Phase 2 –Final Technical Report

Novel Algae Technology for CO_2 Utilization

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Summary

The United States, the world's largest energy user and second largest CO_2 emitter, is heavily dependent on fossil energy. In 2014, U.S. coal burning utilities emitted ~1562 million (MM) tons/year (TPY) of CO_2 into the atmosphere, accounting for 76% of the total US power sector emissions[1]. Hence, reducing the CO_2 emission footprint from coal plants is widely viewed as a key element in mitigating global warming. Despite significant interest, implementation of CO_2 capture technologies has been constrained by the high capture cost which significantly increases the total cost of electricity. Not only is capturing CO_2 with traditional technologies expensive, but generally the CO_2 has little value and additional expense must be incurred for sequestration.

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This project funded by a SBIR grant from the U.S. D.O.E. to Helios-NRG in collaboration with the State University of New York at Buffalo (UB) and Membrane Technology and Research Inc. (MTR) aimed to develop a novel, algae based technology to capture CO_2 from the effluent of coal-based power plants and convert it to renewable bio-fuels and higher value co-products such as animal feed and nutraceuticals with the potential to enable a substantial reduction in the net cost of carbon capture. The Phase 2 project was aimed at further demonstrating the technical feasibility of the proposed multi-stage continuous (MSC) flow CO_2 capture system and the generation of high-value co-products to offset the CO_2 capture cost.

The project was completed and the project objectives were met and exceeded. A first-of-a-kind integrated, laboratory scale MSC process unit was fabricated and tested in a greenhouse. The tests were conducted with the preferred algae species identified in Phase I and simulated flue gas containing contaminants at levels typically present in the post flue gas desulfurization (FGD) stream, including ~12% CO_2, acid gas (SOX, NOX), and a large number of heavy metals. The tests were successful and demonstrated a 25g/m2/day seasonal average algae productivity and an 80% CO_2 capture efficiency. Two new algae species were identified for high-value nutraceutical production. Studies to improve growth rate and nutraceutical content of these algae species were performed and a pathway for further improvements was identified. A new dewatering technology called DeAqua was further advanced. Significant improvement in the performance index was achieved. The anti-fouling membrane was developed and fabricated into a module. The fabricated membrane module was tested with algae slurry and demonstrated improved fouling resistant properties, that can potentially reduce the cost and energy of the critical dewatering step.

Test data were used to simulate operation of the overall process. The preliminary economic analysis was updated and modelled based on a 5000-acre algae farm. To the extent possible, the financial and operating assumptions used were the same as those used in the DOE's 2022 projections for algae technology for CO_2 capture and utilization. The results showed the proposed technology's potential to significantly reduce the cost of CO_2 capture compared to current options and that the high value products generated from the CO_2 captured can make a step change in the cost of carbon capture. Plans to advance the technology to Phase 2B were developed and potential end-user partners were identified.

1. Executive Summary

The United States, the world's largest energy user and second largest CO_2 emitter, is heavily dependent on fossil energy. In 2014, U.S. coal burning utilities emitted ~1562 million (MM) tons/year (TPY) of CO_2 into the atmosphere, accounting for 76% of the total US power sector emissions[1]. Hence, reducing the CO_2 emission footprint from coal plants is widely viewed as a key element in mitigating global warming. Despite significant interest, implementation of CO_2 capture technologies has been constrained by the high capture cost which significantly increases the total cost of electricity. Not only is capturing CO_2 with traditional technologies expensive, but generally the CO_2 has little value and additional expense must be incurred for sequestration.

With support from the U.S. D.O.E., Helios-NRG has been developing a novel, algae based technology to capture CO_2 from the effluent of coal-based power plants and convert it to renewable bio-fuels and higher value co-products such as animal feed and nutraceuticals with the potential to enable a substantial reduction in the net cost of carbon capture.

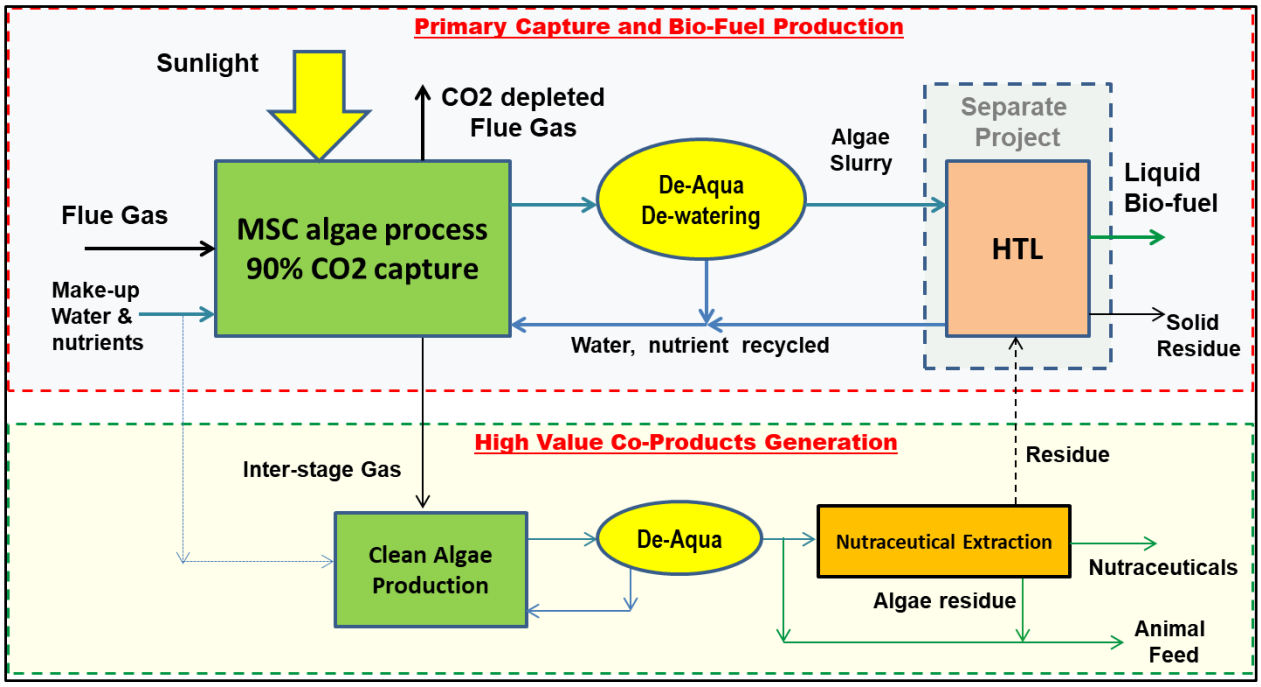


Figure 1: Schematic of overall process for Carbon Capture and Utilization

A schematic of the overall scheme is shown in Figure 1. Flue gas, after de-sulfurization, enters a MSC flow photo-bioreactor (PBR) process where ~90% of the incoming CO_2 is captured and used beneficially to accelerate the algae growth. The algae culture leaving the MSC process is de-watered using a new technology called DeAqua, being developed by Helios-NRG together with the University at Buffalo (UB), to produce an algae slurry with high solids loading at a much lower cost than the state of the art. Over 99% of the culture media is separated out in the DeAqua step and recycled back to the PBR, thus reducing the fresh water and nutrient requirements.

The de-watered algae leaving DeAqua is fed to HTL where it is converted to bio-crude. The waste water from HTL, which contains residual nutrients, and the CO_2 rich gas from HTL are recycled back to the MSC process and used for growing algae. The HTL conversion of algae was validated in an earlier DOE funded SBIR project and is not part of this project.

A portion of the gas stream, taken from an inter-stage location in the MSC process, is used in a parallel algae-culturing operation optimized for high value nutraceuticals and animal feed production. Phase II work showed that an average of ~90% of the heavy metal contaminants (HMC) in the flue gas are removed by each MSC stage. By using the gas after the inlet or second stage of the MSC process, the concern of HMC's in the high value co-products can be mitigated. Depending on the specific location in the MSC from where this gas stream is extracted, the CO_2 concentration can range from ~1-8% which is suitable for several of the algae strains intended for nutraceutical and food applications. The clean algae production will be done in closed systems to ensure purity and quality control and will typically include an induction step to maximize nutraceutical content and use the DeAqua technology for dewatering. Most of this algae production is used for feed applications (e.g., fish, cattle) with a smaller fraction fed to an extraction and separation step where current technologies (e.g., super-critical CO_2) are used to produce unrefined nutraceuticals. The biomass after nutraceuticals extraction, still containing proteins and carbohydrates, can potentially be used for animal feed blends or recycled to the HTL process for bio-fuel production.

Unique features of the proposed technology which will enable a step change in carbon capture costs are as follows:

- High CO_2 capture efficiency by the algae process (~90%)
- Ability to handle high CO_2 concentrations present in flue gas (~12%)
- Very high algae productivity
- Process intensification and cost reduction via use of a continuous flow process
- A low-cost de-watering technology whose slurry product can be directly used in HTL
- Revenue from bio-crude produced from algae which can be used in existing refineries
- Revenue from higher value co-products (animal feed, nutraceuticals) from algae

The Phase I project was aimed at demonstrating the technical feasibility of critical components of the proposed concept such as MSC based carbon capture in the presence of flue gas contaminants, advancing the dewatering technology, and validating potential for higher value co-products. The project objectives were met or exceeded. Phase I demonstrated the ability of two preferred strains of algae to survive and grow in simulated flue gas (CO_2, SOx, NOx) andin the presence of three of the most toxic heavy metals - As, Se and Hg. Three single stage, batch PBR's were used to replicate the conditions in the different stages of the MSC and CO_2 capture efficiency from simulated flue gas of >70% was measured in the lab using artificial light. The algae concentrations and the CO_2 capture efficiency in each stage was shown to be stable over time - critical features of the new MSC process. Significant progress was made in advancing the DeAqua technology. The viability of using a continuous gravity table for the first stage of DeAqua was demonstrated and concentration ratios in the range of 2.-3.5x were achieved. It was demonstrated that the commercial membranes can be co-deposited using bio-adhesive polydopamine (PDA) and super hydrophilic graphene oxide (GO) or poly (ethylene glycol) diamine (PEG) to increase antifouling properties and thus water permeance for algae dewatering. Water permeance meeting or exceeding the Phase1 target of 40 LMH/bar was demonstrated. Five valuable nutraceuticals, including astaxanthin and β-carotene, were found to be present in the algae used for carbon capture and a plan developed to increase their concentrations and production through new species selection and process refinements. Preliminary economic analysis showed that the proposed technology has the potential to lower the net cost of CO_2 capture to $3-23/ton.

The current Phase2 project aimed to build on the success of PhaseI and had the following objectives:

- Design, build and operate an integrated lab scale MSC unit and demonstrate a productivity of 25 g/m2/day and 80% capture efficiency on simulated post FGD flue gas.

- Advance co-production of nutraceuticals through new species, improved culturing techniques and optimizing induction procedures
- Advance DeAqua technology for dewatering algae by achieving higher concentration ratios and reduced residence times in Stage1 and developing an improved fouling resistant membrane module for Stage2.
- Refine techno-economic analysis including measured productivity, improved DeAqua operation and nutraceutical products mprove the confidence level and enable better extrapolation to commercial operation.

Phase 2 is now complete and all the project objectives were met or exceeded as summarized below with greater detail provided in the next section.

MSC technology for carbon capture

A first-of-a kind integrated MSC system comprised of three stages was designed and fabricated. A new control system to regulate gas supply, make-up water and culture harvesting and enable unattended 24/7 operation was developed and implemented. Algae species H1903, which had been qualified in prior Phase1 work, was used for carbon capture studies on simulated flue gas. Controlled tests were first performed in the lab in artificial light, followed by tests in the greenhouse and outdoors on natural sunlight. Make-up water and simulated flue gas continuously enters the system and CO_2 depleted gas and harvested culture are continuously removed from the MSC.

The original MSC program envisioned a counter-current, multi-stage mode of operation wherein the gas and liquid streams flow in opposite directions (Figure 8 in Section 2). A significant breakthough during this project was the development of a new configuration wherein the liquid stream is in cross-flow to the gas stream (Figure 11 in Section 2). While both modes of operation can achieve comparable CO_2 capture efficiencies and algae productivities, Helios-NRG has found that the cross-flow design has some significant operational benefits with respect to operational stability under large fluctuations, contamination and power consumption.

Lab tests with steady, contolled light intensity showed that the algae concentration in each stage was stable and time invariant. This is a key feature of the MSC technology which permits stable CO_2 capture and productivity over time. The specific levels of capture efficiency and productivity can be regulated by varying the gas flow and liquid extraction rates from the process. In general higher gas flows result in higher productivity and lower capture efficiency while higher liquid flow rates can increae both these parameters up to a point.

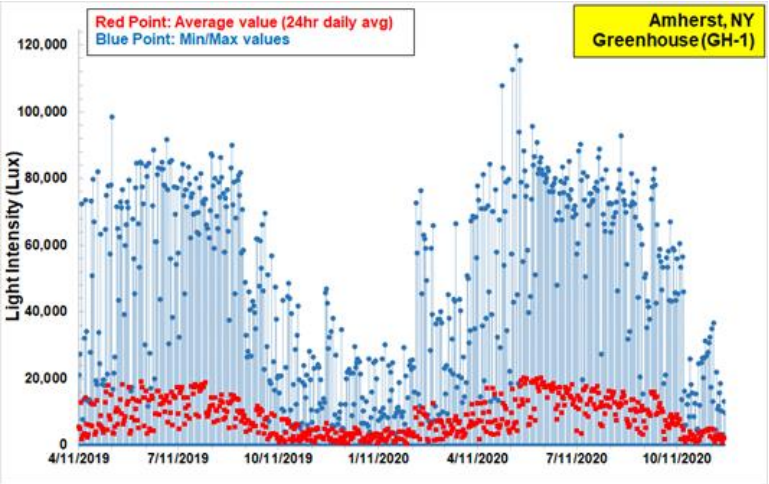


Figure 2: Daily light variation in greenhouse (GH-1)

Operation in natural sunlight increases the light intensity but adds challenges due to the significant light intensity variation from day to day and over the seasons. Figure 2 shows the natural light variation at our test site - blue symbols show the variations during each day and the red symbols show the variation in the daily 24 hr average intensity. It is evident that these is substantial variation from day to day and over the seasons. During the current project significant progress was made in controlling the MSC operation to maintain high performance despite the large light variation by adjusting the gas and liquid flow rates. The new control system enables long term tests to be performed with the MSC system.

Over the course of the Phase II project a large number of long term tests were performed in the lab, greenhouse and outdoors to map the MSC system performance and iteratively improve operation. The results of the test campaign are summarized in Figure 3 which shows the average algae productivity and CO_2 capture efficiency measured in these tests. Over the course of this project the MSC performance was systematically improved and the project targets of 25 g-dry wt/m2/day of algae productivity and 80% CO_2 capture efficiency were exceeded in oudoors operation where light intensity is significantly higher than in the greenhouse.

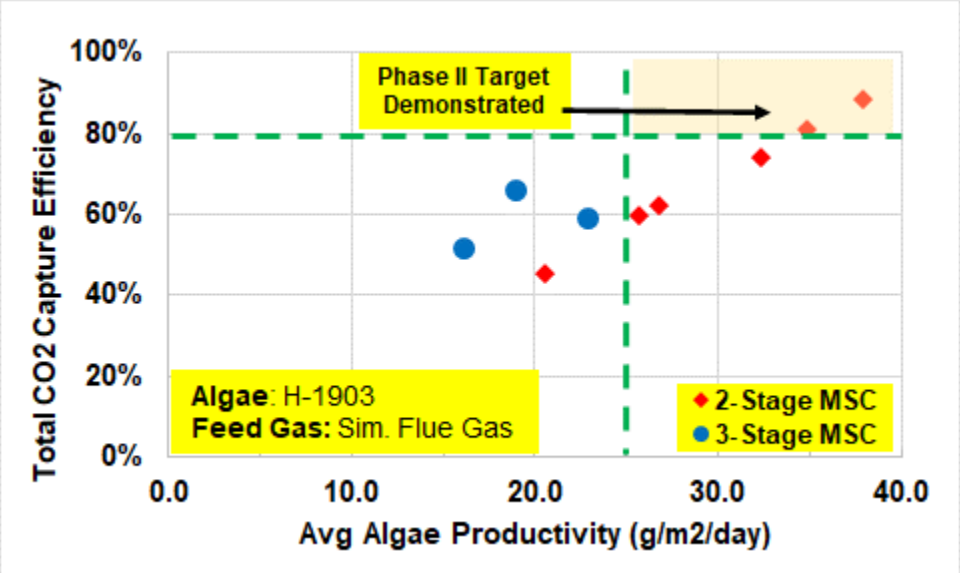


Figure 3: MSC performance Phase II target demonstration

Generation of nutraceuticals

Species H-0816 is well known for the high concentration of Astaxanthin that the induced cells can accumulate. Another species, H-0522, is not well known in the scientific literature, but the availble data indicate that β-carotene and omega-3-fatty acids are present in the induced form of this species and could provide valuable products to generate revenue as well. In order to obtain the nutraceuticals, the algae is first grown under optimal conditions to obtain a large amount of biomass. The next step generally is an induction process which can often greatly increase the amount of nutraceuticals contained within the algae. The final step of extraction results in a more concentrated nutraceutical product. In Phase 1 of this project, nutraceutical levels in algae used for carbon capture were found to be lower than desired. Therefore the two species mentioned above were selected for further analysis here in Phase 2.

