Key results are highlighted with quantitative metrics, and any variances from the original plan are explained.

Task 0: Project Validation

Task 0 Objective: Before initiating R&D activities, this task involved working with DOE's site **verification team** to validate the project's foundational elements – namely, the algal genetic toolset, the pilot-scale cultivation setup, and the chemical processing plans at UCSD. Successful validation was a prerequisite (**Go/No-Go Decision Point GN.1**) to proceed beyond Budget Period 1.

Planned Activities: The team prepared documentation and initial data on the genetic engineering methods intended for the algae strains, demonstrated the capability of the UCSD algae outdoor facility, and outlined the safety and feasibility of the proposed chemical conversion experiments. This included presenting prior achievements by the labs (e.g., earlier successful transformations in algae, preliminary yield data, etc.) as evidence that the project was technically grounded.

Actual Accomplishments: DOE's validation review was conducted in Q1 of the project. The project passed this review successfully. *Milestone M0.1 (Validation of algal genetic tools, pilot systems, and processing) was achieved.* The DOE validation

team confirmed that the genetic modification techniques (for both the cyanobacterium *Synechococcus* sp. PCC 11901 and the green alga strain “402”) were suitable and UCSD’s outdoor algae cultivation facility and bench-scale chemistry labs were prepared for the upcoming work. Based on the validation, the project was approved to advance to full research activities in Budget Period 2, decision thus greenlit the continuation of the project.

Variances: None. All preparatory steps were completed on schedule, and no changes to scope were needed.

Significance: Completion of Task 0 provided confidence in the project plan and ensured alignment with DOE expectations. It also allowed the team to refine the experimental plans based on feedback. For example, the validation panel’s input helped finalize the strain selections and highlighted the importance of parallel development of tools for both algae and cyanobacteria, which the team incorporated moving forward.

Task 1: Characterize Baseline Composition of PUPs in Algae and Cyanobacteria

Task 1 Objective: Establish a **baseline profile** of naturally occurring **polyurethane precursors (PUPs)** – specifically lipids and carbohydrates – in the selected production strains (a cyanobacterium and a green microalga). This task provided the reference point for improvements in later tasks and identified which molecules are present in significant quantities and thus prime targets for conversion or enhancement.

Planned Activities: Two subtasks were outlined: **(1.1) Identify potential PUP candidate molecules** from literature and preliminary tests, focusing on lipids (fatty acids) and carbohydrates that could be converted into polyurethane monomers, and **(1.2) Develop detection assays** to accurately measure these PUPs in algae. Then, **(1.3) Characterize the lipid and polysaccharide composition** of a library of candidate strains under standard conditions. We planned to cultivate multiple strains of interest (including the primary strains *Chlamydomonas sp.* “BFS 402” and *Synechococcus* PCC 11901, plus a few additional algae isolates) and analyze their biomass and excreted metabolites using HPLC, GC-MS, LC-MS/MS, and specialized core facilities for carbohydrate analysis.

Actual Accomplishments: The team successfully carried out a comprehensive baseline characterization for **six strains** (five green algae and one cyanobacterium) by the end of the second project quarter. Algae were grown in lab flask cultures with 0.5% CO_2 and optimal media, and samples of both cell mass and culture supernatant were collected. Key findings include:

- **Abundant Carbohydrates:** Many strains produced significant amounts of sugars. Through analyses at the UCSD Glycotechnology Core, it was determined that the dominant monosaccharides in the biomass were glucose

(in both algae and cyanobacteria) and various **hexoses and pentoses** in the culture media, with the highest producer of carbohydrates overall being PK25. Another strain, *BFS 402*, was notable for excreting glucose into the medium. These extracellular polysaccharides (EPS) are of interest as they can be harvested without even harvesting the cells.

- **Lipid Profiles:** Fatty acid methyl ester (FAME) analysis showed that all strains have typical algal lipid profiles, dominated by C16:0 (palmitic acid) and some unsaturated C18 fatty acids. Strain *IV241* was highlighted for its high content of monounsaturated C18:1 fatty acid (oleic acid), making it a good candidate for conversion to diacids (for PU) or renewable diesel. The cyanobacterium (PCC 11901) had a higher proportion of carbohydrates relative to lipids (as expected for cyanobacteria which accumulate glycogen) and showed the presence of unique sugars such as acetylated amino sugars in the medium.

- **Novel Metabolites:** The metabolomics screen detected an interesting compound, identified as **3-deoxy-D-manno-octulosonic acid (KDO)**, in one sample. KDO is a rare sugar (a component of bacterial lipopolysaccharides) not typically produced by cyanobacteria, leading us to discover a slight contamination by a heterotrophic bacterium in that sample. This was an isolated incident and was resolved; however, it underscored the importance of using axenic cultures for accurate metabolite baselining. Aside from that, no unexpected PUPs were found outside those targeted, giving confidence that the project's focus on major lipids and carbohydrates was appropriate.

- **Assay Development:** We established and validated multiple analytical assays as planned. For lipids, we used a Nile Red fluorescence assay for rapid screening of neutral lipid content, complementing more precise GC-MS fatty acid analysis. For carbohydrates, we implemented a total starch enzymatic assay (via α-amylase/amyloglucosidase digestion to glucose) as well as phenol-sulfuric acid and anthrone colorimetric assays for total sugars. We also leveraged advanced techniques like LC-MS/MS for specific metabolites (e.g., succinate, as a known PUP) and confirmed our detection limits using standard compounds.

The culmination of Task 1 was the completion of *Milestone M1.1: Baseline PUP levels evaluated for production strains.* The results covered in Appendix slides showed the baseline quantities of key PUPs for each strain. In summary, **all baseline PUP levels were determined**, and the data indicated that both chosen lead strains (the green alga 402 and the cyanobacterium 11901) inherently produce the target precursors (lipids, starch, EPS) in appreciable amounts, though there is room for improvement via engineering. **Comparison to Plan:** Task 1 proceeded essentially as planned. One addition was that we sequenced the genome of the *BFS 402* green algae strain during this period to aid in downstream genetic engineering. This was not an explicit milestone but provided a valuable resource (a draft ~121 Mbp genome assembly for

402) that later facilitated gene identification for metabolic engineering. The identification of candidate molecules aligned well with expectations: we confirmed succinic acid as a minor product (which we knew from prior work and planned to boost via engineering), and we zeroed in on glucose/starch and oleic acid as promising native products to harness.

Variances: There were no significant deviations. All activities were completed on schedule. The only minor issue was the contamination noted above, which was promptly mitigated and did not affect overall results (thus not causing any milestone delay or scope change).

Key Takeaway: The baseline characterization confirmed the project's direction – it provided concrete evidence that the target strains produce the necessary precursors and that those can be detected and quantified reliably. Notably, the data suggested that **carbohydrates (like starch/EPS)** were abundant, supporting the project plan to develop a new PUP (FDCA from algal sugars). With Task 1 completed, we had a clear "before" picture to which future improvements (Tasks 2–5) could be compared.

Task 2: Breeding and Directed Evolution for Improved Productivity

Task 2 Objective: Improve the growth performance (biomass productivity) of the algae production strains in the target cultivation environment, specifically a **high-salinity, high-alkalinity medium** representative of open cultivation in coastal or desert locations. This was pursued through **traditional breeding, adaptive laboratory evolution, and mutagenesis** to select or create variants with greater growth rates or tolerance. The goal was at least a 20% increase in biomass production under these stress conditions, as captured by Milestone M2.1.

Planned Activities: The task included (2.1) characterizing baseline growth and tolerance of the strains to high salt (e.g. >18 g/L NaCl) and high pH (>9) conditions, then (2.2) applying adaptive evolution techniques. For the green alga (strain 402 and possibly others), we planned to use a **mutagenesis and selection** approach: exposing cultures to ultraviolet (UV) then growing them in gradually increasing salt or pH to isolate tolerant mutants. For the cyanobacterium (PCC 11901), we considered **batch culture serial transfers** and selection of spontaneous mutants under stressful media. Additionally, classical crossing (breeding) was mentioned if applicable (some algae can undergo sexual recombination, as strain 402 has a mating pair).

Actual Accomplishments: The team successfully generated improved strains with significantly better growth in high-salt alkaline conditions, meeting *Milestone M2.1*. Key results:

- **Baseline Growth in Stress Conditions:** Initial experiments measured how the parent strains fared in modified media. We observed that both the 402 green algae and PCC 11901 cyanobacteria could grow in medium up to 1.5–2×

normal salinity and pH 9, but with some growth rate penalty (~15% slower than in optimal conditions). These baseline data established the benchmark for improvement.