Species H-0816 is a species that grows relatively slowly and has different culture conditions compared to the carbon capture species used in phase 2. Both an appropriate culture vessel and conditions were addressed as outlined in table 1 below to improve the growth and overcome the problem of the algae becoming induced prior to the desired time. During induction two parameters were used simultaneously – decreased nitrogen in the culture and elevated light intensity. Further maintaining the algae in a healthy

state was found to be important. Overall these steps resulted in a decrease in the time of induction from about two weeks to one week. Two methods were used to quantify the induction process, measuring astaxanthin concentration over time and a spectrophometric analysis to accurately track the color change of the culture from green to red). A more quantitative method (High Pressure Liquid Chromatography) for determining the levels of Astaxanthin in algae resulted in 6% w/w which compares with the highest amounts reported by others.

The project also addressed the nutraceutical potential of species H-0522. A process similar to that used for H-0816 was used for the scale up and optimization of the of H-0816 culture. The culture size was scaled up, and the process of induction was decreased from two to one week. The TLC analysis demonstrated that induction increased several carotinoids and fatty acid levels from nearly undetectable levels before induction to higher levels afterwards (see Figure 24B, Table 9 below).

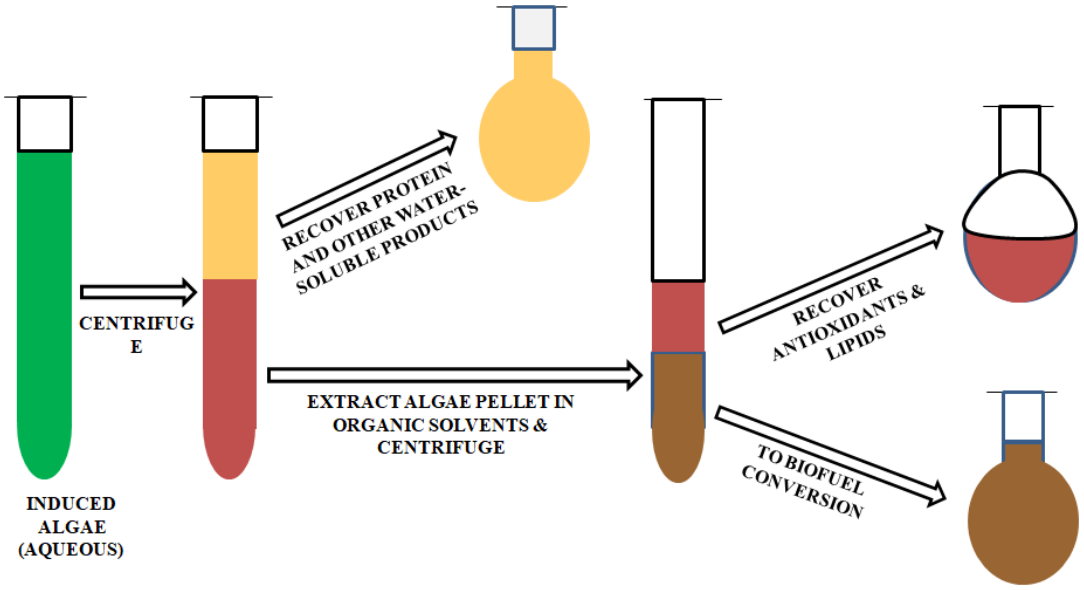


Figure 4. Sequential aqueous/solvent extraction process for increased recovery of high-valued algal products.

Post-induction, the extraction step is important to recover products at maximal levels. This study used a traditional solvent extraction with mechanical cell breakage which is known to be effective. Another method was examined to increase the product potential of the algae. Currently, most of the nutraceutical products are soluble in organic solvents. However, water soluble products like proteins, enzymes and phycocyanins are also have high value potential and these are excluded from recovery by the organic solvent process. A sequental aqueous/solvent process was created (Figure 4) so that initial aqueous fraction allowed recovery ofwater soluble products (i.e. proteins as shown in Figure 23 below). Then the water insoluble frations was extracted by organic solvent and the expected antioxidant and lipid components recovered in the this second step (Figure 24 below). Any remants at the end not used for products could be converted to biocrude. Further development of this sequential two phase extraction process will increase the types of products available for sale at the highest price point and utilize 100% of the algal biomass.

Dewatering Technology

Several design parameters for the Stage 1 gravity table were identified and their impact on algae settling quantified. However, a step change in continuous gravity table operation is difficult without use of chemical flocculants as most of the parameters identified were focused on the settling tank design. This is because the natural terminal velocity of algae quantified by Stokes' Law is very low and any continuous flow velocity into the gravity table will be significantly higher, leading to short-circuiting of the settled algae. A new technique of electro-flocculation aided gravity settling was studied as a method to improve the performance of the DeAqua Stage 1. Design and operational parameters of the electro-flocculation process were systematically studied in batch tests with algae culture. Subsequently, a bench-scale continuous flow electro-flocculation chamber with 4 pairs of electrodes was fabricated. The Stage 1 performance was mapped based on operating conditions of flow residence time, feed algae concentration, current density and field strength and energy-mass requirement quantified. In order to improve algae recovery from the after the electro-flocculation process, the treated algae culture from the chamber is fed through a gas stripping chamber follow by settling in a conical bottom settling tank. The addition of a gas stripping chamber with either air or N2 increased the algae product recovery via elimination of microbubbles formed as a by-product of the electro-flocculation process.

A new performance index was developed for comparison of the results obtained between the various Phases of the overall project. The performance index is defined as the product concentration ratio divided by the overall process time (electro-flocculation exposure and settling time). The performance index achieved in the difference phases of the project is summarized in Table 1. Overall, significant progress was achieved in Phase II compared to that achieved in Phase I of the project. The results greatly exceed the initial project objective for the product concentration ratio and product recovery by a factor of 4x and ~20% respectively. Additionally, >90% of water is removed as compared to ~70% in Phase I, all at reduced energy requirement (~5% of total algae energy content).

Table 1: Performance comparison of advancements for DeAqua Stage 1 gravity table

	DeAqua Stage 1 Performance Specification		
Project	**Product Conc Ratio**	**Performance Index**	**Product Recovery**
Phase I Work	3-6	0.12-0.25	70%
Current Work	20-25	5.0-12.5	80-95%

The DeAqua Stage 2 objective was to double the membrane water permeance for algae dewatering via the optimized membrane surface modification, develop periodic regeneration technique, and demonstrate the performance of the spiral-wound module fabricated using the modified membrane in algae dewatering operation. In this project, commercial polysulfone (PSf) and polyethersulfone (PES) flat-sheet membranes were selected as substrates for surface modification after comparing with several widely used ultrafiltration (UF) and microfiltration (MF) membranes. Hydrophilic materials, including graphene oxide (GO) and sulfobetaine methacrylate (SBMA) was grafted to the membrane surface using the bio-adhesive polydopamine (PDA) to improve antifouling properties. The surface grafting decreases water contact angles and retains high water permeance. Figure 5a demonstrates the improved water permeance after grafting with GO. When challenged with 10 g/L algae solution, the GO modified membrane (GO/PDA/PSf) shows 31% greater water permeance than the pristine PSf membrane. Additionally, the GO modified membrane presents much less attachment of the algae than the pristine PSf after the algae dewatering test (Figure 5b), which demonstrates the enhanced fouling resistance of the GO modified membranes.

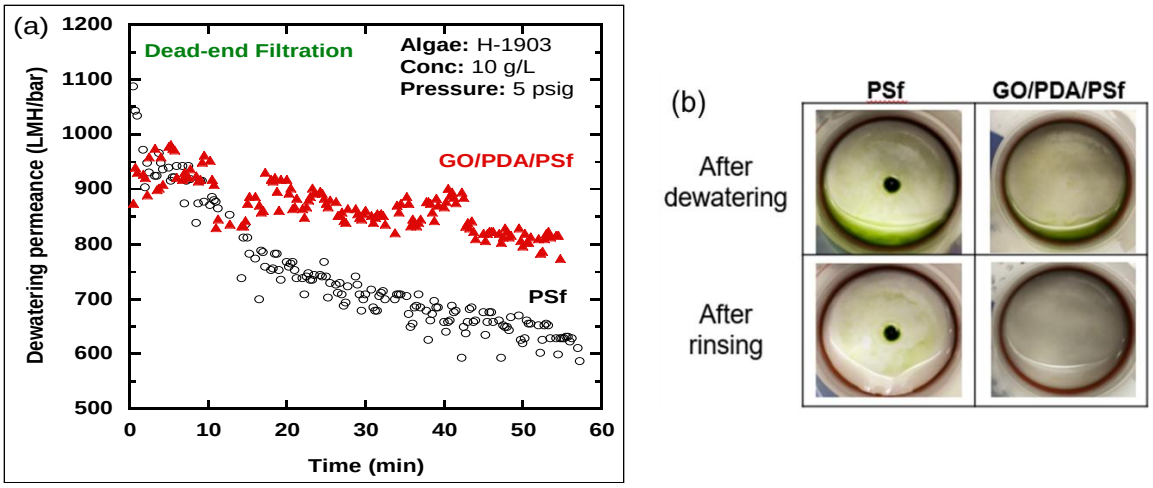


Figure 5. Improved antifouling properties by GO grafting. (a) Comparison of algae dewatering permeance between the GO modified PSf membrane and pristine PSf membrane in a dead-end filtration system. (b) Comparison of images of the fouled membranes before and after rinsing with DI water.

We have also modified PES membrane sheets using SBMA and had them fabricated into a spiral-wound membrane module by Membrane Technology and Research, Inc. (MTR) (Figure 6a). The module shows pure water permeance of 180 LMH/bar. The module was then challenged with algae solutions in various concentrations in a cross-flow filtration system, and the results are shown in Figure 6b. The recovery after each cleaning process is 86% when challenged with 0.1 g/l algae and 60% when challenged with 0.5 and 1 g/L algae. When the module was cleaned using a NaOH solution with pH of 11, the recovery rate increased from 60% to 85%.

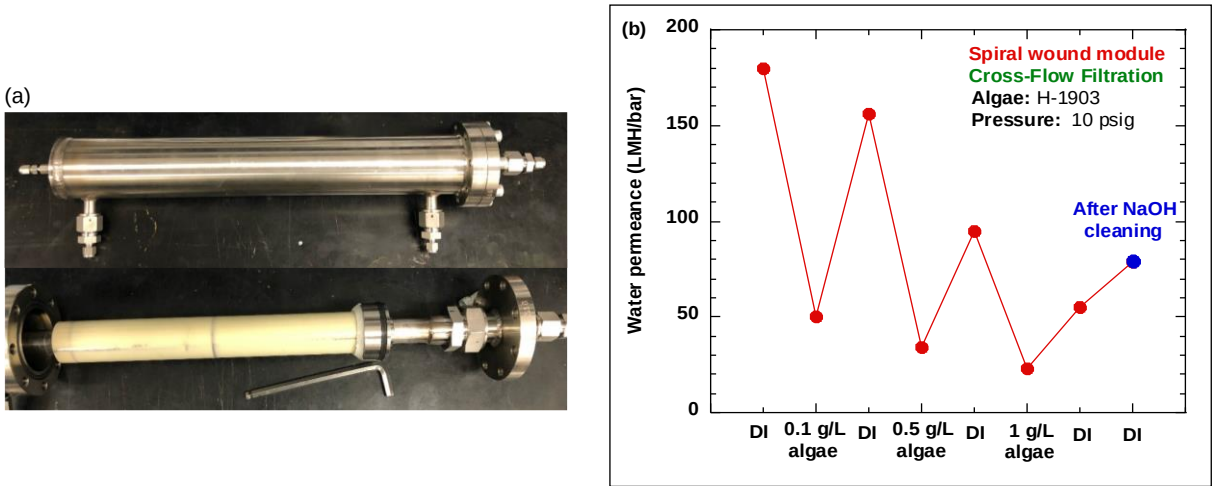


Figure 6. (a) Photos of the module housing and spiral-wound membrane module containing SBMA modified PES membranes. (b) Water permeance profiles versus algae concentration for the module in a constant pressure cross-flow filtration system.

Techno-economic analysis

The techno-economic analysis was modeled based on a future commercial system with 5000-acres of wetted area comprised of parallel MSC systems with each MSC stage comprised of a 10-acre raceway with an inexpensive plastic top cover to minimize contamination, water evaporation and enable high CO_2 capture. Based on measured MSC system performance in outdoors operation, an annual average algae

productivity of 25g-dry wt/m2/day was used for the baseline case. At this base case level of productivity, the 5000-acre algae farm would produce ~187,000 tons-dry algae/yr. and capture ~350,000 tons- CO_2/yr.

In a parallel DOE funded project with the University of Illinois at Urbana Champaign (UIUC), Helios-NRG has demonstrated the ability to replace up to ~80% of purchased nutrients for algae with nutrients from municipal waste water sources. In the base case, 50% nutrient replacement from waste water is assumed. The CO_2 required for the operation is obtained directly from post FGD flue gas from a coal power plant without purification and 90% utilization of the CO_2 by algae is assumed. The culture harvested is dewatered using the DeAqua technology which is estimated to reduce the cost of dewatering by ~$10/ton-algae. 99% of the water removed is recycled back for algae growing.

To the extent possible, the financial and operating assumptions used here are the same those used in DOE's 2022 projections for algae technology for CO_2 capture and Utilization[3,4]. Key assumptions include a 90% onstream factor, 30-year life, equity financing of 40% of total investment with an IRR of 10%, a loan term of 10 years at 8% interest and an operational staff of 100 people.

The algae produced is used to make three products with existing market needs to generate revenue. 50% of the algae produced is used in a hydrothermal liquefaction process (HTL) which has been identified by BETO as an attractive pathway for biofuel production. The baseline case assumes a biofuel price of $3/GGE which requires an algae price of $271/ton-DW. 48% of the algae grown is used for animal (cattle, fish) feed applications – the high levels of protein, lipid and other nutritional compounds in algae makes it attractive for this market. The price of feed varies widely from ~$250/ton for cattle to >$1000/ton for fish, one of the fastest growing segments. The present analysis assumes a blended algae price of $500/ton for this market. 2% of the algae produced is used for nutraceuticals production (e.g., astaxanthin, beta carotene, omega fatty acids, polysaccharides). These are characterized by very high current market prices ranging from $500-5000/kg of product. The algae grown goes through an induction process followed by harvesting and supercritical extraction to generate unrefined nutraceutical products. The blended value of this product is conservatively estimated to be $600/kg. In addition to product-based revenue, there is potential for credits from waste water remediation and CO_2 capture. The base case assumes no credits from either of these while the sensitivity analysis explores the impact of these credits.

Table 2: Production cost and revenue

Algae Production Costs (2014 $/ton dry algae)	
Component	Base Case 25 g/m2/day
Ponds, cover, Innoculum, site infrastructure	257
CO2 Supply	47
Nutrients	11
Dewatering	53
Power & Utilities	32
Labor Costs	65
Total Production cost ($/ton-dry algae)	464
Revenue & Credits (2014 $/ton dry algae)	**Base Case**
Biofuels ($3/GGE) - 50% algae	136
Feed ($500/ton) - 48% of algae	240
Nutraceuticals ($600/kg) 2% of algae	102
WW Credit	0
CO2 Capture credit	0
Total Revenue & Credits	478
Net cost of algae ($/ton)	**(14)**
Net cost of CO2 capture ($/ton)	**(7)**

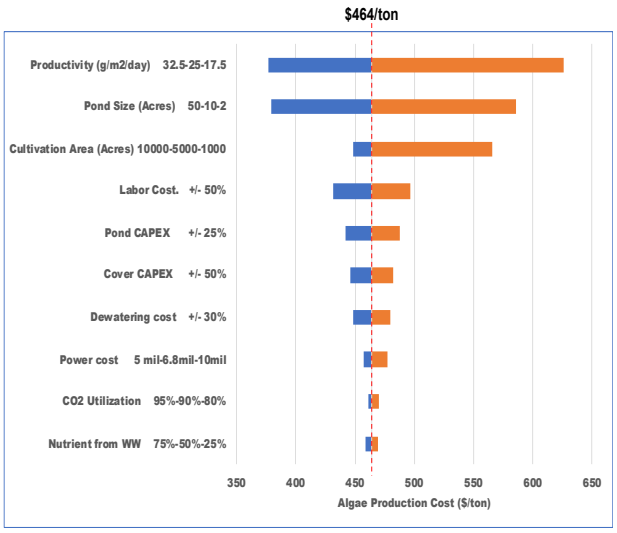


Figure 7: Production cost sensitivity

Table 2 summarizes the costs of algae production and revenue/credit streams for the base case. The net cost of algae production is estimated to be $464/ton which is slightly below the $494/ton in DOE's 2022 projection[3]. This is primarily due to cost reductions from use of waste water nutrients, flue gas vs purified CO_2 and the DeAqua dewatering technology. The total revenue generated is estimated to be $478/ton which enables near cost neutral CO_2 capture (-$7/ton- CO_2). The base case shows that generation of these products has the potential to enable cost neutral operation which would facilitate CO_2 capture on large scale. Figure 7 shows a tornado diagram of the sensitivity of the algae production cost to key parameters. Algae productivity has the greatest impact and a productivity of 32.5 g/m2/day can reduce production cost by ~$90/ton-algae. A similar analysis has been performed to assess the sensitivity of the revenue/credits to key parameters (Figure 44, Section 2). There is significant added revenue potential though tailoring the algae for higher value feed applications and optimizing nutraceutical production. Optimization of these two revenue streams has the potential to increase revenue by ~$110/ton. *Thus, higher productivity combined with product stream optimization can potentially lower the net cost further by ~$200/ton-algae to minus $214/ton-algae. This would result in net revenue positive carbon capture operation.* Remediation credits from waste water can be substantial and amount to over $210/ton algae which makes the profit motive even more compelling.