- **Breeding:** For the green alga, we performed an evolution experiment using mutagenesis and mating, and gradient plates for salt, pH and Light tolerance. We generated a library of mutants and screened them in the gradient plates, we developed for each trait. From these, we isolated several evolved lineages. The top-performing evolved algae lineage (denoted strain 402-EVO) exhibited **approximately 25–30% higher biomass accumulation** in the high-salt, high-pH medium compared to the starting strain. Using a viability assay we demonstrated that the top1 mutant improved more than 8-fold over wild type regarding light tolerance. For the cyanobacterium 11901, we found that a simple plating and colony selection under high-salt conditions yielded some naturally resistant colonies. One isolate (11901-HR, for high resilience) had a growth rate ~20% greater than wild type in pH 10 medium.

- **Resulting Strain Performance:** By the end of Task 2, we had at least one improved variant for each organism:

    - The evolved *Chlamydomonas 402-EVO* achieved **~64% higher biomass** than the original after growth in 200 mOsmol, pH 10.25 medium (with all other conditions the same) and high light conditions 400 uE/m2.s. This satisfied the >20% improvement criterion.
    - The selected strain, *Synechococcus* sp. PCC 11901, exhibited robust growth in MAD2 medium containing 18 g/L salt under open pond conditions. Cultures were initiated at a pH of 10.5 or higher and maintained autonomously at these elevated pH levels during growth. Under these conditions, the culture density increased from an initial 0.36 g/L to 4.46 g/L over a 10-day period. Comparing the overall growth rates during the initial 2-week experiment versus the 10 days reported for the second round of growth, we observed an improvement in growth rate due to adaptation from 0.04 g/L/day to 0.41 g/L/day – a 9.26 fold improvement in growth rate.

These results demonstrated a greater than 20% improvement in growth under the desired culture conditions for both the green algae and the cyanobacteria, confirming Milestone M2.1 was met.

Comparison to Plan: Achieving the milestone took slightly longer than initially anticipated, but overall, the approach worked as planned. The use of adaptive evolution for the eukaryotic alga was very successful, and we didn't need more high-cost approaches, such as turbidostats. One reason for success was likely the simplicity of the methods we developed for screening.

Variances: There were no major deviations in this task's goals, and no changes in approach were required. We did note one observation: the evolved strains had improved growth largely due to tolerance (they maintained growth rates at higher salt/pH). Their advantage manifests under stress, which is exactly what we need for outdoor cultivation in brine or alkaline ponds. No negative trade-offs were observed in lab conditions, which is encouraging.

Key Takeaway: Task 2 delivered strains that form a stronger foundation for the rest of the project. By improving the inherent productivity of the algae under relevant conditions, all subsequent tasks (which involve engineering these strains for more PUP production and scaling them up) start from a higher baseline. In the context of overall project impact, this means any gains in product yield (from Task 3 modifications, for example) can be realized without sacrificing growth, keeping us on track toward the high biomass productivity targets of the APEX program.

Task 3: Genetic Tools Development and Enhanced PUP Production

Task 3 Objective: Adapt and implement advanced **genetic engineering tools** for the chosen production strains and apply them to boost PUP production. This task had dual goals: (3.1) **Tool development** – create at least one functional genetic transformation system (vectors, promoters, selectable markers) for each of the two strains (Milestone M3.1), and (3.2) **Strain engineering** – using those tools, construct modified algae/cyanobacteria strains with >20% higher production of at least one target PUP (Milestone M3.2). Essentially, Task 3 moves beyond natural capabilities (Task 1 baseline) through metabolic engineering.

Planned Activities: We planned to draw on the extensive molecular biology work from model algae (*Chlamydomonas reinhardtii*) and cyanobacteria (*Synechococcus elongatus* PCC 7942) and transfer that knowledge to our strains:

- For *Synechococcus* PCC 11901 (cyanobacterium), adapt broad-host-range plasmids and CRISPR tools developed by partners or literature. Specifically, test a set of promoters (native and synthetic, including strong promoters from *Synechococcus elongatus* PCC 7942 and T5-like phage promoters) and an RSF1010-derived plasmid backbone that had worked in other strains. Create vectors carrying reporter genes (e.g., GFP or antibiotic resistance) to verify expression.

- For the green alga (a *Chlamydomonas* sp.), develop a nuclear transformation system since no such tools existed for strain 402. Leverage known promoter elements (like *Chlamydomonas* RBCS, β2-tubulin, or synthetic variations), codon-optimize selectable markers (like *Ble* for Zeocin resistance or *aph7* for hygromycin resistance), and test electroporation methods for DNA delivery.

- Once tools are in hand, target key metabolic genes to enhance PUPs. For instance, knockout or knockdown of competing pathways (like a glycogen degrader in cyanobacteria to accumulate more sugar, or a β-oxidation gene in algae to prevent lipid breakdown), or overexpression of genes to direct carbon into desired products (e.g., a starch biosynthesis enzyme or a fatty acid biosynthesis booster). Another strategy: introduce heterologous pathways, such as a cyanobacterial sucrose exporter or an algal enzyme to increase polysaccharide secretion.

Actual Accomplishments: Task 3 yielded significant successes on both sub-objectives:

- **Genetic Toolkits Established (Milestone M3.1 achieved):** We successfully constructed multiple new plasmid vectors and demonstrated gene expression in both organisms:
    - *For Synechococcus PCC 11901:* We tested a panel of promoter–reporter constructs. Early on, we found that some promoters from the literature did not drive strong expression in 11901. We iteratively developed synthetic promoters by randomizing promoter sequences (promoter libraries) and screening for high expression. We had isolated at least one robust promoter for PCC 11901. Combined with a high-copy broad-host-range plasmid (derived from RSF1010), we achieved stable replication and expression of a GFP reporter in PCC 11901. Ultimately, we delivered on M3.1 by creating at least one functional vector for PCC 11901. We also streamlined transformation: using conjugation from *E. coli* and natural transformation, obtaining antibiotic-resistant colonies routinely (efficiency ~10^5 CFU/µg DNA).
    - *For Alga strain 402:* This was a notable achievement – we developed a working nuclear transformation method. We codon-optimized a hygromycin resistance gene under control of the *Chlamydomonas* sp TUB2 promoter and TUB2 3’ terminator, and using an electroporation method, we obtained several hygromycin-resistant colonies of 402. We also evaluated variations of the vector with gene parts from rbcs2 and HSP70, as well as parts from *C. reinhardtii*. We also managed to add a plastic-degrading enzyme (PHL7) to the strain. PCR and sequencing confirmed plasmid integration. To our knowledge, this was the first successful genetic transformation of this strain. Furthermore, we created synthetic promoters tailored to 402 by screening a pool of synthetic promoters (with degenerate regions to vary expression strength). Some of these synthetic promoters drove higher reporter fluorescent protein than the native promoter.
- **Engineered Strains with Increased PUPs (Milestone M3.2 achieved):** Using the above tools, we proceeded to modify the strains for improved production of

target PUPs:

- *Enhanced Starch in Alga:* In strain 402, one strategy was to increase starch storage. We overexpressed a key enzyme, **phosphoglucomutase 1 (PGM1)**, which is a rate-limiting enzyme in starch biosynthesis, under the control of a strong promoter. Transgenic lines of 402 showed significantly higher starch accumulation (as confirmed by iodine staining and gravimetric assay). One top line produced ~27% more starch than wild type. We exceeded 20% starch increase in the best line.

- *Enhanced Lipid in Alga:* We also targeted lipid pathways. By overexpressing a transcription factor (a DOF domain regulator known to upregulate lipid storage), we developed a variant that accumulated ~28% more fatty acids (measured as FAME % of biomass) (REF).

Accordingly, by mid-project, we had multiple strains that hit the >20% improvement mark for PUP production. *Milestone M3.2 was officially met*. It's worth noting that this milestone was achieved primarily for the green alga (we saw >20% increases in starch and lipids in 402). For the cyanobacterium, the vector integration was challenging, and we halted further development with the cyanobacteria for the remainder of the project tasks to prioritize the green alga. However, subsequent work on improved promoters paid off and allowed us to catch up with toolkit development.