In summary, the work done to date indicates the potential to achieve cost neutral carbon capture. Further improvements, have the potential to transform carbon capture to a net profitable business proposition (~$100/ton- CO_2) which can incent large scale adoption without legislative credits.

Table 3 summarizes the actual accomplishments in the project to-date

Table 3: Actual accomplishments vs Initial Goals

Objective	Project Target	Phase2 Results
1	Design & construct 1st integrated MSC unit	3 Stage MSC unit built with control system for unattended operation
	Demonstrate stable operation	Stable, 100 day operation achieved
	Test in natural sunlight	Unit tested in greenhouse and outdoors
	Demonstrate algae productivity of 25 g-dry wt/m2/day & 80% capture efficiency on simulated flue gas	Algae productivity & capture efficiency targets achieved in natural sunlight on simulated flue gas
2	Qualify 2 new nutraceutical species	Two new species (H-0816, H-0522) selected
	Develop culturing and induction procedures	Culturing & induction techniques developed; growth rate improved 3X
	Quantify nutraceutical content	High levels of 3 nutraceuticals confirmed by outside lab
3	Increase stage1 concentration ratio by 3x	Stage 1 concentration ratio of 20x achieved
	Achieve >70% recovery from Stage1	>90% recovery achieved in Stage1
	Optimize Stage2 membrane & make module	2 spiral wound, anti-fouling modules fabricated
	Achieve 20LMH/bar with module in dewatering tests	20LMH/bar achieved using Stage1 product (10 g/L) in dewatering
4	Refine TEA based on Phase 2 results & identify path to further improvement	TEA modeled at 5000acre size including Phase2 results
		Cost neutral CO2 capture (production costs offset by prod revenue)
		Revenue positive operation possible (higher productivity; product opt)

2. Detailed discussion of work and accomplishments by task:

The current Phase2 project had the following objectives:

- Design, build and operate an integrated lab scale MSC unit and demonstrate a productivity of 25 g/m2/day and 80% capture efficiency on simulated post FGD flue gas.
- Advance co-production of nutraceuticals through new species, improved culturing techniques and optimizing induction procedures
- Advance DeAqua technology for dewatering algae by achieving higher concentration ratios and reduced residence times in Stage1 and developing an improved fouling resistant membrane module for Stage2.
- Refine techno-economic analysis including measured productivity, improved DeAqua operation and nutraceutical products mprove the confidence level and enable better extrapolation to commercial operation.

Task 1: Design, build, operate MSC system (Obj 1, Helios)

Subtask 1.1- Design the MSC system

The objective of this subtask is to develop a lab-scale design for the integrated 3-stage Multi-Stage Continuous (MSC) system enabling 25g/m^2/day algae productivity and 80% CO_2 capture efficiency. In Phase 1 of the project, single-stage PBR test replicated the conditions the different MSC stages. A key requirement for this project was to transition the PBR design to top lit operation, mimicking large-scale algae cultivation operations. The key design and operating parameters were determined using Helios proprietary model. A preliminary MSC performance simulation was conducted for a counter-current operation (Figure 8) where gas and liquid flow counter-current. In counter-current operation flue gas enters Stage 3 where algae concentration is highest, while fresh media enters Stage 1 where algae concentration is lowest.

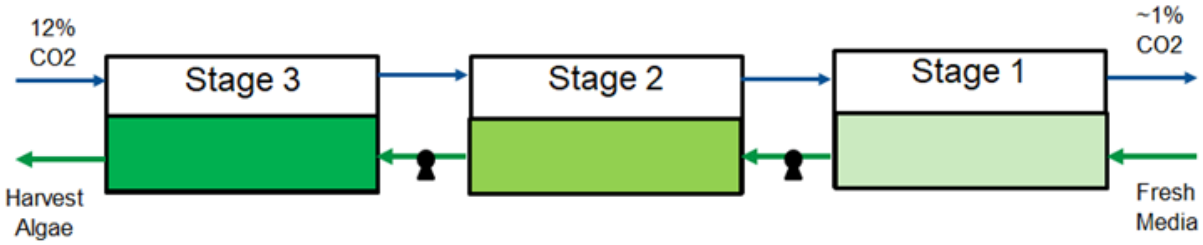


Figure 8: Counter-current configuration of MSC system

In the following simulations, a preset PBR geometry design where the top area for each stage be ~0.30m^2 and a fixed incident light intensity of 17,000 Lux. Key design variables include culture depth, culture volume distribution among stages, flue gas flow rate, inter-stage gas concentrations, culture flow rate, and the algae concentrations in the stages. The impact of gas and liquid flowrates on MSC algae productivity and CO_2 capture efficiency is shown in Figure 9.

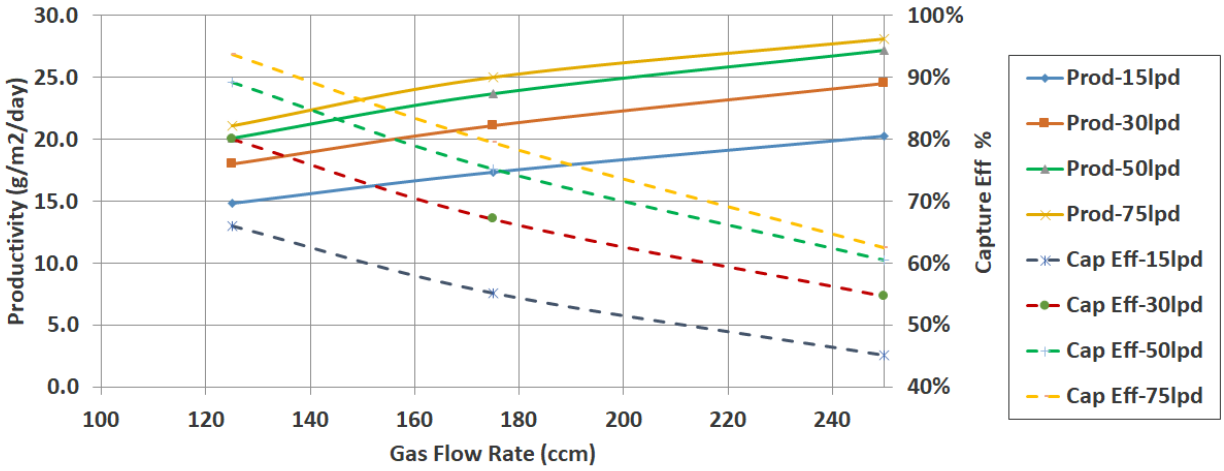


Figure 9: Impact of gas and liquid flow on MSC performance

In general, the algae productivity and CO_2 capture efficiency are inversely related, both strong function of the MSC performance. As seen in Figure A, an increase in gas and liquid flow results in higher algae productivities, but have a tradeoff in CO_2 capture efficiency. A balance in gas and liquid flow is necessary to achieve high algae productivity and CO_2 capture efficiency. Another key design parameter was the depth of each PBR stage, and is elucidated in Figure 10.

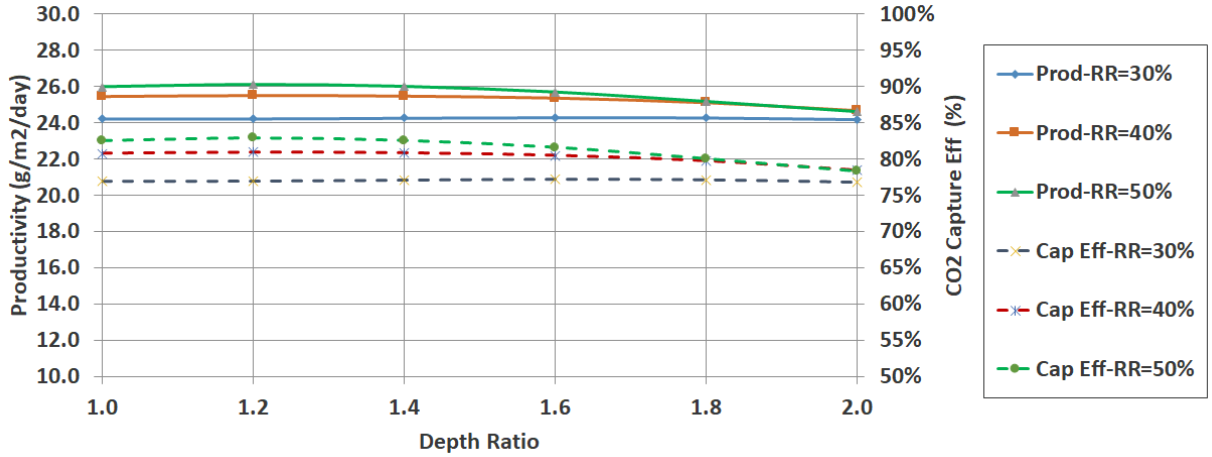


Figure 10: Impact of stage depth ratio on MSC performance

The stage depth ratio (DR) was defined as the ratio of PBR depth between Stage2/Stage3 and Stage 1/Stage2 where the depth of Stage3 was set to 10cm. The replacement ratio (RR) in Figure 10 was defined as the ratio between culture flowrate and overall MSC culture volume. The impact of PBR depth on the MSC performance is a weak function where the change in algae productivity and CO_2 capture efficiency is minimal despite a large depth ratio. Overall, this enables the possibility for a single PBR design for the MSC system, simplifying future scale-up work.

A key consideration in the overall design of the counter-current MSC system is that the gas and liquid flow directions are opposite, resulting in the requirement of the liquid to flow against the PBR pressure gradient from gas pumping, a potentially highly complex operation. As the liquid flows between stages, the potential for cascading contamination is high, resulting in poor MSC performance. In order to address

the potential flaws of the counter-current system, Helios has designed a new crossflow MSC configuration, shown in Figure 11.

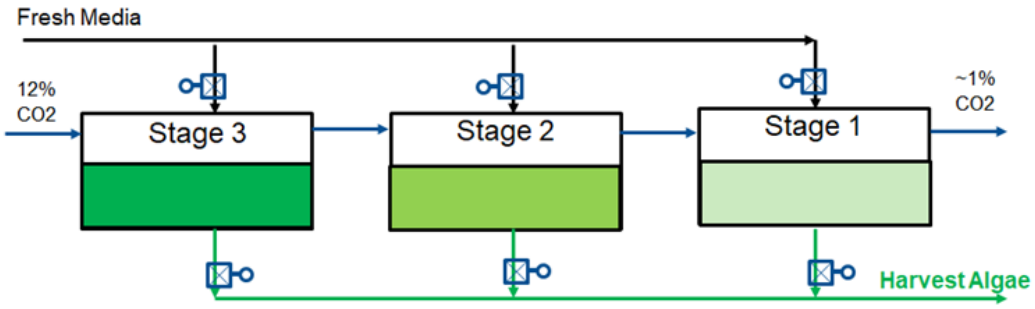


Figure 11: Crossflow configuration of MSC system

In the crossflow MSC configuration, flue gas is still pumped between stages while the liquid flows in/out of each stage independently, avoiding culture pumping and potential cascading contamination in the system. Overall, this will enable a simpler operation. The impact of the culture flow in the two MSC configuration on its performance is summarized in Figure 12.

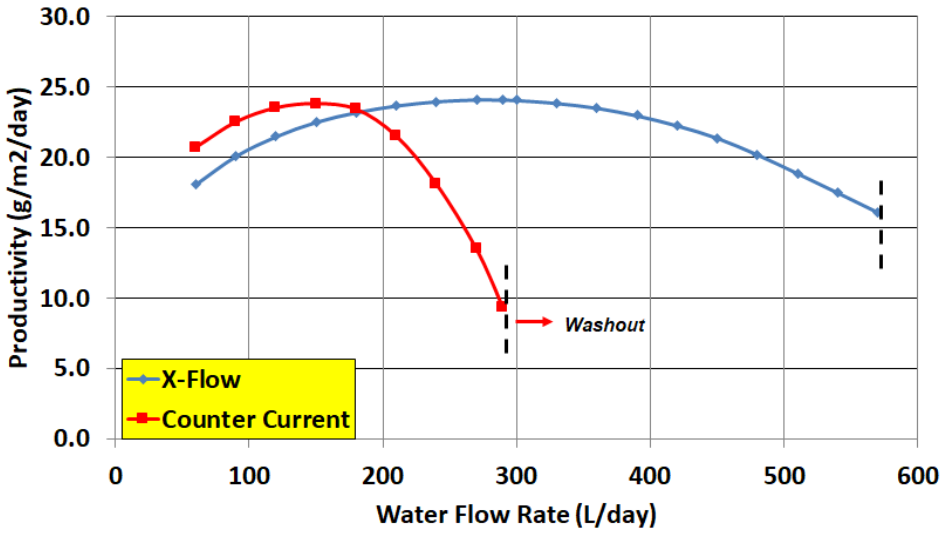


Figure 12: Impact of culture flowrate for different MSC configuration on algae productivity

The impact of culture flowrate was simulated on the counter-current and crossflow MSC configuration. As above, a higher culture flowrate resulted in an increase in MSC performance. However, unnecessarily increasing culture flowrate indefinitely would result in operational instability as predicted by the Helios model. This is as at higher culture flowrates, the potential for algae concentration washout is increased. However, the crossflow configuration has a larger operational flowrate range as compared to the counter-current configuration for a fixed MSC system.

Based on the preliminary model simulations, the PBR depth of each MSC stage was determined to be ~6-9in to maximize sunlight utilization, with an additional 3in as disengagement height to prevent fouling of the top cover by algae spray. The initial design of the PBR was determined to be of a top lit rectangular design with top area of each stage be ~0.30-0.45m^2. The rectangular tank allowed for design of an outer flat lip such that a sealing mechanism could be installed for air tight operation.

Subtask 1.2- Build the MSC system

The design of the single-stage PBR from Subtask 1.1 was finalized such that each stage of the MSC system be ~100L. The PBR tanks were custom-made to size via fiberglass molds with top lips to accommodate the top cover sealing mechanism. The top cover was made of a thin clear acrylic sheet to allow for maximum light entry into the system. The sealing mechanism for the top cover allowed for pressurization of gas such that the gas can be transferred between stages without additional pumping. The gas pressure in each of the stages were measured for its potential use in the techno-economic analysis. The ports for the gas distribution, culture flow and sampling, and submersible pump was made through the tanks. Preliminary shake-down tests were conducted to check the single-stage for leaks, culture mixing and gas distribution prior to integration of the 3-stage MSC system as shown in Figure 13.

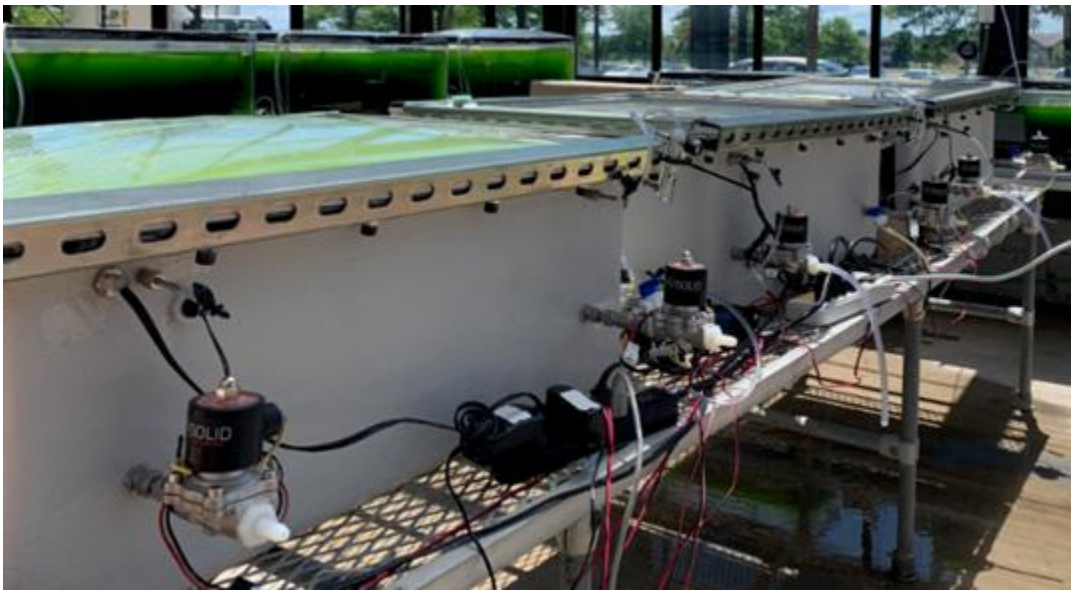


Figure 13: Integrated 3-stage MSC system test setup with PBR-F

The culture flow from the tank was controlled via timed solenoid valves while the inlet media flow was regulated with a solenoid valve activated with a float valve, which was set to the desired level of 9in culture depth. The timed solenoid valves and float valve in each of the MSC stages were connected to a liquid flow control system designed and fabricated by a vendor company (Tresca Design, LLC). The liquid flow control system enabled an automated periodic/continuous liquid withdrawal from the individual MSC stages, activation of a sterilization system (pump, water filtration, and UV sterilization unit), and filling of the fresh culture media to the required PBR volume, regulated by the float valve. Valve calibrations for the installed liquid flow control system in the interconnected MSC system was performed to determine the impact of timed solenoid activation on the product culture flowrate.

Algae growth test with the in the first automated MSC system test has been setup with the feed gas pressurization concept. Preliminary results of the short-term algae growth test in the MSC unit has shown difficulty in maintaining high growth rates due to culture health, possibly due to instability/intermittency of the gas pressurization system in the subsequent stages of the MSC system, leading to poor CO_2 capture efficiencies. The cornerstone of the MSC system is the continuous liquid transfer, with algae concentration measured over time. The algae concentration in both MSC stages equilibrates once the system has reached steady state operation as seen in Figure 14, as predicted by Helios proprietary MSC algae simulation for a fixed daily liquid transfer rate.

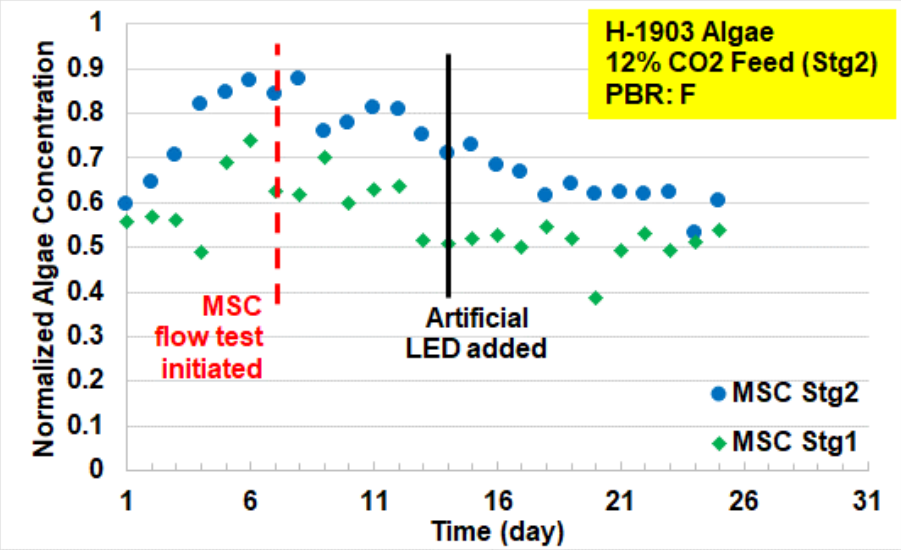


Figure 14: Algae concentration of 2-stage crossflow MSC operation

Subtask 1.3- Validate stable performance of continuous MSC system

Helios has been actively culturing algae in the greenhouse with the objective of demonstrating an integrated operation of the Multi-Stage Continuous System (MSC) capable of achieving an algae productivity of 25 g/m2/day. While many factors are important in the performance of the MSC system, one of the most important is the intensity of the incident sunlight. Helios has been actively measuring the internal and external light intensity variation at the greenhouse facility in use, averaging the measurements throughout the day. Figure 15 shows that the intensity of sunlight inside the greenhouse varied extensively during each day as well as from day to day. Table 4 shows the comparison of the average monthly light intensity for 2019 and 2020 while Table 5 summarizes the internal and external light average light intensity on a quarterly basis.

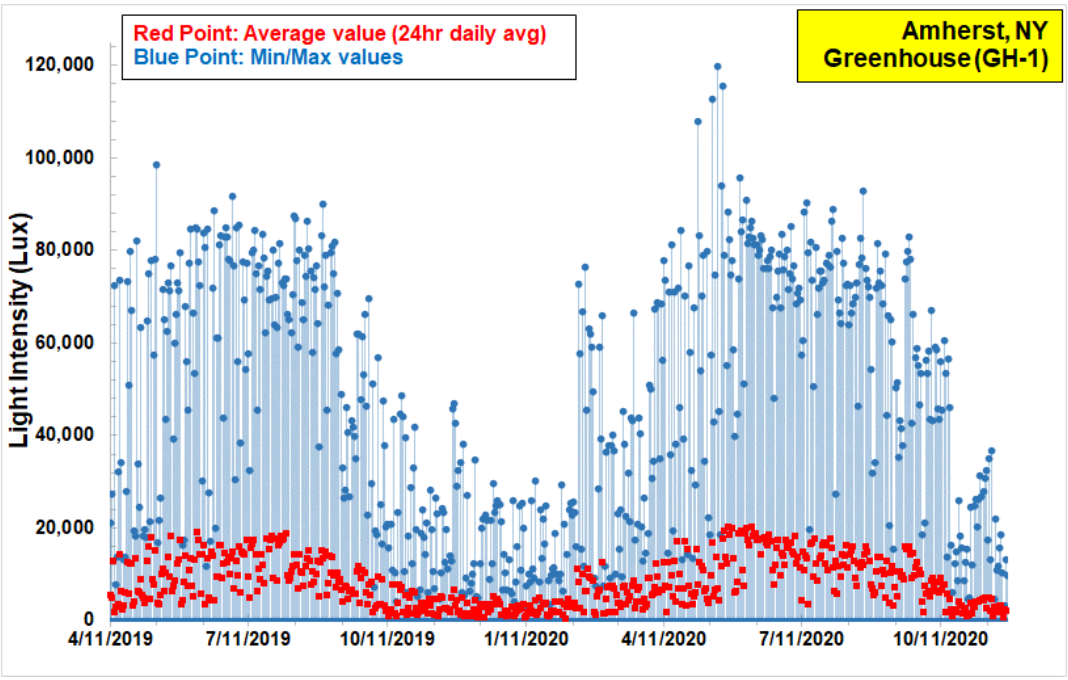


Figure 15: Daily light variation in greenhouse (GH-1)

Table 4: Monthly average light intensity inside the greenhouse (GH-1)

Month	Avg Light Intensity (Lux)	
	FY 2019	FY 2020
January		2236
February		4874
March		4963
April	6893	8211
May	9423	10888
June	11306	15577
July	12981	13675
August	12144	11437
September	7808	9795
October	3909	4637
November	3002	3006
December	2354	

Table 5: Internal and external quarterly average light intensity for greenhouse (GH-1)

	GH-1 Avg Light Intensity (Lux)	
	Internal	External
Q2 2019	9207	
Q3 2019	10978	19717
Q4 2019	3088	5890
Q1 2020	4024	7027
Q2 2020	11582	17401
Q3 2020	11636	14819
Q4 2020	3821	8088

The optimal algae growth period at the test site in WNY was during the months of May-August, as seen in Table 4. The quarterly average light intensity external to the greenhouse is substantially higher (~2x) than indoor due to lack of infrastructure blockage and losses through the glass, as summarized in Table 5. The substantially lower light intensity available inside the greenhouse potentially limits the overall performance of the MSC tests conducted.

The PBR's were set up to mimic a 3-stage crossflow MSC system with algae concentrations of 0.50, 0.45, and 0.40g/L respectively. Several tests were conducted with acid gases and heavy metals levels representative of post FGD flue gas. In prior work, Helios has demonstrated such that the acid gas (ppm levels) and heavy metal (ppb levels) contaminants have little to no impact on the algae growth. Despite lower light intensity inside the greenhouse (GH-1), several tests with varying MSC stages and replacement ratios were successfully conducted at the greenhouse and test matrix is shown in Table 6.

Test #	PBR Type	Location	Simulated Flue Gas Added Contaminants	MSC Replacement Ratio (RR%)	# of MSC Stages
1	F	GH-1	N/A	8.0%	2
2	F	GH-1	N/A	10.6%	3
3	C	GH-1	N/A	13.6%	2
4	C	GH-1	SOX/NOX + 5HM	15.0%	2
5	C	GH-1	SOX/NOX + 5HM	9.9%	3

Table 6: MSC test matrix in the greenhouse (GH-1)

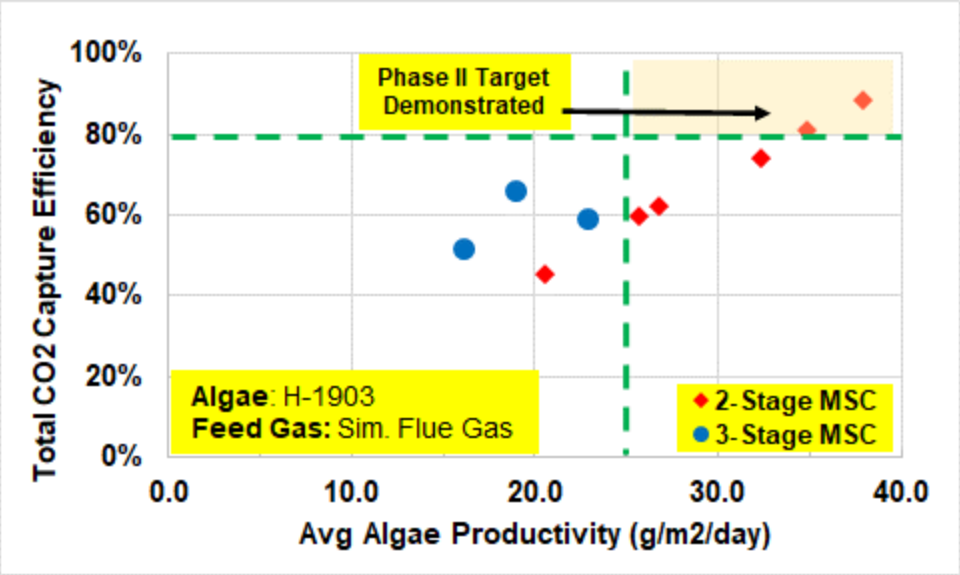


Figure 16: MSC performance Phase II target demonstration

A large number of tests with 2-stage and 3-stage MSC systems were conducted in the greenhouse as shown in Figure 16 at varying sunlight intensity and MSC operating conditions. The Phase II target of 25g/m2/day algae productivity and 80% CO_2 capture efficiency in simulated flue gas was demonstrated in the 2-stage MSC system, as seen in Figure 16 above. In general, the overall MSC performance is affected by a multitude of parameters such as incident light intensity, gas/liquid flowrate and PBR design. While those parameters have first-order impact on the MSC performance, the culture health could also significantly impact the algae productivity and CO_2 capture efficiency, though more difficult to control as operating in the greenhouse is more challenging due to significant variation in light and temperature.

Test #1 and #2 with the integrated MSC PBR-F in Table 6 were conducted in lower incident light intensity (~9500 – 11000 Lux) and thus did not demonstrate the project's productivity and CO_2 capture efficiency targets. However, it is well documented that higher light intensities result in increased algae productivities. Though not part of this project, Helios have been systematically mapped the impact of light intensity on algae productivity in both laboratory and an outdoor greenhouse (GH-2) setting where higher light intensities are possible as shown in Figure 17.

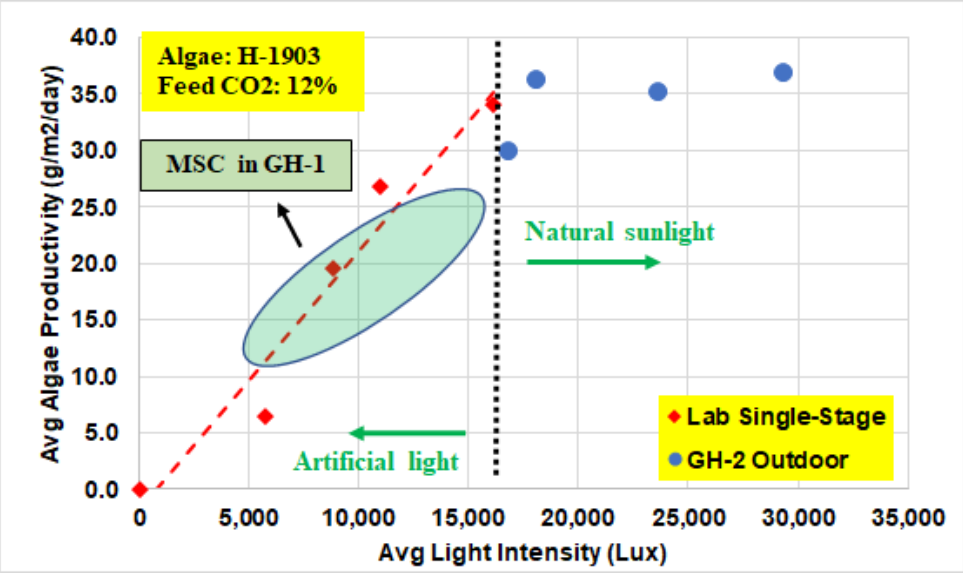


Figure 17: Impact of light intensity on algae productivity

The increase in light intensity clearly results in an increase in algae productivity. However, it is important to note that the increase in algae productivity will eventually plateau despite a further increase in light intensity. Despite having access to higher light intensities at a separate location, the integrated 3-stage MSC PBR-F as shown earlier could not be tested outdoors (at GH-2) due to the electricals not being designed for outdoors operation and hence a 2-stage version was tested.

Task 2: Optimize Nutraceuticals production (Obj 2, Helios)

Subtask 2.1- Select and demonstrate new algae species for nutraceutical production

The products focus of the Phase 2 project has been nutraceuticals. Unlike other products like biofuels and feed which are large markets, nutraceuticals have a much smaller market volume, but are of extremely high value. Due to the nutraceutical being a small percentage of algae relative to feed and biofuels, they can be obtained and sold without affecting the great amount of algae biomass remaining and still available for these larger markets. Two species were selected for this project – one well known for the production of astaxanthin, H-0816 (*Haematococcus pluvialis)*, and the other a less studied possible source of several types of nutraceuticals, H-0522 (*Eustigmatos vicsheri)*. These two species will be produced within the culture system based on their different attributes. These species will not be used for carbon capture, but instead grown in a clean stream where the products can be free of contaminants undesirable in animal feed or for human consumption. In the Phase2 project, the nutraceutical algae species were grown without the added flue gas constituents and other harmful biological and chemical entities present in municipal sewage from which growth nutrients could be obtained for algae growth at scale.

Both species studied here grow at rates slower than the species (H-1903) used for carbon capture, are much more susceptible to shear forces during culturing, but settle at a faster rate. Further, H-0816 in particular grows best at lower light levels. Therefore, a modified PBR design and relevant growth conditions needed to be studied.

Species Selection

Species H-0816 is well known for its ability to produce large amounts of Astaxanthin, a valuable nutraceutical. However, culturing of this species is difficult and it tends to grow slowly compared to many others species. A series of experiments were conducted to improve the productivity of the species so that more product could ultimately be obtained (Table 7). In addition to a slow growth rate, the algae can spontaneously change form in to an induced state when exposed to suboptimal growth conditions. For example, the growth media with less Nitrogen leads to a faster transition to induction. The induced state is when the cells lose their motility, stop dividing, increase in size and become packed full of Astaxanthin (changing color to red, see below). Two growth media with more Nitrogen, BBM/3XN and F2/3XN were compared to see if either was more advantageous to growth. Cell growth in either media performed similarly and with elucidation of the optimizing other steps, the growth rate improved. Since F2/3XN is being used for other species in the lab, this formula was selected. Because these cells are larger than many other species, and get even larger if they start to induce, keeping the cells suspended in culture proved more difficult (Figure 18). A new low shear method was developed and implemented. Next, impacts of light and CO_2 levels during growth were examined. High levels of light induce the algae to stop multiplying and start making Astaxanthin and other products including lipids (see below). Because the two nutraceutical species grow slower than the species used in carbon capture, CO_2 levels less than the 12% representing coal flue gas are sufficient (see below).