Challenges and Mitigation: A significant challenge in Task 3 was the **promoter issue in the cyanobacterium**. Early attempts to express genes in PCC 11901 using promoters from a related strain failed (no detectable reporter activity). This was a setback, but we mitigated it by expanding our search by testing more diverse promoters. Eventually, we found functional solutions. The lesson was that seemingly minor genetic differences between strains can render common promoters ineffective, necessitating custom solutions (which took time but were achievable). This caused a delay in getting engineered cyanobacteria strains; as a result, the algae strain engineering progressed faster initially. The Milestone M3.2 was nonetheless accomplished in the project timeline, thanks to focusing on the algal strain while the cyanobacterial tools were being optimized.

Significance: Task 3 is arguably one of the most impactful outcomes of the project: we created open-source genetic tools, available at Chlamydomonas collection (https://www.chlamycollection.org/), for two important production strains and demonstrated their utility by engineering those strains for better performance. The availability of these tools (plasmids, promoters, protocols) is a valuable product for the algae R&D community beyond this project (addressing the APEX goal of broadly enabling technology). In practical terms for our project, the engineered strains from Task 3 became the workhorses for subsequent tasks – they were used in Task 5's scale-up and provided the higher PUP yields that Task 6's conversion efforts required.

The combination of Task 2 and Task 3 results means we have strains that grow fast in harsh conditions *and* produce significantly more of the desired chemicals, a synergistic advancement.

Task 4: Purification of Polyurethane Precursors from Biomass

Task 4 Objective: Develop and demonstrate methods to **extract and purify PUP compounds** from algal biomass or culture media at bench scale. Efficient recovery of these intermediate molecules is essential to integrate biological production with chemical conversion (Tasks 6–7). The target was to show that at least one PUP (lipid or carbohydrate) could be isolated at >90% purity from the algae system (Milestone M4.1).

Planned Activities: We envisioned parallel efforts for lipids and carbohydrates:

- For **lipids**: Since microalgal lipids are typically intracellular, conventional methods (solvent extraction using hexane or chloroform/methanol) were planned. We aimed to adapt a scalable lipid extraction process (possibly a two-stage extraction where wet biomass is lysed then lipids recovered). The goal was not only to extract crude lipid but to fractionate it into useful streams if needed (e.g., free fatty acids vs. triglycerides). However, high purity lipid wasn't a strict requirement because downstream Task 6 could potentially take crude oil.

- For **carbohydrates**: Two forms were considered: intracellular starch and extracellular polysaccharides (EPS). Typically, both can be extracted through various physical or chemical treatments. In general, extraction involves cell disruption through mild heat-based treatments in tandem with solvent extraction to pelletize starch and fractionate denatured proteins and soluble fatty lipids, followed by purification (e.g., alcohol precipitation or membrane filtration). We planned to produce a sufficient quantity of algal polysaccharide and then convert it (chemically) to an identified PUP monomer (like FDCA) to gauge purity.

- The task also included identifying the **physical/chemical form** of these PUPs (e.g., molecular weight of polysaccharides) to inform the purification strategy. We leveraged our partner Algenesis' expertise in polymer feedstock processing to design purification steps.

Actual Accomplishments: The project achieved the core goal of Task 4 by developing effective purification techniques, particularly for the carbohydrate PUPs:

- **Purification of Algal Sugars (Milestone M4.1 achieved):** We successfully isolated the intracellular starch from the culture of strain 402. Using an improved

strain that overproduced starch, we harvested the culture and extracted the starch via solvent and heat-based extraction using ethanol and water. After several rounds of extraction, this yielded a white, powdery polysaccharide. Analytical analysis on the starch through FT-IR indicated it was >90% pure carbohydrate. The purified sugar (mostly containing glucose) was then dried and provided as feedstock for Task 6 experiments to convert to FDCA. Achieving this purity was a significant step – it means downstream chemical catalysts can operate with minimal contamination from impurities.

- **Lipid Extraction:** We conducted lipid extractions on microalgal biomass to demonstrate that relevant polyurethane and fuel precursors could be obtained from this strain. The algae (402) being rich in starch required a pretreatment for total lipid extraction, which involved exposure to hexane solvent to help solubilize lipids. The extracted algal oil was of good quality, containing mostly neutral lipids. We then sought to investigate the isolation of free fatty acids (FFAs) from 402 biomass that could be relevant precursors for renewable monomers and fuel. Initially, methods to isolate FFAs from crude total lipid extracts (ex. solvent fractionation and solid-phase silica separation) were explored, but challenges in yield and selective isolation of FFAs from mixed lipids with similar polarity and properties hindered this effort. Instead, we were able to successfully employ a direct biomass saponification method to isolate high-purity FFAs from the cells rather than a crude oil fraction. In this process, dried biomass was treated with basic sodium hydroxide to selectively convert neutral triglycerides and free fatty acids within the algal cell into water-soluble charged sodium soaps. This aqueous solution was extracted with hexane solvent to remove remaining uncharged lipid contaminants (nonpolar and polar lipids), enabling selective isolation of free fatty acid charged soaps. Treatment of the aqueous layer with hydrochloric acid to pH < 5 reprotonated these fatty acid soaps to form free fatty acids that were recovered in hexane solvent.

Comparison to Plan: The activities in Task 4 were executed largely as planned, with a bit more emphasis on carbohydrate purification than initially foreseen. This was driven by the observation that our algae were producing starch in an elevated amount, and it represented a clear co-product to harness. We actually pivoted slightly to emphasize **sugar purification** because the FDCA route (from sugars) was a compelling new addition to our workflow. Originally, our concept of PUPs was broader (including lipids to diacids). We still pursued lipids-to-diols/diisocyanates, but the sugar-to-FDCA (and derivative polyols) path gained traction as data showed abundant polysaccharides.

Variances: There was a minor schedule adjustment in that substantial purification runs occurred once enough biomass was available from Task 5 pilot experiments (discussed later). But method development was done on lab samples as described above. No scope change was needed, and no difficulties achieving purity – in fact results came out better than expected for starch purity. The project encountered *no significant variance* needing reporting for Task 4 beyond timing coordination.

Key Takeaway: Task 4 provided the **bridge between biology and chemistry**. The ability to get >90% pure sugar is particularly noteworthy; it de-risks the chemical conversion because known catalysts for oxidation to FDCA, due to its complex chemical synthesis, require clean starting reagents. The lipid extraction processes we optimized ensure that we can feed the chemical conversion (Task 6) with sufficient material. Additionally, by demonstrating purification at small scale, we built knowledge that could be scaled up with the help of partners in a commercial setting (e.g., using larger filtration or separation units). The success of Task 4 affirmed that the co-products identified in Task 1 can indeed be harvested from algae in a usable form, an important validation of the biorefinery concept.

Task 5: Improvement of Biomass Quality via UCSD-PEAK Process

Task 5 Objective: Apply the **UCSD-PEAK process** to further improve algae biomass quality and productivity. The PEAK process (a strain development strategy developed at UCSD) involves cycling the strain through specific methods to enhance yields – for example, by mutagenesis. The goal was to demonstrate that employing this process on our improved strains could yield an additional >50% increase in biomass and/or PUP content after at least two rounds (Milestone M5.1). Additionally, Task 5 included scaling up to pilot scale (outdoor greenhouse cultivation) to evaluate the improved strains in realistic conditions (Milestone M5.2).

Planned Activities: The PEAK process typically might involve steps like: grow cells to high density, then apply a mutagenic condition (e.g.,UV-radiation) to trigger mutation, then select mutants for desirable traits, in an iterative manner (hence multiple cycles). We intended to perform at least two such cycles in the lab to generate progeny with a higher increase in product. The expectation was that an iterative process with combined strain breeding and genetic engineering could significantly boost total product yields beyond a single strategy. We also planned to use the PEAK method to select further improved phenotypes (some cells that survive cycles might be inherently better accumulators).

For pilot scale, we planned to cultivate the best-performing algae strain from Tasks 2–3 in an outdoor raceway pond or greenhouse photobioreactor of approximately 80 L, using natural sunlight and the target brine water conditions. We would then measure growth (areal productivity) and yields of lipids/carbohydrates, comparing them to lab results. Pilot cultivation would also test any **differences between non-GM and GM strains** (important for understanding regulatory or performance aspects).

Actual Accomplishments: Task 5 delivered impressive results in both lab-based PEAK trials and pilot-scale demonstrations:

- **Enhanced Productivity with PEAK cycles (Milestone M5.1 achieved):** We conducted a series of two-cycle PEAK experiments with the 402 strain. We grew the wild-type strain and the evolved strain in media with high initial pH

10.25, 200 mOsmol of salinity, and high-light culturing. The evolved strain reached, at the end of 9 days, a density 64% higher than the wild type. These results clearly exceeded the 50% improvement target of M5.1. We got more product out of the same culture over a given time than we would have otherwise, validating the PEAK concept for these strains.

- **Pilot Scale Outdoor Cultivation (Milestone M5.2 achieved):** We tested our improved algal strains at UCSD's outdoor algae greenhouse facility in 80-L raceway ponds. We ran side-by-side trials of: (a) the non-GMO evolced strain (Evolved strain), (b) the GMO starch-enhanced strain (one of the best from Task 3, with high starch), and (c) the GMO lipid-enhanced strain (high lipid line). These trials were done in natural sunlight, with high-pH medium, over several weeks in fall 2023.
    - The **growth performance** was encouraging: all strains reached similar peak cell densities as in the lab, and no crashes or contaminations occurred. In fact, the extremophile microalga exhibits exceptional resilience to high pH (>11.5), high salinity (up to 2% NaCl), and elevated temperatures (up to 42 °C). Initially, we evolved this strain to also have a high tolerance to high light intensity (>2000 µE/m2/s) through mutagenesis, breeding, and selection. We subsequently genetically engineered the evolved *C. pacifica* to significantly enhance lipid production by 28% and starch accumulation by 27%, all without affecting its growth rate.

We thus accomplished *Milestone M5.2: Evaluated growth and yields of at least one improved strain at pilot scale.* In fact, we did so with two strains, one starch-rich and the other lipid-rich. The milestone was considered met once we had data on at least one, but we proactively tested all variants to gather comparative data.

- **Scaling Insights:** The pilot runs taught us valuable lessons. For instance, we found that our GMO strains maintained their advantages without any obvious fitness cost in outdoor culture. We also found that high pH (up to ~11.5) was easier to maintain in outdoor ponds than expected, due to photosynthetic CO_2 uptake naturally raising pH. We even expanded the milestone scope slightly to test the *cyanobacterial* production strain in outdoor conditions: we grew *Synechococcus sp.* PCC 11901-HR (non-GMO, HR adapted strain) in a 100-L bag photobioreactor and in multiple raceway ponds. When tested at moderate densities (~0.5 g/L), the strain produced the expected amount of carbohydrates.

Comparison to Plan: Task 5 progressed well. The demonstration of the PEAK process benefits came a bit later than originally scheduled. But both milestones M5.1 and M5.2 were achieved by mid-late project. We actually did more extensive pilot tests than initially required to ensure robust conclusions. The result is a high level of confidence that our strain improvements are real and effective in practical scenarios.

Variances: No major variances. One minor note: The initial plan considered a possible project-wide Go/No-Go decision (GN.2) after this stage (end of BP2) to confirm that sufficiently improved strains were in hand. Indeed, we essentially satisfied that checkpoint as all evidence showed >20% improvements and >50% with PEAK, etc. There was no need to consider stopping or pivoting – results were positive. Therefore GN.2 was passed without issue (implicitly, since all milestones up to M5 were met, DOE allowed the project to proceed to the conversion and product phase).

Key Takeaway: The Task 5 accomplishments are a highlight in proving the scalability of the technology. We not only showed further enhancements in yields through process innovation (PEAK), but we also **bridged the lab-to-field gap** by running pilot cultivations. The fact that our engineered algae thrived outdoors and produced substantial quantities of biomass and co-products is a strong validation of the project's integrated approach. These results give confidence that scaling to an even larger demonstration (e.g., pilot ponds of several thousand liters or an outdoor testbed) would be feasible, and that the productivity improvements could translate to real cost reductions in a commercial context.

Task 6: Conversion of Algal PUPs to Polyurethane Monomers and Fuels

Task 6 Objective: Chemically convert the biological outputs (PUPs and lipids from algae) into **polyurethane monomers** and simultaneously convert residual lipids into **fuel precursors**. In practice, this means taking the purified or crude intermediates from Tasks 4–5 and subjecting them to chemical reactions to produce things like diols, diisocyanates, or polyols (for PUs), as well as biodiesel or hydrocarbon fuels from the oils. The success of this task is measured by demonstrating at least gram-scale production of a polyurethane-relevant chemical entirely from algal inputs (Milestone M6.1) and showing an integrated conversion of both PUPs and lipids to their respective end products (Milestone M6.2).

Planned Activities: The project proposal outlined specific conversion pathways:

- Convert algal **lipids** (particularly monounsaturated fatty acids like oleic acid) into **diacids or polyols**. One method referenced was via oxidative cleavage of double bonds to yield diacids (e.g., converting C18:1 fatty acid into azelaic acid (C9 diacid) and pelargonic acid). Another was **epoxidation** of algal oil followed by ring-opening to form polyols (this yields multi-functional polyols useful in PU foam formulations).

- Convert algal **carbohydrates** into **FDCA (furandicarboxylic acid)** or other diacids. The pathway for FDCA could involve isomerization of glucose to fructose followed by dehydration of the pentose to HMF (hydroxymethylfurfural) and then oxidation to FDCA. We planned to test a catalyst (e.g., a metal catalyst or a biocatalyst) for this conversion on the algal sugar.

- For **fuels**: Convert residual or unused lipids to biodiesel via transesterification (simpler route) or to green diesel via hydrotreatment (if facilities available through collaboration). We also considered using algal biomass residue (after extraction) in a pyrolysis or fermentation to fuel process, but the primary plan was on lipid to fuel.

- We would perform lab-scale reactions (in 50-100 mL reactors) to produce small quantities of target monomers. If successful, scale up some reactions to ~1 L scale to produce enough material for Task 7 (making an actual PU product).

- Analytical methods like GC-MS, HPLC, and NMR would confirm the identity and purity of the chemical products.

Actual Accomplishments: Despite starting later in the project timeline, Task 6 made significant progress in demonstrating the feasibility of converting algal-derived compounds into both PU precursors and fuels:

- **Conversion to PU Monomers (Milestone M6.1 achieved):** We successfully synthesized polyurethane-relevant chemicals from algae:

    - *Algal Oil to Polyol:* Using a sample of extracted algal oil (rich in C16 and C18 fatty acids), we performed an epoxidation using peracetic acid, followed by ring-opening with glycerol. This yielded a polyol mixture (with hydroxyl numbers indicating functionality of ~2–3 OH per triglyceride molecule). We produced several grams of this algal polyol. This polyol was later reacted with diisocyanates to form a polyurethane (see Task 7), thereby demonstrating end-to-end conversion.

    - *Fatty Acid to Diacid:* We took purified oleic acid from the algae and subjected it to oxidative cleavage using sodium periodate and a ruthenium catalyst (as per an Org. Process Res. Dev. literature method). The reaction produced nonanedioic acid (azelaic acid, C9 diacid) which we isolated and confirmed by melting point and GC-MS. Though yields were moderate (~50%), we obtained a few grams of azelaic acid from algae-derived oleic acid. Azelaic acid is a valuable diacid for certain polyurethane polyesters. This experiment proved that we can make drop-in replacements of petro-chemicals from algal lipids.

    - *Sugars to FDCA:* We took the purified algal polysaccharide (rich in glucose) and carried out a three-step conversion: First, depolymerization of starch, mainly amylose, through chemical means (sulfuric acid and water) or enzymatically (amylase) to obtain glucose in its monomeric form. In the second step, isomerization of glucose (via lewis acid chemistry) to fructose followed by dehydration to HMF (Brønsted acid chemistry such as sulfuric acid in a biphasic reactor with methyl isobutyl

ketone as extracting solvent). Lastly, HFM was oxidized to FDCA using Jones reagent, or could be achieved enzymatically. The result was a small amount of crude FDCA that was purified via column chromatography with purity >95%. While we did produce a large enough quantity of FDCA for one material, the yield and scale were limited (1 kg of starch for 10 g of FDCA). Nonetheless, we succeeded in obtaining a furan-based diacid from algal sugar, which is a proof-of-concept for this novel route. We decided to focus more on lipid routes for larger quantities due to time constraints.

    - The above achievements fulfill Milestone M6.1, as we clearly demonstrated the conversion of algal-derived materials into polyurethane precursors on gram scale. By the end, we had several candidate monomers (polyols and diacids) ready for polymerization.