Table 7: Steps in culture improvement of species H-0816

Culture of Species H-0816 for Maintainence of Growth Stage (Green, Motile)			
	Method	Result	Color/ Growth
Culture Media	BBM - 3N media	good growth	green
	F2 - 3N media	good growth - used for other species	green
	Low 1x Nitrogen Media	growth slowed and turned red sooner	red
Culture Mixing	submersible pump	damaged cells	poor
	slow speed paddle	kept cultures mixed and cells suspended (6 - 30 RPM)	green
Culture Add-ins	1 - 3% CO2 addition	good growth	green
	Lower light 6-10K LUX	good growth, interplay with cell density and culture depth	green
	Higher light 12-18 LUX	tendancy for growth to slow and cells change color	red
Culture Density	High cell density > 0.8 g/l	tendancy for growth to slow and cells change color	red
	Low cell density <0.05 g/l	lag in growth getting started	poor

Subtask 2.2- Maximize nutraceutical production in algae

Induction:

Species H-0816 for nutraceuticals

Once a sufficient amount of algae is grown, it is ready for transition to an induction phase whereby the nutraceutical content is significantly elevated due to cell stress. In the case of H-0816 algae species, the induction results in a color change from green to red as a signal that Astaxanthin has been synthesized (Figures 18 and 19). While a number of factors have been reported to cause induction, this study has focused on three – low nutrient level (Nitrogen), high light intensity, and adding salt to these freshwater algae species. All three methods did cause induction individually, but combining nutrient depletion and high light intensity together shows promise. Further modifications to the induction process were developed to keep the cells in their best condition as the process goes forward. The improvements include a step to remove the nutrients from the media, and then replacing just the phosphate, but not the Nitrogen.

Trace elements were also replaced, gentle mixing established and CO_2 feed continued. Initial experiments with a single parameter induction and without other process improvements showed that induction can take as long as two weeks. Combining two methods simultaneously combined with other process improvements decreased the length of time needed to 1 week (Figure 19).

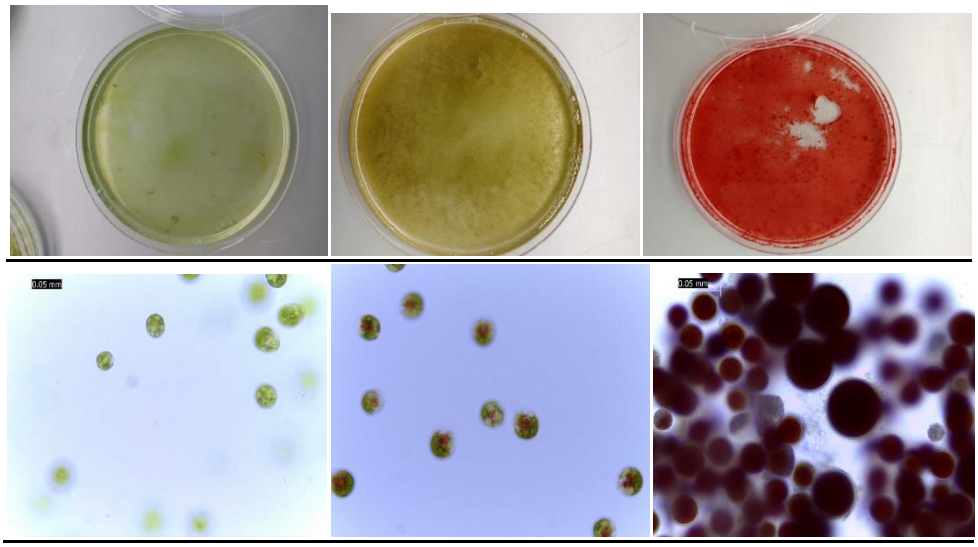


Figure 18: Top row left to right – 100 mm diameter culture plates of green, motile stage; start of induction; and completion of induction (red). Bottom row left to right – Micrographs of motile green cells of size 25-30 microns diameter; cells starting induction, some red of size of 25-30 microns in diameter; and fully induced cell all red, of size 30-50 microns in diameter.

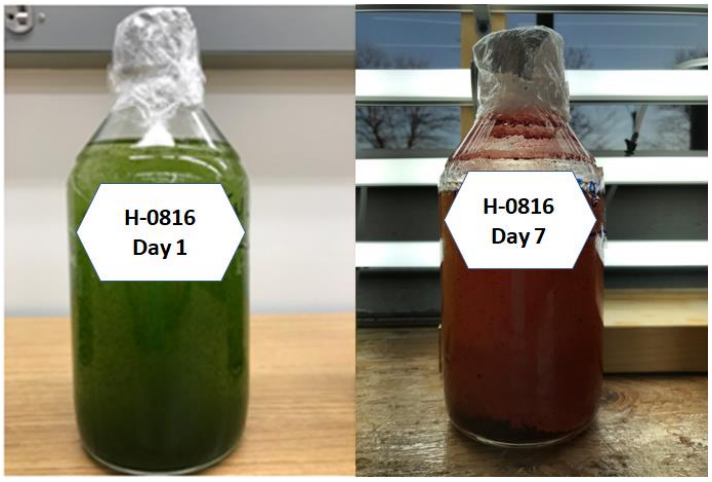


Figure 19: Culture of H-0816, left – growth stage (green); right – induced (red)

Figure 20 shows a convenient analytic tool that will help to monitor induction over time for species H-0816. When the algae are green and growing well, when its absorbance ratio is measured at a frequency of 730 nm over 350 nm, the ratio is a number is 0.4. This number will increase over time as induction develops and the ratio value will eventually reach 1.2 when the process is complete. Another way to more definitively monitor induction is to extract the algae and qualify the sample via thin layer chromatography (TLC) – see below.

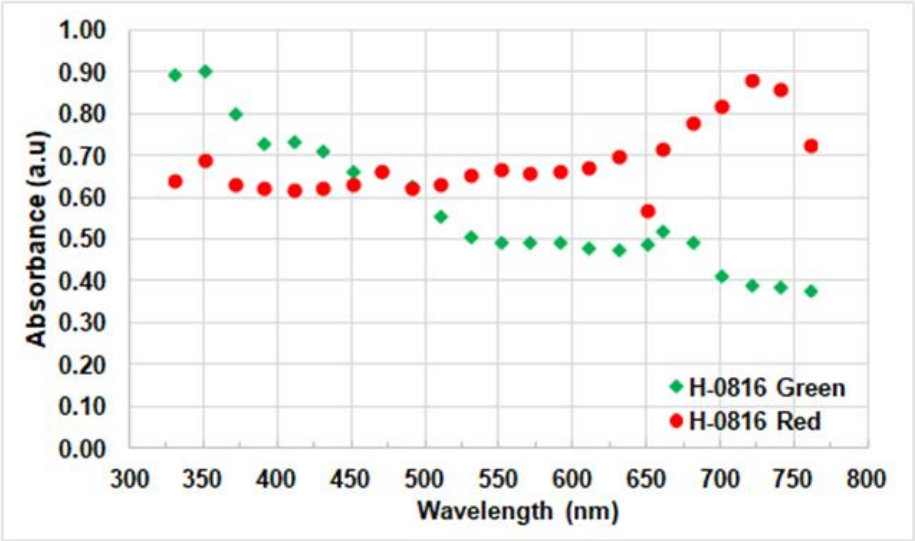


Figure 20: Tracking of induction of H-0816 by ratio of absorbance at 730 nm/350 nm. Ratio for green cells is 0.4 and for red cells is 1.3

Species H-0522 for nutraceuticals

The second species, H-0522 was selected for its purported features of high levels of □-carotene and poly unsaturated fatty acids (PUFA) as value-added products. All the prior work described above in improving the growth and induction of species H-0816 was utilized to help advance the work on the second species more rapidly. Induction in this species is demonstrated by a color change from green to a yellow-orange (Figure 21). The improved process as describe above were again linked with the simultaneous reduction of Nitrogen and high light conditions and induction was accomplished within a one-week period.

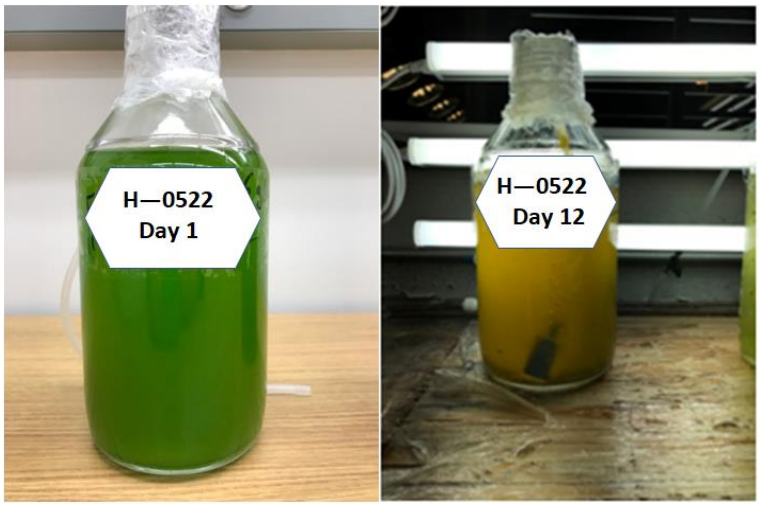


Figure 21: Cultures of species H-0522, left - growing stage (green); right - induced (yellow-orange)

Extraction Method:

Once a large amount of algae is induced, it is subjected to extraction to begin the down-stream preparation of the nutraceuticals for wholesale users. Much of the work here has relied on a long used commercial method of solvent extraction combined with mechanical cell disruption. This tried and true method was conducted here with 3:1 hexane:isopropanol in a bead mill. The algal material soluble in the solvent fraction was shown to contained chlorophyll, colored antioxidants, and the lipids of the cell including any high-valued PUFAs (see Figure 24 and Tables 8 and 9). At present this crude solvent soluble fraction obtained from algae, plants, krill and fish is used for animal/aquaculture feed, food additives, and supplements sold for human health. The alternative commercial product could be by incorporating the entire algal biomass into a food product for animals or humans. The thin layer chromatography (TLC) analysis of induced species H-O816 shows the presence of Astaxanthin, small amounts of carotenoids, and fatty acids. For species H-0522 the products identified include carotenoids and small amounts of Astaxanthins, and fatty acids. While the solvent extraction process is extremely efficient and not too costly, there are health concerns of solvents contaminating the products and their disposable are problematic environmentally.

We have advanced the extraction process to two steps termed a aqueous/solvent system (Figure 4). This is a process being developed as an additional method to increase the value and assortment of products obtainable from the induced algae. Typically, after the crude hydrophobic fraction is obtained, the remaining material is sold for lower value animal feed or thrown away. Helios-NRG envisions increasing the number of nutraceuticals and amounts recovered by first extracting this hydrophilic fraction as it contains proteins and other water-soluble materials with value. This two-step or aqueous/extraction method was tested on species H-0522 and H-0816 with the results shown in figure 22. For the species H-0522 a sample of the aqueous phase was shown to contain proteins by SDS-polyacrylamide gel electrophoresis and then staining the proteins (figure 23. The different bands observed on the gel represents a number of prevalent proteins(darker) and less abundant (lighter bands) in the aqueous fraction. The protein bands are separated by molecular weight with the larger ones towards the top of the gel and the smaller proteins towards the bottom. Proteins, such as enzymes that can be used in biochemical processes and growth factors which may stimulate plant growth would be examples of high-value products. By comparing the results for induced and uninduced algae, it is shown that there may be some differences in quantity of some of the proteins between the two sources. This initial step shows promise for obtaining and identifying specific valuable proteins/enzymes prior to the second step being carried out where the hydrophobic products already described would be recovered (Figure 24) by organic solvents and analyzed by TLC .

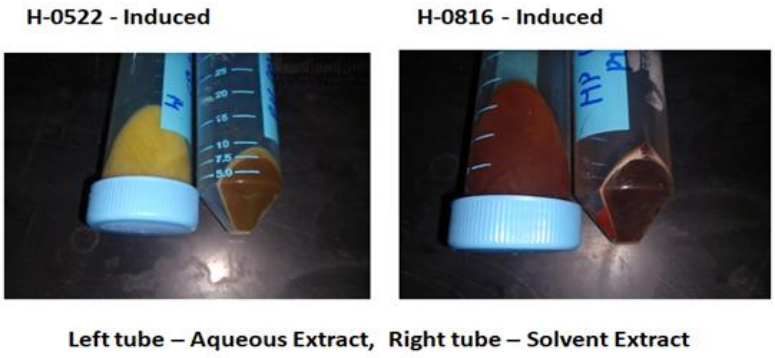


Figure 22: Samples of two phases of extracts from the induced nutraceutical algae

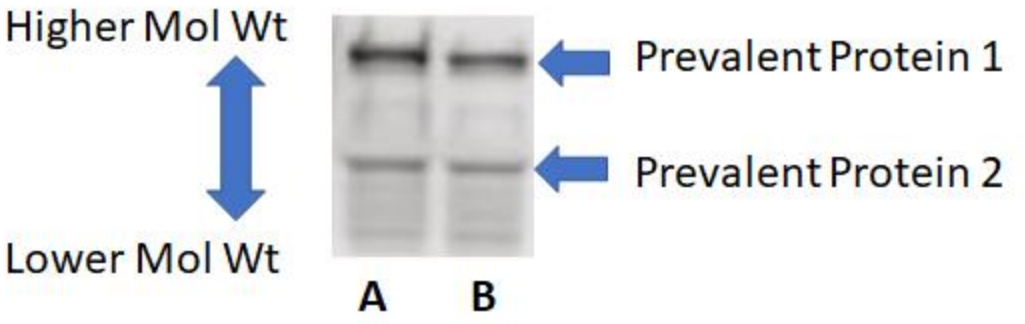


Figure 23: Demonstration of proteins in aqueous extraction as analyzed by SDS gel electrophoresis; Species 0522, A – induced, B - growing.

In Figure 24, the TLC identifies two categories of products in the organic fractions of the two species. Species H-0816 and H-0522 contains a group of antioxidants – astaxanthin, carotene and other carotenoids. It also contains fatty acids where PUFAs have a greater value. To more specifically identify and quantify these products the extracts were subjected to high pressure liquid chromatography (HPLC). In table 8, the results of the astaxanthin analysis for the induced H-0816 is presented. The test shows that the induced algae contains about 6% wt/wt of total astaxanthin and the most prominent form is the complexed compound of the mono-ester. Then for the second species, both the antioxidants and lipids were quantified (Table 9). Several nutraceuticals were found, astaxanthin, carotene, lutein, zeaxanthin, but at lower levels than in H-0816. The lipids of interest, fatty acids, were at 15% wt/wt, with a significant amount of omega-fatty acids. Finally, a type of extraction that is becoming more common in commercial operations is supercritical CO_2 extraction. This is as efficient as solvent extractions and provides the further benefit of the ultra-cold process protecting the chemistry of labile material being obtained which will have much greater value when intact. This would likely be a part of a future project by Helios-NRG and would be connected with additional fractionation processes to widen the number and value of nutraceutical species.

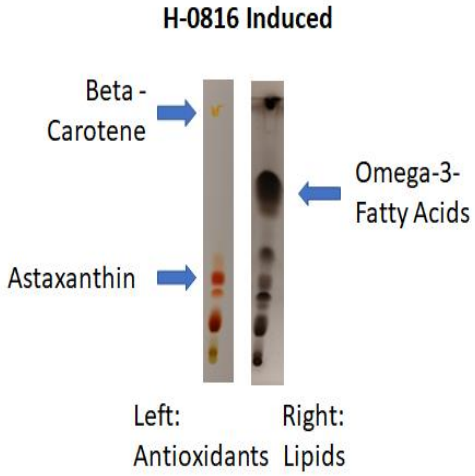


Figure 24A: TLC of induced H-0816

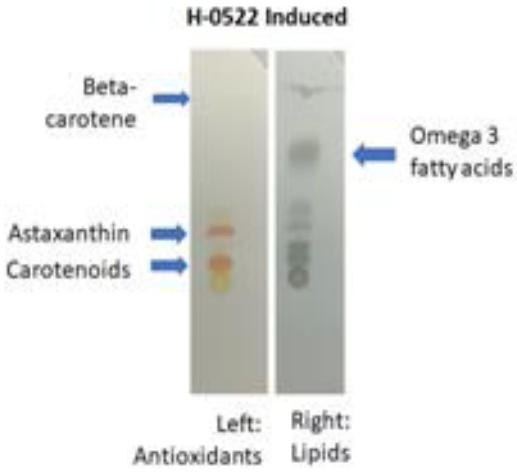


Figure 24B: TLC of induced H-0522

Note: Left lane of each panel showing visible antioxidants and right lane of each panel stained for lipids

Table 8: Astaxanthins in induced H-0816

Astaxanthin Content of H-0816	
Component	%
Mono-esters	4
Di-esters	2
Total	6

Table 9: Nutraceuticals in induced H-0816

Induced H-0522	
Component	%
Astaxanthin	0.08
Beta - Carotene	0.003
Lutein	0.004
Zeanthin	0.014
Total Carotenoids	0.021
Saturated Fatty Acids	15
Omega 3 Fatty Acids	0.6
Omega 6 Fatty Acids	2
Total Fatty Acids	12

Subtask 2.3- Determine optimal gas draw point for nutraceutical algae

CO_2 and growth curves/Potential impact of flue gas contaminants

The primary MSC system has stages designed to operate at specific CO_2 feed concentrations and results in each stage capturing CO_2 with lower levels of CO_2 exiting each stage. Along with the decreased CO_2 level exiting each MSC stage, prior work and measured data has shown that algae can remove heavy metals from the media. This essentially results in the inter-stage MSC gas streams to be depleted of the heavy metal contaminants, and thus a cleaner gas stream suitable for nutraceutical algae production. Inter-stage gas options for the nutraceutical algae production could be the last stage (Stage1 of a 3-Stage MSC system like in Figure 8 and Figure 11). The inter-stage MSC gas exiting Stage 1 would have ~1% CO_2 and would be the cleanest stream in regards to HM or exiting Stage 2 which would have ~3% CO_2, but a slightly higher HM content. Therefore, if the nutraceutical species could be grown in this stream, it might be suitable for animal and human consumption. The experimental results outlined in Table 10 show that both nutraceutical species H-0816 and H-0522 do grow at sufficient rates, although slower than a fast-growing species which increases its growth rate when more CO_2 is fed into the culture (H-1601).

Table 10: Growth rate (in terms of slope of growth curve) of three species of algae fed CO_2 concentrations representing reduction by MSC as exiting of stage 1 and 2.

CO2 feed	1%	3%
Species	Slope of Growth Curve	Slope of Growth Curve
H-0816	0.02	0.02
H-0522	0.02	0.02
H-1601	0.05	0.1

An additional experiment was conducted to extend of cleanup of the post FGD flue gas by utilizing mimicked stages of the MSC to remove HM. The estimated amount of five HM listed in Table 11, were added to a culture of species H-1903 (used for carbon capture) based on the expectation of the solubility and amounts coming from coal-based flue gas feeding CO_2 to the algae. The culture was then grown to algae concentration exceeding Stage 3. The algae was then collected and the amount of HM captured up by the algae was measured (Table 11) by an external laboratory. The recovery of HM at levels of 70 to essentially 100% in a single-stage indicates that across several MSC stages, a clean inter-stage gas suitable for feed into the nutraceutical algae production would be feasible. This would ensure a clean nutraceutical algae production, free from HM contamination.

Table 11: Fraction of HM in culture media captured in algae

Heavy Metal	% Captured by Algae
As	100
Cr	100
Cu	100
Hg	70
Se	85

Task 3: Advance DeAqua Stage1 (Obj 3, Helios)

Subtask 3.1- Develop continuous flow gravity table

The objective of this subtask was to further advance the performance of the continuous flow gravity table from that achieved in Phase I to a minimum concentration ratio of 4-5x while achieving a recovery ratio of >80%. In parallel, the process was to be improved to have a residence time reduced to <8hr. The concentration ratio is defined as the ratio of the concentration of the product microalgae to feed algae concentration. Recovery ratio is defined the ratio of mass recovered in the concentrated product microalgae compared to the initial feed mass. In Phase I, several design parameters were identified and their impact on algae gravity settling summarized as seen in Table 12.

Table 12: Summary of gravity table design parameters identified

Design Parameter	Effect on Gravity Settling H-1903	Comment(s)
Tank Width	Low	
Tank Depth	Low	
Tank Material	High	Plastic materials desirable as compared to glass
Tank Shape	High	Conical bottom desirable for collection
Culture pH	Low	
Algae Mobility	Potentially High	Ways to reduce mobility to be studied
Environment	Moderate	Dark environment desirable N2 degasification desirable

In this project, the strategy was to utilize the design parameters identified previous to improve gravity settling of algae. However, a step change in continuous gravity table operation is difficult without use of flocculant as most of the parameters identified were focused on the settling tank design. This is because the terminal velocity of algae is very low and any continuous flow velocity into the gravity table will be

significantly higher, leading to resuspension of the settled algae. The terminal velocity can be quantified via Stokes' Law and was modeled in Figure 25.

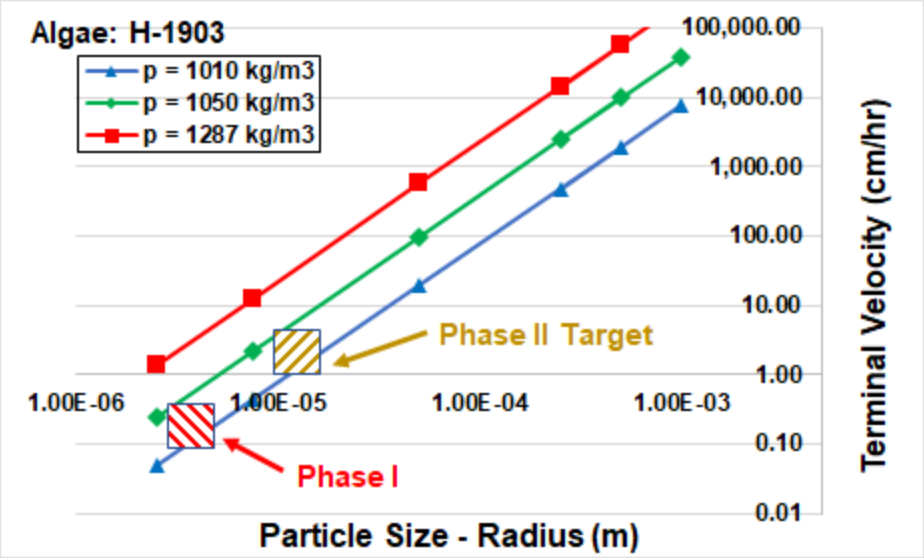
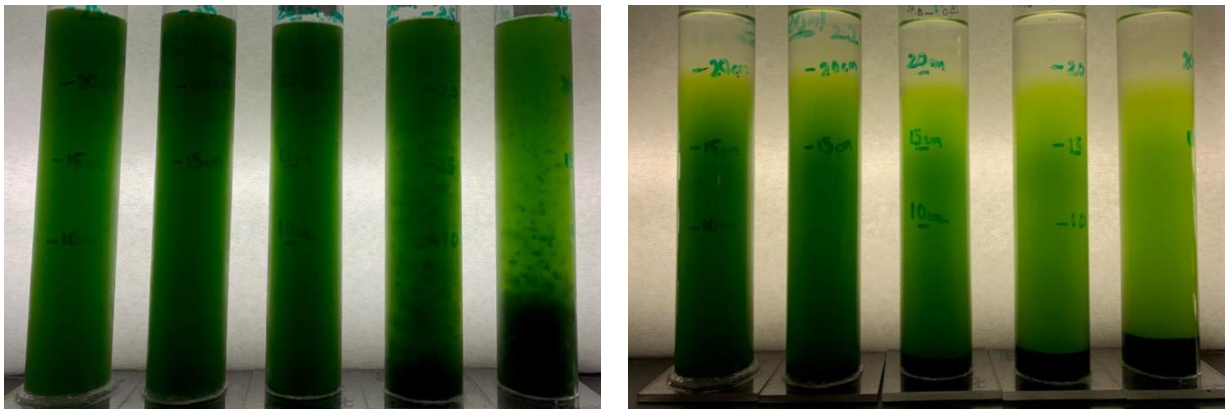


Figure 25: Algae terminal velocity estimation

Algae terminal velocity increases with increasing algae density and particle size. While the exact particle size (radius) and density is difficult to quantify, both remain strong variables affecting terminal velocity. However, manipulation to the algae particle size is easier than varying the inherent particle density. This can be achieved via addition of chemical flocculants, though less desirable due to potentially high material cost, toxicity issues and high internal media recycle. Alternatively, seeding of settled algae cells into freshly harvested culture can be potentially beneficial to improve algae particle size. A batch experiment was conducted to determine the impact of increased particle size on terminal velocity. Previously settled seed was recycled to the freshly harvested cultures at varying volume% to increase its particle size as seen in Figure 26 and its impact of gravity settling quantified in Figure 27.



Control (0 vol%) → 4 vol% at 0 hr **Control (0 vol%) → 4 vol% at 24 hr**

Figure 26: Batch experiment of recycled settled algae into freshly harvested culture

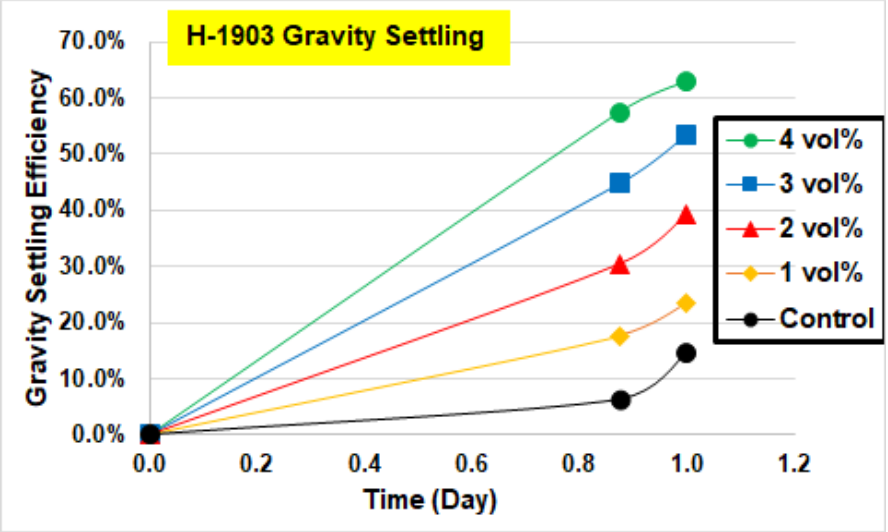


Figure 27: Impact of recycled settled algae on gravity settling

Seeding freshly harvested algae culture with previously settled seed (larger clusters) improves gravity settling significantly. This is because the larger clusters can potentially scavenge and aggregate individual microalgae, improving terminal velocity as seen with higher gravity settling efficiency. This technique is potentially promising for continuous gravity table operation as it defines a method to manipulate algae particle size for improving terminal velocity. However, in large-scale operation, this may have limitations as the total residence time required significantly exceeds 8hr for moderate settling efficiency and product recovery. This technique is also highly dependent on the obtaining sufficient quantities of the previously settled seed and the operational control may be limited.

Electro-flocculation has the potentially to be a highly controlled algae dewatering process. It is low cost, quick, and highly efficient (>90%)[5]. Many metal electrodes have been extensively studied for electroflocculation with aluminum (Al) being one of the best candidates due to its high conductivity and low cost. Through the electro-flocculation reaction, only a small concentration of ions is ionized into the medium to produce larger algae aggregates, promoting settling. This is theorized to have negligible impact on the growth of microalgae as compared to chemical flocculation. Preliminary performance mapping test on key operation parameters such as chamber exposure duration and applied voltage of the algae electro-flocculation process is shown in Figure 28 and Figure 29 respectively.

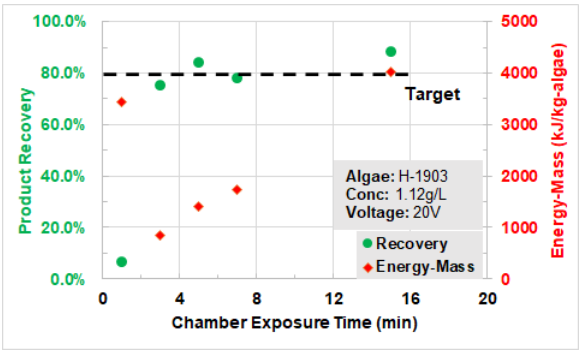


Figure 28: Impact of varying exposure time on algae electro-flocculation performance

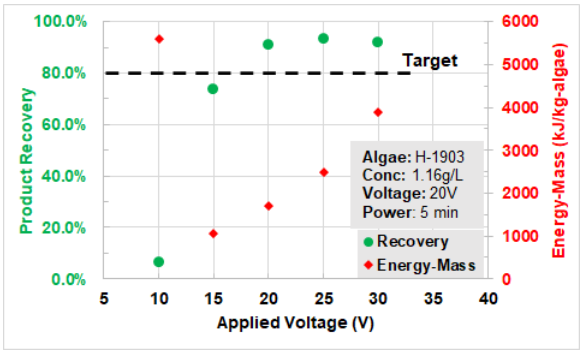


Figure 29: Impact of varying voltage on algae electro-flocculation performance

In Figure 28 and Figure 29, at short chamber exposure time (1min) at a fixed voltage and low applied voltage (10V) at fixed chamber exposure time, the algae product recovery is very poor due to inability to produce large algae flocs. As expected, increasing the electro-flocculation exposure time and/or the applied voltage improves the product recovery. However, at prolonged exposure time and high applied voltage, the energy/mass requirement increases for little gain in product recovery. In the test system, an exposure time of 3-5min and voltage of >15V was adequate to achieve high product recovery (>80%).

The spacing of electrodes in electro-flocculation chamber is an important design parameter. To elucidate the impact of the electrode spacing on algae dewatering performance, the process can be operated in two modes: (1) Constant voltage, (2) Constant current as shown in Figure 30 and 31 respectively.

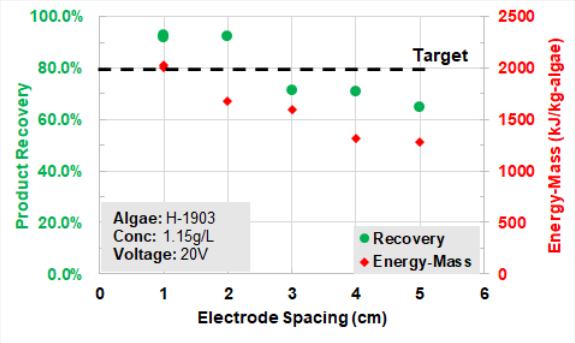


Figure 30: Impact of electrode spacing on electro-flocculation performance at constant voltage

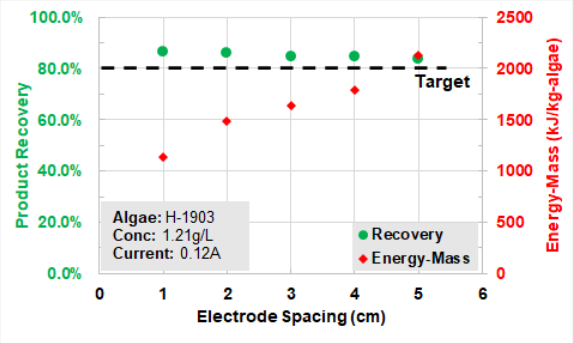


Figure 31: Impact of electrode spacing on electro-flocculation performance at constant current

As seen in Figure 30 and 31, at increasing electrode spacing, operating under fixed current as compared to fixed voltage is highly desirable as the high algae product recovery is maintained. At an increased electrode spacing for a fixed voltage operation, the field strength and current density decreases due to the high electrical resistance of the algae culture. This results in poor ion transport to the culture medium in a fixed electro-flocculation chamber design and thus reduced algae product recovery. However, while operating at fixed current ensures high algae product recovery, the increased in energy-mass requirement is due to the requirement of a higher voltage application to compensate the high resistance of the algae culture. In summary, for a fixed design of the electro-flocculation chamber, it is essential to reduce the spacing between electrodes to ensure high algae product recovery and lower energy-mass requirement.

Subtask 3.2- Develop technique to increase algae recovery from gravity table

The goal in this subtask is to further reduce the loss of algae product in the top fraction, increasing overall algae recovery in bottom fraction. In Subtask 3.1, at increased voltage or current, it was observed that the algae flocs formed in 2 distinct layers, a settled layer at the bottom and a floating layer at the top. This multiple layer formation is undesirable as it complicates the efficient recovery of algae product. Based on the batch tests learnings in Subtask 3.1, a continuous flow electro-flocculation chamber was designed with 4 pairs of aluminum electrodes, each with 1in electrode spacing.

In order to improve algae recovery from the gravity table process, the electro-flocculated algae culture from the chamber is fed through a gas stripping chamber follow by settling in a conical bottom settling tank. The addition of a gas stripping chamber with either air or N2 increases the algae product recovery via elimination of microbubbles formed as a by-product of the electro-flocculation process. The schematic of the continuous flow gravity table process is shown in Figure 32.

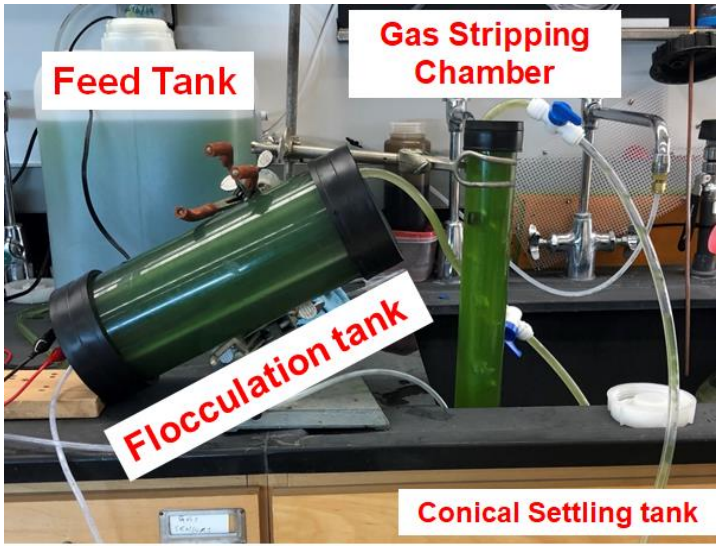


Figure 32: Bench setup of continuous flow electro-flocculation aided gravity table process

The preliminary performance of the continuous flow electro-flocculation aided gravity table was conducted based on several key parameters. In all continuous flow systems, the flow residence time is an important parameter affecting the overall performance. Performance mapping tests for varying flow residence time for the electro-flocculation chamber is shown in Figure 33 and its further impact on Al liberation into algae culture is shown in Figure 34.