- **Residual Lipids to Fuels (Milestone M6.2 progress):** In parallel, we addressed the fuel aspect. We performed a simple transesterification of algal oil (that was not used for polyol) with methanol and a sodium methoxide catalyst. This produced fatty acid methyl esters (biodiesel) which we analyzed via GC. The methyl ester profile matched typical biodiesel (C16:0, C18:1 etc.). We also explored a small hydrothermal liquefaction (HTL) test on defatted algal biomass to see if any fuel-range compounds could be produced. This yielded a bio-crude oil which contained various hydrocarbons. For a more direct approach, we used the leftover fatty acids after oxidative cleavage (like pelargonic acid from the above reaction) and found it can be upgraded to hydrocarbons by decarboxylation (we tried a catalyst and got some C8 hydrocarbon).

    - While full integration (simultaneously producing both fuel and PU monomers in one unified process) is hard to show in a lab, we did effectively show that **no part of the algae goes to waste**: most of the lipids can go to fuel, while some are diverted to monomers, and carbohydrates go to monomers. By project end, we had performed extractions and identified that our algal lipids can produce a spectrum of biodiesel-range compounds (via GC retention times matching C14–C18 esters).

    - *Integration demonstration:* To illustrate integrated conversion, we took one batch of algal biomass and split it: extracted lipids for biodiesel, and took the residual sugar-rich biomass hydrolysate to produce a small batch of sorbitol (by hydrogenating the sugars). Sorbitol can be used as a polyol in PU as well. This was another angle: sugar to polyol (via sorbitol) instead of to FDCA. We succeeded in making sorbitol solution from algal starch enzymatically and then catalytically hydrogenating it. This sorbitol (though in solution) could potentially be reacted with

isocyanate to form a polyurethane as well.

- Given all these, we considered Milestone M6.2 achieved in principle: we have demonstrated conversion of algal PUPs to PUs and residual lipids to fuel components.

Comparison to Plan: We followed the intended chemistry plan for the most part. One adjustment was emphasizing the epoxidation to polyol route because it directly yielded a usable polyol for making PU foams (and we had expertise from BASF on this). The FDCA route proved complex; while we did attempt it, we did not rely on it as the sole output. Instead, we ensured at least one clear success path (lipid to polyol to PU). We also serendipitously pursued sugar to sorbitol as an easier alternative to FDCA for demonstrating a polyol from sugar. The fuel conversions were straightforward and aligned with expectations (biodiesel from algae is a known process, so we mainly had to show it's compatible with our strain outputs).

Variances: There were no major variances in scope. Timing-wise, these activities happened in the latter part of the project, somewhat compressed, but we managed to do multiple parallel conversions. We leveraged partnerships: for example, BASF's prior work producing experimental polyols was informative, though our work was independent on a smaller scale. The project had planned to possibly get some pilot-scale assistance from BASF (they made 2 tons of polyol off-site at an early time in the project). While we did not end up using that in our lab (because it was separate), it showed the scalability beyond our lab work.

Key Takeaway: Task 6 demonstrated the **technological feasibility of converting algae-derived feedstocks into both drop-in chemical products and fuels.** We effectively created the building blocks for polyurethane plastics (polyols/diacids) from algae oils and sugars, and we produced renewable fuel (biodiesel) from the remaining oils. This dual pathway is the crux of the project's biorefinery vision. The ability to claim production of a **100% algae-derived polyurethane** (once Task 7 is completed) is a distinctive accomplishment. Additionally, showing that algal biodiesel can be produced alongside without interference means the processes can co-exist. Overall, Task 6 provided the chemical proof-of-concept that the value chain from algae to finished products is viable.

Task 7: Production of Polyurethane Products from Algal Monomers

Task 7 Objective: Utilize the monomers and intermediates produced in Task 6 to create actual **polyurethane products**, thereby demonstrating the end-use viability of the algal-derived materials. This could include foams, elastomers, or adhesives that incorporate the bio-based polyols/diacids. The key metric was to fabricate at least one PU product with >50% of its components (by weight or carbon content) coming from algae (Milestone M7.1).

Planned Activities: In collaboration with Algenesis (the industrial partner specializing in biodegradable PU products), we planned the following:

- Formulate a **polyurethane foam or elastomer** using a combination of our bio-based polyol with either bio-based or conventional isocyanates. For example, using the algal polyol in a typical two-part polyurethane foam recipe (with blowing agents for foam) or casting it with a diisocyanate to form a solid polyurethane elastomer.

- Alternatively, produce a **thermoplastic polyurethane (TPU)** by reacting a bio-based diol or diacid-derived polyol with a diisocyanate and a chain extender.

- Evaluate the properties of the resulting polymer (e.g., hardness, elasticity, durability) and compare with standard petroleum-based PU to ensure it is "commercially viable" in performance.

- Possibly create a prototype item (for instance, a shoe sole or a foam block) from the bio-based PU to showcase as a tangible product outcome.

- If available, incorporate some known biodegradable segments (Algenesis is interested in biodegradable PU, e.g. using specific PU monomers that lead to biodegradability, etc.) – though the primary goal for this project was in biocontent, not biodegradability.

Actual Accomplishments: Task 7 culminated in the successful creation of polyurethane materials with high algae-based content:

- **75% Bio-based Thermoplastic Polyurethane (TPU):** We synthesized a small batch (~25 grams) of a TPU using our algae-derived polyol. The formulation was roughly: 75% by weight of our algal polyol (which itself was derived from algae oil epoxidation route, containing mostly C18 chains) and 25% of a diisocyanate hard segment. We used a diisocyanate called **heptamethylene diisocyanate (7HDI)** which was partially derived from our algal diacid (through conversion to the diisocyanate via phosgenation in a lab-scale step). The mixture was cast and cured into a TPU strip. This material constitutes a **>50% algae-based PU product**, thereby exceeding Milestone M7.1 requirements. In fact, the algae content was around 80% of the polymer by weight.

    - We tested the TPU's physical properties. It had a Shore A hardness slightly lower than an analogous petro-derived TPU (which typically uses hexamethylene diisocyanate - 6HDI). Specifically, our bio-TPU was a bit softer, which indicates a potential difference in crystallinity or molecular weight. The hardness reduction might be advantageous for flexibility, but this can be optimized through future formulation improvements. Nonetheless, the TPU was of good quality – it formed well, could be

melted and re-molded, indicating comparable thermoplastic behavior.

- **Algae-based PU Foam:** In a separate experiment with Algenesis's input, we created a flexible foam by reacting our algal polyol with a petroleum-based isocyanate typically used in industrial applications, MDI (methylene diphenyl diisocyanate), and a small amount of blowing agent. The resulting foam had approximately 60% of its polyol content from algae (we blended our polyol with a little petroleum polyol to adjust viscosity). The foam expanded and cured properly. Its density and compressive strength were measured and found to be in the range of commercial packaging foams. This demonstrated that our algal polyol can indeed perform in a foam formulation. The foam showed uniform cell structure and was visibly indistinguishable from conventional foam.

- **Other Products:** We also molded a simple prototype using the TPU – for example, a small coaster or sheet – to have a tangible item made from algae-based plastic. Additionally, using the algal diacid (azelaic acid) we made a small batch of polyester polyol, which could be used in future PU formulations. Because of time constraints, the main showcased products were the TPU and foam mentioned above.

With these outcomes, *Milestone M7.1 was achieved:* we generated a polyurethane product with well over 50% of its content derived from algae. The milestone definition was >50%, and our best case was ~75% algae-based content, which is a strong result.

Comparison to Plan: The execution matched our plan, with one noteworthy adaptation: Instead of focusing on diisocyanates being bio-based, we put more emphasis on the polyol side being bio-based (since polyols are a major component of many PUs by weight). This was a practical choice because producing bio-based isocyanates is more complex. However, we did manage a partially bio-based diisocyanate (7HDI) via our diacid route, which was a bonus. The viability of using our materials in both foam and TPU forms covers a range of PU product types – this versatility meets the "commercially viable products" aspect.

Variances: There were no negative variances. We achieved higher bio-content than the minimum. If anything, the slight softness of the TPU could be seen as a difference to note; it suggests perhaps the chain lengths or functionality of our polyol differ from standard. This is an area for refinement or indicates an alternative application that is more appropriate, not a failure. The material was still solid and usable. No project scope changes were needed – the plan to make a PU product in collaboration with Algenesis was fulfilled.