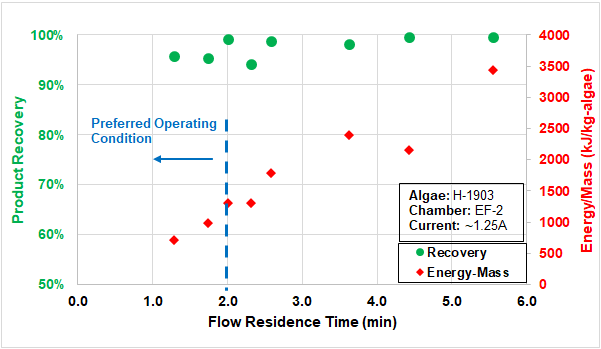


Figure 33: Impact of flow residence time on electro-flocculation performance

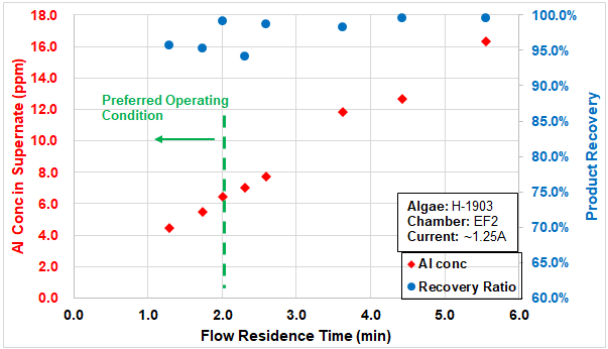


Figure 34: Impact of flow residence time on Al liberation into algae culture

The increased in flow residence time in the electro-flocculation chamber enables high product recovery (>90%), which greatly exceeds Phase II objective. However, at long flow residence time, the energy-mass requirements increase for little gain in product recovery, an undesirable mode of operation due to excess utilization of energy. The aluminum ion liberation into the algae culture was quantified via Faraday's Law. As seen in Figure 34, the long flow residence time results in a higher concentration of aluminum ions into the algae culture. Based on this study based on the preliminary design of the electro-flocculation chamber, it was determined that < ~2min flow residence time provided optimal performance.

The impact of algae feed concentration on the electro-flocculation aided gravity settling is elucidated in Figure 35. This is an important parameter as this process is a direct downstream process from the main MSC system and serves as a potential optimization parameter.

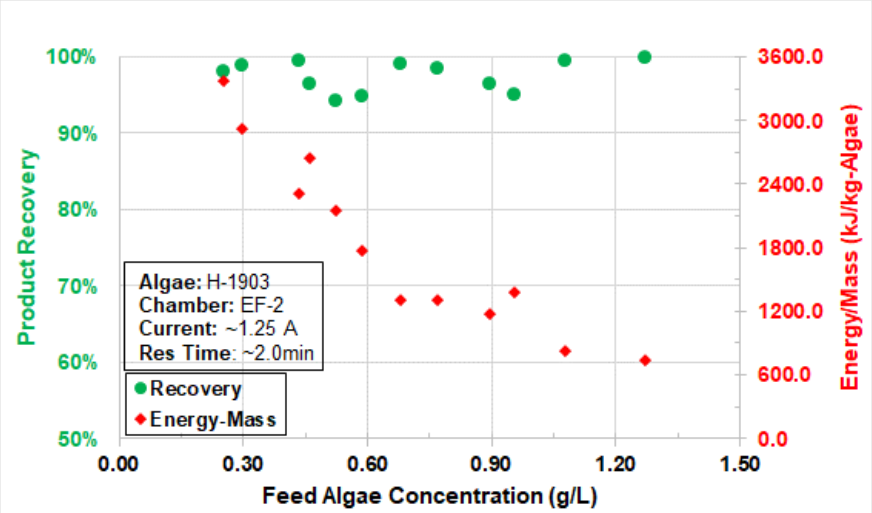


Figure 35: Impact of algae feed concentration on electro-flocculation performance

At the constant current operation, the energy/mass requirement decreases as feed algae concentration increases. This is because the electrical power is efficiently utilized for dewatering a higher concentration of algae as compared to electrolysis of water, typical of lower concentration of algae. However, based on prior work in the development of the MSC system, the feed algae concentration would be ~0.4-0.6g/L from the MSC system due to optimal growth and productivity. In most tests, a product concentration ratio of 20-25x was achieved, significantly exceeding the Phase II project objective.

A new performance index was utilized for comparison of the results obtained between the various Phases of the project. The performance index is defined as the ratio between the product concentration ratio and overall process time (electro-flocculation exposure and settling time). The impact of overall process time on the algae product recovery and performance index is shown in Figure 36.

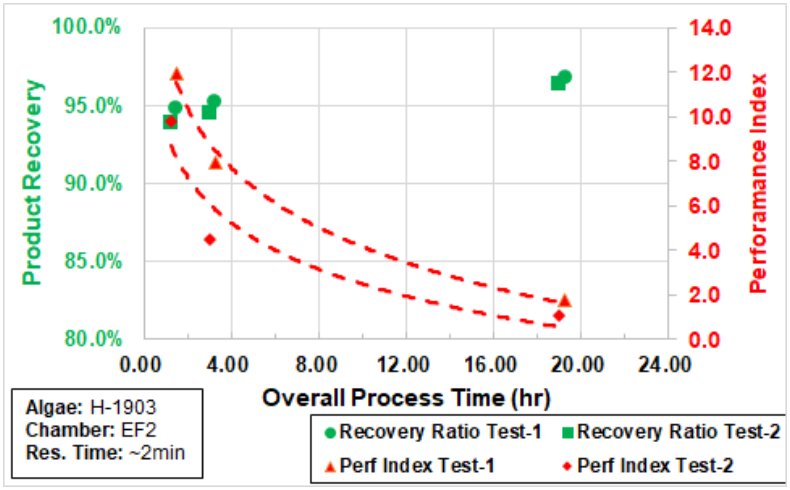
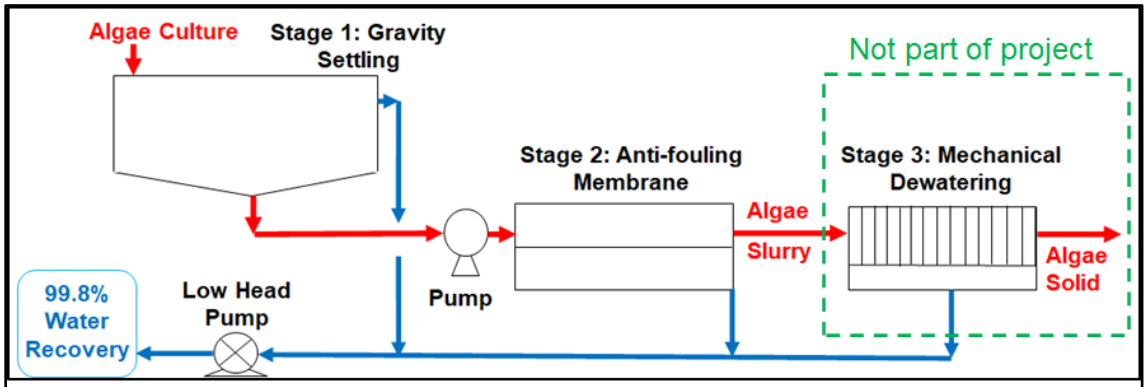


Figure 36: Tradeoff between algae product recovery, performance index with overall process time

In general, the performance index and the overall process time to achieve >90% recovery ratio are inversely related. To achieve recovery ratios >95%, a longer gravity settling time is required. This is undesirable as for long processing time, the settling tanks would need to be sized larger, resulting in higher capital costs. An overall processing time of 4-6hr was determined to be a good operating point

representing good product recovery together with high performance index. It is also important to note that the overall algae culture health has a significant impact on the dewatering performance. Presence of other bio-contaminants generally lowers the separation efficiency (concentration ratio and recovery ratio) of the DeAqua Stage1 process.

Advancement in the DeAqua Stage 1 gravity table resulted in major improvement in the dewatering step. The updated process schematic for the DeAqua process is shown in Figure 37 and the comparison between the performance index for the gravity table process for the difference phases of the project is summarized in Table 13.



DeAqua Stage	Initial Target		Current Work	
	Product Conc Ratio	Water Recovery (%)	Product Conc Ratio	Water Recovery (%)
1 - Gravity Table	~5	~80.0	~25	~96.0
2- Anti-fouling Membrane	~12	~18.3	~8	~3.5
3- Mechanical Dewatering	~10	~1.5	~3	~0.3

Figure 37: DeAqua process including performance of the various dewatering steps

Table 13: Progress in DeAqua Stage 1 gravity table performance

Project	DeAqua Stage 1 Performance Specification		
	Product Conc Ratio	Performance Index	Product Recovery
Phase I Work	3-6	0.12-0.25	70%
Current Work	20-25	5.0-12.5	80-95%

Overall, significant process was achieved in Stage 1 of the DeAqua process as compared to Phase I of the project. The performance greatly exceeds the initial project objective for the product concentration ratio and product recovery by a factor of 4x and ~20% respectively. Additionally, >90% of water is removed as compared to ~70% in Phase I, all at reduced energy requirement (~5% of total algae energy content). Future work will focus on reducing the energy/mass requirement of the electro-flocculation process via culture additives. Further study on the utilization of the water recycle in the algae growth stage will determine more refined electro-flocculation operating conditions.

Task 4: Advance DeAqua Stage2 (Obj 3, UB, MTR)

Our objectives of developing membranes for algae dewatering were to double the water permeance in algae dewatering via the optimized membrane surface modification, develop periodic regeneration technique, and

demonstrate the performance of the spiral-wound modules fabricated using the modified membrane. The details are shown below.

1. Membrane modification to improve antifouling properties for algae dewatering

1.1 Selection of commercial membranes for modification

In this project, state-of-the-art commercial ultrafiltration (UF) and microfiltration (MF) membranes of polysulfone (PSf) and polyethersulfone (PES) were investigated, such as PES 0.05 (MF), Nylon 0.2 (MF), and PSf 100 (UF). Fig. 38 shows the water permeance of PES 0.05 as a function of time when challenged with 1 g/L algae solution at a feed pressure of 5 psi. Water permeance decreases rapidly at the beginning due to fouling and then reaches a steady state. This behavior can be modeled by the cake-formation model, i.e., algae and other organic matter form a cake on top of the membrane, increasing transport resistance for water.[6,7]

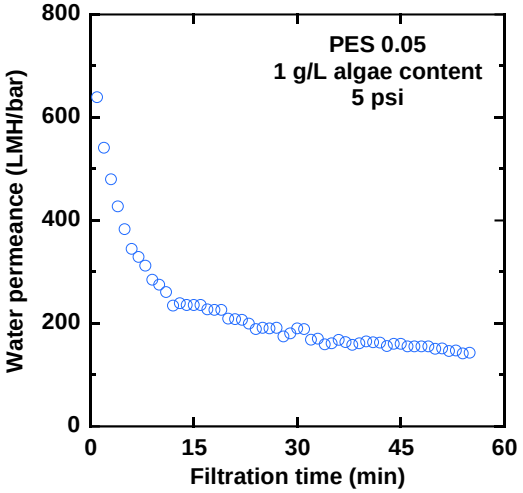


Figure 38: Water permeance of PES 0.05 as a function of filtration time when challenged by 1 g/L H-1903 algae solution at 5 psi.

Table 14 compares the pure-water permeance and steady-state permeance of an algae solution for PES, Nylon, and PSf membranes. Although the pure-water permeance of PSf UF membrane is significantly lower than the MF membranes (PES and Nylon), the PSf UF membrane exhibits much higher water permeance when challenged with 1 g/L algae solution than the MF membranes, presumably because of the larger pore size and more severe internal fouling in the MF membranes. Hence, the PSf 100 was chosen for further surface modification to improve antifouling properties and thus dewatering performance.

Table 14. Pure-water permeance and steady-state permeance of 1 g/L algae solution for PES 0.05, Nylon 0.2, and PSf 100.

Membranes	Water permeance (LMH/bar)	
	Pure-water	1 g/L algae solution
MF: PES 0.05	1220 ± 70	186 ± 9
MF: Nylon 0.2	8100 ± 500	142 ± 9
UF: PSf 100	700 ± 20	320 ± 10

1.2 Regeneration technique for flat-sheet membranes

As fouling is inevitable, the membrane regeneration technique is important to remove the deposited foulants on the membrane surface and recover the water permeance. Fig. 39 shows the effect of membrane cleaning on the permeance recovery of the PSf 100 membrane through multiple cycles of dewatering-cleaning experiments in dead-end permeation cells. The PSf 100 membrane was first tested with pure-water and then 10 g/L algae solution at 5 psi till the water

permeance became steady. The membrane was then cleaned by water-rinsing (WR) or chemical cleaning using a NaOH solution of pH ~8.5 (CC), followed by another cycle of pure-water permeation-algae dewatering-cleaning. As shown in Fig. 39, the pure-water permeance decreased after the first cycle due to the irreversible fouling (caused by internal fouling). After that, both cleaning methods of WR and CC can recover the pure-water permeance by almost 100%.

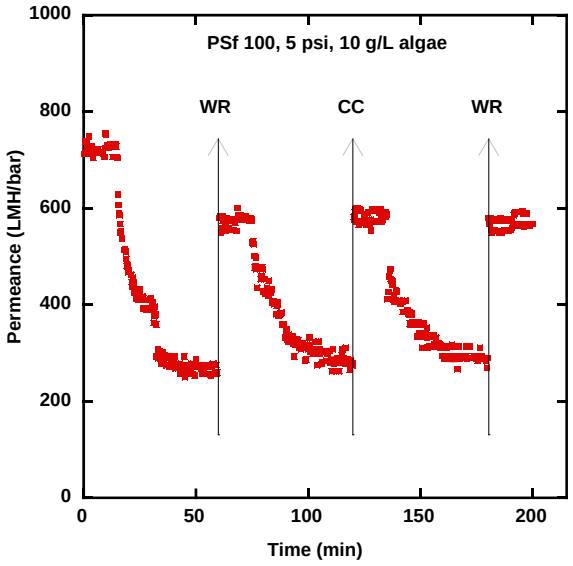
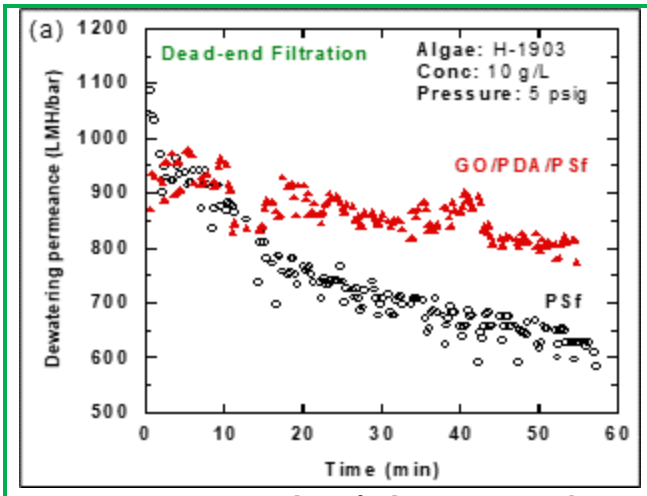


Figure 39: Pure-water permeance and steady-state water permeance of 1 g/L algae solution of PSf 100 during multiple cycles of pure-water permeation-algae dewatering-cleaning.

1.3 Membrane surface modification to improve algae dewatering performance

Hydrophilic materials, including graphene oxide (GO) and sulfobetaine methacrylate (SBMA), were grafted onto the membrane support using the bio-adhesive polydopamine (PDA) to improve antifouling properties.[8,9] These modified membranes were examined for algae dewatering. The following procedures were developed to graft GO onto the membrane surface. First, a PSf-100 membrane was coated with 1 g/L dopamine for 2 h. Second, the GO was activated using two catalysts of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). Finally, the PDA-modified membranes were immersed in the activated GO solution for 2 h for the GO to react with the PDA. The surface grafting decreases water contact angles and retains high water permeance.

Figure 40a demonstrates the improved water permeance after grafting with GO. When challenged with 10 g/L algae solution, the GO modified membrane (GO/PDA/PSf) shows 31% greater dewatering permeance than the pristine PSf membrane. Additionally, the GO modified membrane presents much less attachment of the algae than the pristine PSf after the algae dewatering test (Figure 40b), which demonstrates the enhanced fouling resistance of the GO modified membranes.



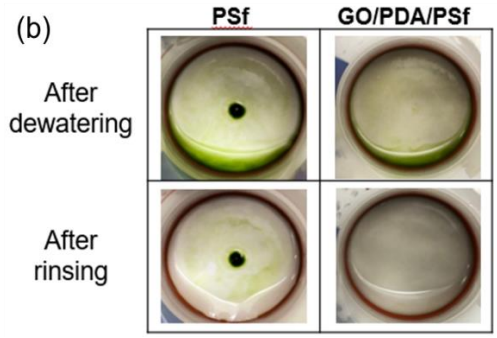


Figure 40: Improved antifouling properties by GO grafting. (a) Comparison of algae dewatering permeance between the GO modified PSf membrane and pristine PSf membrane. (b) Comparison of images of the fouled membranes before and after rinsing with DI water.