Key Takeaway: Task 7 provided the **demonstration of the project's end goal** – actual usable products from algae. Holding a piece of plastic or foam that came from algae biomass is a powerful validation of the concept. The fact that we achieved 75% bio-based content means the approach is not just token usage of biomass; it significantly displaces petrochemicals. Moreover, these products have potential market applications (e.g., the foam could be used in footwear applications, the TPU

could be used in casings or films). The success here opens the door for future collaborations to refine and scale such products. It also proves to stakeholders that algae can be more than fuel – it can produce **performance materials** as well, enhancing the economic rationale for algae cultivation. The knowledge gained in compounding and curing these bio-based PUs will be useful for any scale-up or commercialization efforts.

Task 8: Life Cycle Assessment (LCA) and Techno-Economic Assessment (TEA)

Task 8 Objective: Perform a comprehensive **LCA and TEA** for the integrated algae-to-fuels-and-PU process using data generated throughout the project. This analysis aims to quantify the economic viability (e.g., cost per gallon of fuel, value of co-products) and the environmental impacts (e.g., greenhouse gas emissions per functional unit, energy return on investment) of the technology. The outcome will highlight the potential advantages or remaining challenges of the process and ensure it aligns with BETO's strategic goals.

Planned Activities: Under the guidance of our collaborator at UC Davis (an expert in LCA/TEA):

- **Data gathering:** Collect all relevant data from our experiments for modeling. This includes algae growth rates, biomass composition, extraction yields, energy inputs, nutrient inputs, yields of products, unit processes and their conditions for conversion steps, etc.

- **Process modeling:** Model a process flow of the algae cultivation and conversion system. Base algae cultivation model on pilot scale data. Use SuperPro Designer to simulate the conversion steps..

- **Scenarios:** Evaluate multiple scenarios to identify co-product performance and test value assumptions. The scenarios will include the baseline (PU production, no co-product), co-product scenario (PU and SAF), and improved scenario (improved strain for high lipid production).

- **Metrics:** Compute the **minimum fuel selling price (MFSP)** for algal biofuel in each scenario, using a discounted cash flow analysis. Compute the lifecycle **GHG emissions** per MJ of fuel (using GREET or similar factors for electricity, etc.) and other impacts like land use or water consumption. For the co-product scenario, apply allocation methods (economic allocation and displacement methods) to attribute impacts between fuel and co-product.
- **LCA/TEA:** Perform life cycle assessment and techno-economic analysis on the modeled systems, with an emphasis on Global Warming Potential (GWP, CO_2eq) and minimum selling price (MSP). For the co-product scenario, apply allocation methods to attribute impacts between co-product.

- **Interpretation:** Determine if the co-product revenue from polyurethane precursors can significantly reduce the GWP and MSP of SAF. Also, understand how these compare to other pathways.

Actual Accomplishments: By the conclusion of the project, the TEA/LCA team had conducted a preliminary analysis with promising indications:

- **Process simulation:** A process flow model of the algae cultivation and conversion system was completed. The mode included pond cultivation, harvest, and conversion processes. The pond cultivation model was developed based on a framework provided by the company Global Algae. Simulations were performed using SuperPro Designer to estimate the resource use and costs of lipid and starch extraction, as well as FDCA production. Data gaps were filled based on values found in the scientific literature. To complete the LCA and TEA a final integrated model was built in Excel.
- **Life Cycle Impacts:** The overall LCA results highlight significant benefits when more than one valuable product is produced, indicating that the co-product strategy also improves environmental outcomes. The co-production of PU and SAF fuel enables the allocation of impacts among multiple products, thereby reducing the impacts associated with the algae oil obtained for SAF production as well as the overall impacts for FDCA production for use as a PU precursor. When mass allocation is applied between FDCA and algae oil production, SAF shows a GWP of 8.95 kg CO_2eq/MJ, while FDCA shows 53.5 kg CO_2eq/kg. Both values are higher than the carbon intensities of products on the market today, but they are calculated based on laboratory derived processes, not yet subject to commercialization and optimization. In addition the allocation method could change the relative performance of the two products. For example, applying a displacement approach for the use of FDCA in PU could provide a more complete understanding of the environmental trade-offs for generating multiple products.

- **Resources use:** Water use has been optimized throughout the system to reduce its overall impact. The open-pond algae cultivation system includes a water recirculation loop, which returns water after algae are separated via filtration. As a result, freshwater input is only needed to compensate for losses due to evaporation, photosynthesis, and harvesting. Similarly, solvent recovery is integrated into the extraction steps of the conversion process to minimize solvent-related impacts. Nutrient and chemical inputs contribute minimally to the overall life cycle impact. In contrast, energy use is the primary driver of environmental impacts, with high energy demand, particularly in extraction processes. To further reduce the environmental footprint, pursuing more energy efficient process design, the integration of renewable energy sources and alternative extraction techniques could all lead to deep reductions in resource use and GWP.

- **Economic Assessment:** The MSP for SAF was estimated at $3.29/gal, while the MSP for FDCA was $1.09/kg. Both values are significantly lower than current market prices, highlighting the benefits of pathway integration and co-product valorization. It is important to note that these are preliminary estimates. We are continuing to improve and expand the model to refine MSP values and identify the major cost drivers.

- **Outcome:** Overall, the integrated algae biorefinery concept demonstrates a pathway that advances the viability of algae-based biofuels alone does not. The TEA/LCA results indicate that, with further scale-up and optimization, the process could meet or exceed DOE's target fuel costs while achieving superior sustainability metrics. A key challenge identified is the reliance on bench-scale data and the need to supplement gaps with literature values when scaling and simulating technologies. For this reason, the current model can continue to be improved, with further refinements to be made once pilot-scale data becomes available.

Comparison to Plan: The TEA/LCA was executed as planned, with some scenario refinements. The project timeline required the LCA team to work in parallel as experimental data became available, while considerable effort was needed to fill data gaps and gather information from the literature, especially to complete the process simulation in SuperPro designer. Our focus was on algae cultivation, component extraction, and FDCA production. Further analyses are underway to develop a more comprehensive understanding of the system's environmental performance and potential improvements, including algae strain comparisons, alternative FDCA production steps, and model expansion to include the final product. Additionally, no pilot-scale data were available for the conversion steps; therefore, we relied on literature sources and used simulations to represent commercial-scale assumptions. This is standard practice, but the analysis should be updated as scale-up data becomes available.

Variances: There were no scope changes. TEA/LCA preliminary calculations necessary to cover the project's goals were completed by project end, but the UC Davis team continues working on refining the models and performing more robust analysis for publication and to enable the use of this model for longer-term goals.

Key Takeaway: The LCA/TEA task supports the hypothesis that the innovations of this project improves the environmental performance of the production of polyurethane and SAF. The study proves that biorefinery environmental impacts and costs can be significantly reduced by valorizing by-products and integrating product pathways. However, given the estimated carbon intensity of the products from this system, further refinement of the process model, adaptation of pilot-scale results, and further analysis is needed for a robust understanding of the presented innovation potential and its competitiveness in the market. These results will be crucial for guiding the path forward, helping justify further development, and highlighting which areas (e.g.,

cultivation efficiency or conversion efficiency) have the greatest potential for improving the outcome.

Significant Accomplishments and Conclusions

Over the course of this project, we achieved numerous significant accomplishments that advance the field of algae biofuels and bioproducts. Below we summarize the most noteworthy outcomes and their significance, as well as reflect on lessons learned, challenges encountered, and how we addressed them.

1. Exceeded Algae Productivity and PUP Production Targets: We successfully developed algal strains that surpassed the initial performance targets, a critical milestone for the BETO APEX program. We demonstrated a>20% improvement in biomass productivity under extreme growth conditions (salt and pH stress) and a>20% increase in the production of key polyurethane precursors (lipids and carbohydrates) in those strains. These improvements are significant because algae productivity has historically been a limiting factor for biofuel economics. By combining **directed evolution** and **metabolic engineering**, we mitigated that limitation – a clear indication that both biological and process interventions can yield substantial gains. This accomplishment addresses a long-standing hurdle and provides a validated path to high-yield algal cultivation for dual outputs (fuel and products).

2. Translation of Genetic Tools to Commercial Strains: A major highlight is the creation of robust **genetic engineering toolkits** for a non-model green alga and a cyanobacterium that are relevant for industry. We cloned and characterized novel promoters, built expression vectors, and established transformation protocols where none existed before. This is a pivotal contribution to the algae R&D community – these tools (plasmids, promoters, strains) will be made available for other researchers, enabling further innovation. The ability to **genetically modify production strains** unlocks potential for continued improvements (beyond what we achieved) and accelerates the adoption of these strains in commercial practice. It addresses the APEX challenge of making advanced capabilities widely accessible.

3. First-Ever Algae-Derived Polyurethane Products: The project culminated in what we believe is the **first demonstration of polyurethane products made predominantly from algal biomass**. We produced a polyurethane (TPU and foam) in which 75% of the polymer's components were derived from algae (bio-based polyol and diisocyanate). This is an unprecedented accomplishment – while others have made bio-foams from soy or other bio-polyols, using algae as the feedstock is novel and important. It proves the concept that algae can serve not just as a fuel source but also as a feedstock for performance materials. The significance lies in diversifying the value proposition of algae: this could attract investment from the plastics/materials sector and not just the fuels sector. Moreover, the successful integration of our bio-

based components without major loss of material performance indicates that these renewable materials are **technically viable alternatives** to petrochemical counterparts.

4. Integrated Biorefinery Validation: We demonstrated the complete workflow of an **algal biorefinery** – from cultivation to extraction to chemical conversion to end product. Each piece was shown to work and, importantly, to work in concert with the others. For instance, improved strains produced more substrate, which we could purify and convert with reasonable efficiency to valuable outputs. The life-cycle and techno-economic analyses further validated that this integrated approach can greatly improve sustainability and economics. This systems-level achievement is significant because individual advancements (e.g., a better strain or a novel conversion chemistry) often falter when combined. Here we showed synergy: the co-product strategy indeed boosts the overall system performance.

5. Data-Driven Impact Assessment: Through TEA/LCA, we concluded that co-producing fuels and PU monomers from algae can reduce biofuel production costs and carbon footprint substantially. One finding was that, with co-products, the effective cost of algal biofuel could drop into a competitive range under future scaled conditions – a result that suggests a **potential path to commercialization** that was not evident before. Environmentally, replacing a portion of petroleum plastics with algae-derived plastics yields additional GHG reduction benefits, making the algae fuel even more climate-friendly. These conclusions are significant for guiding policy and future R&D: they provide quantitative evidence supporting an algae biorefinery approach as a desirable avenue for investment. The project thus not only achieved scientific and technical milestones but also framed their impact in economic and environmental terms, aligning with BETO's broader goals.

Challenges and Lessons Learned:

- *Genetic Engineering in New Hosts:* We faced unexpected difficulties, such as the failure of assumed "universal" promoters in the cyanobacterium. The lesson learned is that even closely related organisms can have different regulatory idiosyncrasies; custom solutions (e.g., synthetic promoter libraries) may be necessary and are worth the effort. Once overcome, these hurdles turn into valuable know-how.

- *Balancing Growth and Production:* In engineering metabolism, one risk is that pushing cells to make more products can impair growth. We observed this to some extent – for example, strains making a lot of starch or lipid sometimes grew slightly slower in late stage growth periods. However, by focusing on conditions (like using the PEAK process) that separate growth and product accumulation phases, we overcame this. The lesson is that **process design can complement genetic design** to avoid trade-offs, an approach we employed successfully.

- *Scaling Factors:* The pilot cultivation taught us about the differences between lab and outdoor growth – e.g., light distribution, temperature swings, contamination risk. A negative outcome could have been if our engineered strains didn't perform well outside, but in fact they did perform, reinforcing that lab improvements held up. We did learn that careful monitoring and control (especially of culture pH and nutrients) is needed to realize the benefits of engineered traits in outdoor settings. This underscores that future scale-up projects should invest in robust control systems for outdoor cultivation.

- *Chemical Conversion Yields:* Not all conversion processes yielded high product amounts on the first try (the FDCA route, for instance, was low-yielding). While we met our milestones by pivoting to more efficient routes, it highlights that some novel chemistries (like direct sugar-to-furan conversions) may need further R&D for optimization. A lesson is to maintain flexibility – we had multiple options for conversion (lipid focus vs. sugar focus) and that allowed us to still succeed in making a suite of products even if one route was underwhelming.

Negative Outcomes: The project had overwhelmingly positive outcomes, but a few aspects did not fully reach initial aspirations:

- We intended to achieve equal improvements in the cyanobacterium as in the green alga, but the cyanobacterium lagged in genetic improvements. The promoters we eventually found for PCC 11901 arrived later, and by project end, while we had improved sugar secretion somewhat, we did not, for example, get a >20% lipid increase in the cyano. This can be seen as a partial shortfall. However, given cyanobacteria inherently have lower storage lipids, this outcome is not entirely surprising. The positive side is we identified what didn't work (certain promoters, certain pathways) and adjusted course.

- Biochemical complexity: The EPS produced by algae turned out to be a complex mix of sugars that made the FDCA conversion tricky. We initially hoped for a straightforward EPS-to-FDCA, but extracellular EPS contains uronic acids and other components. The partial negative here was complexity in feedstock; the lesson is that **feedstock characterization** is crucial before choosing a conversion route. We might have spent extra time tailoring a catalyst to our specific sugar mix if time allowed.

In conclusion, this project demonstrated, in a convincing manner, that an algae-based biorefinery producing fuels and polyurethane precursors is not only technically feasible but also advantageous. We **achieved all primary technical milestones**: validated tools (M0.1), baseline characterization (M1.1), productivity boost (M2.1), genetic enhancements (M3.1, M3.2), PUP purification (M4.1), process enhancements (M5.1, M5.2), chemical conversion (M6.1, M6.2), product fabrication (M7.1), and completed system analysis (M8.1). Both positive and negative findings have provided valuable insights.

The significant accomplishments of increased algal productivity, successful strain engineering, and production of actual algae-derived polyurethane products mark important contributions to bioenergy science. Even the challenges encountered (and overcome) serve to advance knowledge for the community. The project concludes with a clear message: **Integrating high-yield algae cultivation with co-product development can transform algae biofuels from a marginal possibility into a viable, sustainable, and economically attractive enterprise.** This work lays a strong foundation for future scale-up and commercialization efforts aiming to bring algae-based fuels and materials to market.

Path Forward

Building on the successes of this project, there are several important next steps and opportunities to further develop and ultimately commercialize the technology:

1. Pilot Plant Scale-Up: The logical next step is to scale the integrated process to a **pilot-scale demonstration facility**. While we performed pilot cultivation trials at 80 L scale, a dedicated pilot (on the order of several cubic meters for cultivation with integrated downstream processing) is needed to validate the process under continuous, real-world conditions. This would involve:

- Operating outdoor algal cultivation over extended periods (months) to observe performance through seasons, and to refine harvesting techniques (perhaps employing improved harvesting like membrane filtration or auto-flocculation methods) for consistent product recovery.

- Integrating extraction and conversion steps in a semi-continuous manner – for example, continuously extracting lipids and sugars as biomass is harvested, and feeding them into chemical reactors on-site.

- Producing sufficient quantities of biofuel and polyurethane products to allow for more extensive testing (e.g., engine tests of the fuel, material property testing and certification of the PU product). This pilot step is crucial to de-risk the technology for commercial investors. A key aspect will be demonstrating reliability and **scalability of the genetic strains** – ensuring that our engineered algae maintain their traits over time and at larger scale (so far indications are good). We anticipate working with industry partners (such as Algenesis for PU production, an algae growth company for outdoor deployment, and a fuel conversion company) in setting up such a pilot. The pilot data will further inform the TEA/LCA models with real operational data.

2. Strain and Process Optimization: Although we achieved significant improvements, there is room to make the strains and processes even better:

- **Strain optimization:** Continue iterative improvement of the algae via advanced methods such as *genome editing* (now that we have CRISPR/Cas tools), or *adaptive laboratory evolution* specifically targeting product yield (e.g., evolving strains on selective pressures that favor high lipid accumulation). Also, exploring the algae's ability to use alternative feedstocks like waste CO_2 or nutrients from wastewater could reduce costs.

- **Genetic stability and regulatory approval:** Ensure the genetic modifications are stable. For commercial outdoor use, especially of GM algae, regulatory approval will be needed – this means conducting ecological risk assessments and possibly incorporating biological containment strategies (such as auxotrophic mutants that cannot survive outside cultivation). Path forward includes starting dialogues with regulatory bodies (EPA, USDA) early, using our pilot as a test case for safe deployment of GM algae.

- **Process intensification:** Investigate methods to further intensify production, such as **continuous cultivation** (chemostats or semi-continuous harvesting) to keep algae in optimal growth phase for co-product accumulation. The PEAK process could be refined (how many cycles is optimal, can it be automated). Additionally, implementing **automation and control** (pH control via CO_2 injection, automated harvesting triggers) will be important to maximize productivity.

- **Downstream improvements:** Work on improving the chemical conversion yields. For instance, develop a more efficient catalyst for converting algal sugars to FDCA or other monomers so that this pathway becomes economically attractive. Partnering with catalysis experts or leveraging new advances (e.g., engineered enzymes for bioconversion of sugars to polyols or diacids) could improve this step. On the fuel side, exploring hydroprocessing of algal oils (possibly in partnership with a refinery) could produce drop-in hydrocarbon fuels that meet ASTM standards.

3. Product Development and Commercial Partnerships: On the polyurethane side, further development is needed to bring products to market:

- Work with companies in the PU industry (foam manufacturers, footwear companies, automotive foam suppliers, etc.) to test the algae-based polyols in their specific formulations. The foam we made with Algenesis is a good start; the next step is perhaps a **prototype product trial** – for example, Algenesis could produce a batch of algae-based flip-flop midsoles (they have experience with algae foams for flip-flops). Feedback on processability and performance will guide any needed tweaks (like adjusting the chain length or functionality of our polyols to match industry standards).

- Investigate the **biodegradability** of the algae-based PU products. Algenesis's interest lies in compostable plastics – it would be valuable to test if our PU foam

or TPU is biodegraded by microbes (since the soft segments are bio-based, they might be more susceptible to breakdown). If yes, that's a selling point (a compostable shoe sole, for instance, made from algae). If not, research could pursue incorporating biodegradable linkages.

- Protecting intellectual property and forming joint ventures: as we move towards commercialization, ensuring freedom to operate and/or patents on key innovations (e.g., unique genetic strains or specific processing methods) might be necessary. While we emphasized open tools, certain aspects like the PEAK process or novel catalysts might be protectable. Engaging the university tech transfer and possibly spinning out a startup or licensing to partners could be part of the path.

- **Market analysis and business model:** Use the TEA results to identify what market prices we need for co-products for the process to be profitable. For example, if our algae polyol can fetch a premium price in niche markets (like biodegradable specialty foam in athletic wear), initially targeting those markets could subsidize fuel production until economies of scale are reached. Engaging with potential off-takers for both the fuel (e.g., sustainable aviation fuel blend users) and the PU products now will help shape the business case and attract funding.

4. Address Remaining Technical Risks: Some technical risks remain and should be addressed in parallel:

- **Contamination control** in large-scale algae cultivation: We mostly worked with monocultures. Scaling up, the risk of contamination (we saw an instance of bacterial contamination affecting metabolites) can increase. Developing a robust control strategy will be critical. Our high-pH, high-salt strategy itself is a form of contamination control (few organisms survive those conditions), which is a strength to continue leveraging.

- **Nutrient recycling:** To improve sustainability, a path forward is implementing nutrient recycling (e.g., using residual biomass or spent media to recover nutrients like nitrogen and phosphorus). We could integrate an anaerobic digester to process spent biomass after extraction, generating biogas (another fuel) and nutrient-rich liquor that can fertilize new algal growth. We did not focus on that in this project, but it's a logical extension to close the loop.

- **Scaling conversion technology:** The chemical conversion steps used lab reagents (e.g., periodate, or bromine in oxidation) that are not practical at scale. The path forward includes researching more scalable, green chemistries. For example, using oxygen and a metal catalyst for lipid cleavage (instead of periodate), or using a heterogenous catalyst for HMF to FDCA (like those used in the production of bio-PET). We may seek collaboration with catalysis research groups or DOE consortiums focusing on bio-renewable chemical

conversion to tackle this.

5. Funding and Collaboration Opportunities: To undertake the above steps, pursuing additional funding and partnerships will be key:

- Private sector partnerships could provide cost-share or direct investment; for instance, companies like BASF or others in the polymer industry might co-fund scaling of the polyol production if it aligns with their sustainability goals.

- Engaging with consortia such as the Algae Biomass Organization, or DOE's Agile BioFoundry (which focuses on bio-manufacturing), might offer resources and collaborative platforms to accelerate development.

- On the academic side, publishing our results (the genetic tools, the production data, etc.) will invite collaboration and independent validation. We plan to prepare manuscripts for peer-reviewed journals on the genetic engineering breakthroughs and the integrated process LCA, which can attract interest from other researchers to build upon our work (for example, someone might take our engineered algae and try producing a different bioproduct, broadening the impact).

In summary, the path forward is focused on **scaling up and derisking** the technology, **optimizing** both biology and chemistry for efficiency, and **aligning** the project outcomes with real-world market and regulatory requirements. The ultimate goal is to move this innovation out of the lab and into practice – where algae farms are producing not just biofuel, but also the raw materials for the shoes on our feet or the foam in our seats, all in a sustainable loop. This will require continued interdisciplinary effort, strong partnerships between academia, industry, and government, and a careful eye on both the technical and economic factors identified in this project. Given the success so far, we are optimistic that these next steps will bring algae-based fuels and products to the cusp of commercialization, fulfilling the promise of this project.

Products

This project has yielded a number of products and outputs, in terms of knowledge, tools, and tangible items:

- **Publications:**

Publications / Presentations:

FY23Q1

Diaz, C. J., Douglas, K. J., Kang, K., Kolarik, A. L., Malinovski, R., Torres-Tiji, Y., Molino, J. V., Badary, A., & Mayfield, S. P. (2023). Developing algae as a sustainable food source. *Frontiers in Nutrition*, *9*(January), 1–21. https://doi.org/10.3389/fnut.2022.1029841

FY23Q3

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Diaz JD, Tessman, M, Schereiman A, Gunawan N, Simkovsky R, Burkart M, Mayfield S. (2023). Green Biodegradation: Analysis of Potential Polyurethane-degrading Enzymes and Their Secretion in Chlamydomonas reinhardtii. AlgalBBB. 12-14 June 2023.

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FY23Q4

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FY24Q1

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FY24Q4

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- **Intellectual Property:** Given the APEX program's spirit of enabling technology, we are making the genetic tools openly available at Chlamydomonas collection. However, any industry partner interested in exclusive use might prompt a patent filing.

- **Data and Software:** We have generated extensive datasets: baseline composition data for multiple algae strains, growth curves under various conditions, and conversion reaction yields. These data will be made available as supplementary information in publications. For instance, the metabolomic profiles of strains and the promoter sequence library results are compiled in papers that are shared. The TEA/LCA model will be provided to BETO and can be made available for others as a case study of an algae biorefinery scenario.

- **Genetic Constructs and Strains:** One of the most important “products” is the **engineered algal strains and their genetic toolkits**. We have:

    - *Chlamydomonas sp. strain 402-EVO-PGM1 (High Algal Starch)* – a strain with high starch accumulation, and *402-EVO-DOF (High Algal Lipid)* – a strain with high lipid content. These strains, along with their baseline parent, are available at Chlamydomonas collection.

    - Plasmids such as pJPCx1 (for algal transformation), among others, were deposited as key plasmids in a public repository at the Chlamydomonas collection.

- **Collaborations and Networks Formed:** Through this project, a strong collaboration was formed between UC San Diego, UC Davis, and Algenesis. This has led to new joint efforts (e.g., UCSD and Algenesis are discussing a follow-on project to develop a fully biodegradable shoe sole from algae PUs). The partnership with UC Davis brought students and researchers together, and several graduate students have been trained in the interdisciplinary aspects of this project, contributing to the human capital in bio

- **Other Outputs:** The project fostered the training of young scientists – at least 6 graduate students and 8 undergraduate researchers participated, gaining hands-on experience in algal biology, analytical chemistry, and techno-economic analysis. The collaboration between UCSD and UC Davis resulted in cross-disciplinary knowledge exchange, and our team has presented this work in DOE BETO meetings and to industrial stakeholders to accelerate technology transfer. We will also submit the required Final Scientific/Technical Report to DOE’s OSTI repository to ensure public dissemination (with an OSTI ID to be provided upon submission).

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