The membranes were also modified using other hydrophilic materials such as sulfobetaine methacrylate (SBMA) and poly(ethylene glycol) diacrylamide (PEGDAm). Fig. 41 compares the water permeance of the three membranes when challenged by DI water and 10 g/L algae solution at 5 psi. The surface modification decreases water permeance, and the permeance is much lower for the algae solution than pure water. Although the pure-water permeance of pristine PES is the highest among all the membranes, the SBMA-modified membrane exhibits the best algae dewatering performance (~500 LMH/bar), demonstrating the improved antifouling properties.

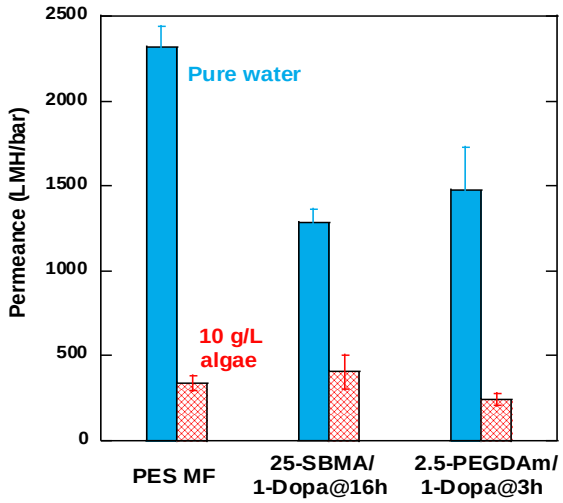


Figure 41: Effect of surface modification of PES membrane on algae dewatering when challenged by 10 g/L algae solution at 5 psi.

2. Demonstration of bench-scale membrane modules for algae dewatering

To demonstrate the surface modification to improve long-term algae dewatering performance, commercial polyvinyl chloride (PVC) hollow fiber membrane modules (HFMs) was utilized as an example and then fabricated bench-scale spiral wound membrane modules. The module preparation and characterization are described below.

2.1. Commercial PVC HFMs for algae dewatering and regeneration

The PVC HFMs were surface-modified using SBMA. Table 15 compares the dewatering permeance of both pristine and SBMA-modified PVC HFMs when challenged by different concentrations of algae solutions. When the algae concentration increased 50 times from 0.1 g/L to 5 g/L, the water permeance of both modules decreased by nearly 80%. However, the SBMA-modified module always showed higher permeance than the pristine one.

Table 15. Comparison of the permeance with various algae solutions for the pristine and SBMA-modified PVC HFMs.

Algae content (g/L)	Permeance (LMH/bar)	
	Pristine module	SBMA-modified module
0.1	91	101
0.5	59	61
5.0	16	23

The efficiency of three cleaning methods to recover water permeance was evaluated, including DI water rinsing (at 2 L/min), DI water backwashing (at 0.55 L/min for 100 min), and chemical rinsing (using NaOH solution at pH of 11 for 1h). The results are summarized in Table 16. The SBMA-modified HFMs exhibited lower pure-water permeance but similar permeance of algae solutions than the pristine HFMs. After dewatering, both modules were subjected to three cleaning methods. NaOH cleaning works best in regenerating the pristine HFMs, while the backflushing shows better regeneration performance than others.

Table 16. Effect of three cleaning methods on the water permeance of the pristine and SBMA-modified PVC HFMs. The modules were tested with pure water, 4.8 g/L algae solution, and then pure water again after cleaning.

PVC HFMs	Pure-water permeance (LMH/bar)	Algae solution (LMH/bar)	Pure-water permeance after cleaning (LMH/bar)		
			Water rinsing	Backflushing	NaOH cleaning
Pristine	278	155	166	149	176
Modified	224	150	127	140	129

2.2. Bench-scale spiral-wound membrane modules containing the modified membranes

PES membrane sheets were modified using SBMA and GO, respectively, and had them fabricated into two spiral-wound membrane modules by Membrane Technology and Research, Inc. (MTR) (Figure 42a).

The test of the SBMA-modified module has been completed, which shows pure water permeance of 180 LMH/bar. The module was then challenged with algae solutions in various concentrations in a cross-flow filtration system, and the results are shown in Figure 42b. Increasing algae concentration makes the fouling more severe. The recovery after each cleaning process is 86% when challenged with 0.1 g/l algae and 60% when challenged with 0.5 and 1 g/L algae. When the module was cleaned using a NaOH solution with pH of 11, the recovery rate increased from 60% to 85%.

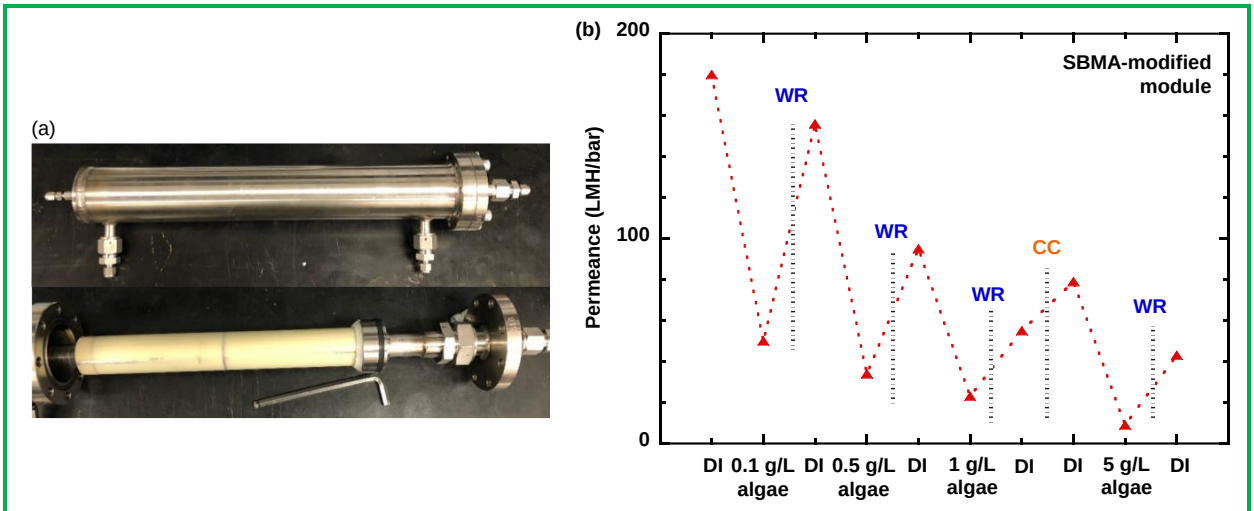


Figure 42: (a) Photos of the module housing and spiral-wound membrane module containing surface-modified PES membranes. (b) Water permeance profiles versus algae concentration at 10 psi for the SBMA-modified module in a constant pressure cross-flow filtration system.

Task 5: Refine Techno-economics (Obj 4, Helios/UB)

Table 17 summarizes the basis for the techno-economic assessment performed for the CO_2 capture and utilization technology developed in this project.

Table 17: Basis for Analysis

Parameter	Project Base Case
Size of algae farm (wetted pond area) - acres	5000
Pond (Raceway) size - acres	10
Total Area of facility - acres	7600
Land cost ($/acre)	3000
Pond Cost ($/acre)	31700
Water recycled	99%
% nutrients from waste water	50%
CO2 utilization	90%
CO2 type used	Flue gas
Operating days /year	330
Facility Life yrs	30
Electricity price (c/kWh)	6.8
Net dewatering efficiency	99%
Algae conc after dewatering (g/L)	200
Fraction of algae used for biofuels	50%
Fraction of algae used for animal feed	48%
Fraction of algae used for nutraceuticals	2%

To the extent possible, the assumptions used here are the same those used in DOE's 2022 projections for algae technology for CO_2 capture and Utilization[3,4]. This recognizes the high quality of work and the depth of analysis inherent in that extensive work, but is also intended to enable easier comparisons with the results presented therein.

Production of Algae Biomass:

Carbon capture and algae production is carried out using the MSC system described in earlier tasks. Each stage of the MSC is comprised of a raceway with an inexpensive plastic top cover to minimize contamination, water evaporation and enable high CO_2 capture. For purposes of the TEA, each raceway is assumed to be 10 acres in size (670m long x 30m channel width) with a paddlewheel to circulate the culture at a velocity of 20 cm/s. The pressure in the headspace above the culture is nominally ambient and hence the plastic cover is not a loadbearing element. The power required for paddle wheel operation is ~12kW per raceway. Nutrients from municipal waste water is expected to a be an attractive source for culturing algae since there~17,000 waste water treatment plants in the US and the N and P compounds in waste water must be removed prior to discharge into the environment. The centrate stream produced by the waste water treatment plants, which is concentrated in N and P, represents a valuable source of nutrients for algae. In a parallel DOE funded project with the University of Illinois, Helios-NRG has demonstrated the ability to replace up to ~80% of the purchased nutrients for algae culturing with the nutrients in the waste water centrate stream. In the present analysis 50% nutrient replacement from waste water is assumed. The CO_2 required for the operation is obtained directly from post FGD flue gas from a coal fuel power plant - the basis for the current project. This is different from the earlier work[4] wherein the CO_2 is purified at the power plant and supplied to the algae growing operation. 90% utilization of the CO_2 from flue gas by algae is assumed based on the performance achieved in this project. The culture harvested is assumed to have a concentration of 0.5 g/l and is dewatered to 200 g/l using the DeAqua

technology discussed in earlier tasks. Based on the advances made in this project, this technology is estimated to reduce the cost of dewatering by ~$10/ton-algae. The water removed is recycled back to the algae growing operation and 99% recycle is assumed in current analysis.

Perhaps the most important parameter influencing algae economics is the productivity of the system. Helios-NRG has achieved an algae productivity of 36 g-dry wt/m2/day coupled with 88% CO_2 capture efficiency in outdoors operation of the MSC system in Buffalo in summer. The present analysis is hence based on a summer productivity of 35 g/m2/day and we have estimated the productivity during other seasons to reflect the variation in duration and intensity of sunlight available (Table 18). At this level of productivity (base case), the 5000-acre algae farm would produce ~187,000 tons-dry algae/yr and capture ~350,000 tons- CO_2/year.

Table 18: Seasonal and average annual productivity used in analysis

Season	Baseline algae productivity g-DW/m2/day
Summer	35
Spring	28.5
Fall	24.9
Winter	11.7
Annual Average	**25.0**

Table 19 summarizes the estimated costs for the algae culturing operations based on the assumptions in Table 17. A simple, plastic top cover has been added to enable efficient MSC operation. Since this is a new feature, we have costed this element conservatively amounting to ~40% of the cost of the pond. It is apparent that the ponds, cover, inoculum and dewatering dominate the capital cost of the facility. The total installed cost of the 5000-acre facility is estimated to be $447MM, about 15% higher than that estimated by Davis[4]. Most of these costs are relatively independent of algae productivity.

Table 19: Capital Costs for algae growing and dewatering facility (2014$)

Component	Base Case (MM $)
Ponds	158.5
Pond Cover	57.5
Inoculum	16.1
CO2 delivery	6.5
Make-up water + On-site circulation	7.2
Storage	5.3
Dewatering	43.9
Warehouse, site dev, Added piping	21.5
Total Indirect Costs	90.9
Fixed Capital - Land	22.8
Working Capital	17.5
Total Capital Investment (MM $)	**447.7**

The facility was assumed to have a 90% onstream factor and a useful life of 30 years. Equity financing of 40% of total investment is assumed with an Internal Rate of Return of 10%. The loan is assumed to be for 10 years at an interest rate of 8%. In addition to the annual capital related charges, there are operating costs such as for electricity, water, nutrients and labor to run the facility. The present analysis assumes a total staff of 100 people comprised of a plant manager, 4 engineers and a variety of personnel for operation, maintenance and site support. All the labor and most of the electricity represent fixed costs that are independent of algae productivity. Hence the total annual cost to operate the facility is to a great extent independent of algae productivity. However, the total algae biomass produced per year depends directly on productivity. For this reason, as shown later, the cost of algae production is very sensitive to algae productivity.

Product Revenue and Credits:

The present analysis assumes that the algae is used to generate the following three products with existing market needs.

- Biofuels – 50% of the algae produced is used in a hydrothermal liquefaction process (HTL) which has been identified by BETO as an attractive pathway for biofuel production. The price at which algae can be sold into this application depends on the desired biofuel price which is market dictated. The baseline case assumes a biofuel price of $3/GGE which requires an algae price of $271/ton-DW. A biofuel price of $2/GGE will require algae price of $116/ton-DW while a $4/GGE biofuel price translates to an algae price of $426/ton-DW.
- Animal Feed – The high levels of protein, lipid and other compounds (e.g., carotenoids) in algae makes it attractive for blending into animal feeds. The present analysis assumes that 48% of the algae grown is used for animal feed applications. The value of algae in these applications depends on both the nutritional profile of the algae as well as the price point of the blended feed. As a point of reference, soybean has a price typically in the $390-650/ton range. Cattle feed represents the largest segment of this market with a price ~$250/ton while fish meal, one of the fastest growing segments, carries a much higher price in the $1000-1600/ton range. The present analysis assumes a blended algae price of $500/ton for this market.
- Nutraceuticals – The algae selected for this market segment contains high levels of valuable components such as astaxanthin, beta carotene, omega fatty acids, polysaccharides etc. These are characterized by very high current market prices ranging from $500-5000/kg of product. The present analysis assumes that 2% of the biomass produced is used for nutraceuticals production and it has a nutraceutical concentration of 3%. Levels up to 6% have been measured in the present project. As discussed in earlier tasks, the algae grown goes through an induction process to increase the nutraceutical content which is followed by harvesting and supercritical extraction to generate unrefined nutraceutical products. The blended value of this product is conservatively estimated to be $600/kg which supports a price of ~$ 5100/ton for the specialized algae used in this application.

In addition to product-based revenue, there is potential for credits from at least two sources:

- Waste Water Credits: Waste water treatment plants incur significant costs to remove nitrogen and phosphorous compounds down to mandated levels prior to discharge. Based on data from a number of municipal waste water treatment plants around the country, researchers at University of Illinois at Urbana Champaign have estimated that the value of the removal of N and P from municipal waste water by algae to be in the range of $210-600 $/ton-algae DW. Our base case does not include any credits from waste water remediation, however a case including $210/ton-

algae DW is shown in the sensitivity analysis to indicate the significant added potential for cost reduction.

- CO_2 Capture Credits: At present there is no CO_2 capture credit in the US and future credits are speculative. For this reason, the base case does not include any CO_2 capture credit. However, a case with a capture credit of $20/ton- CO_2 is shown in the sensitivity analysis for illustration.

Base Case Economics:

Table 20: Economics of algae production and revenue/credits for the base case

Algae Production Costs (2014 $/ton dry algae)		
Component	Base Case 25 g/m2/day	
Ponds, cover, Innoculum, site infrastructure	257	
CO2 Supply	47	
Nutrients	11	
Dewatering	53	
Power & Utilities	32	
Labor Costs	65	
Total Production cost ($/ton-dry algae)	464	
Revenue & Credits (2014 $/ton dry algae)	**Base Case**	**Case2**
Biofuels - 50% algae	136	136
Feed - 48% of algae	240	240
Nutraceuticals - 2% of algae	102	102
WW Credit	0	210
CO2 Capture credit	0	0
Total Revenue & Credits	478	688
Net cost of algae ($/ton)	**(14)**	**(224)**
Net cost of CO2 capture ($/ton)	**(7)**	**(118)**

Table 20 summarizes the costs of algae production and revenue/credit streams for the base case. It is seen that the cost of ponds, cover, inoculum and related site infrastructure dominates the cost of algae production. The net cost of algae production is estimated to be $464/ton which is slightly below the $494/ton in DOE's 2022 projection[1]. This is primarily due to cost reductions from 50% use of waste water nutrients (vs purchased), use of flue gas vs purified CO_2 and use of the DeAqua dewatering technology developed in this project.

These costs are offset by significant revenue from the algae supplied for biofuels, animal feed and nutraceutical applications. In the base case, the total revenue generated is $478/ton which exceeds the cost of algae production and enables near cost neutral CO_2 capture (-$7/ton CO_2). While the actual revenue numbers will be dictated by the product distribution, the market price of products and many other factors, the base case shows that generation of these products has the potential to enable cost neutral or net revenue positive operation which would make it feasible to deploy CO_2 capture on large scale.

A second case (Case2) is also shown which highlights the significant added benefit if waste water treatment credits can be obtained. At a credit of $210/ton-algae, the low end of UIUC's estimate, the net cost of algae production drops to -$224/ton resulting in -118 $/ton of CO_2 capture. This would make deploying the proposed technology for CO_2 capture a profitable business proposition without legislative credits.

Sensitivity Analysis:

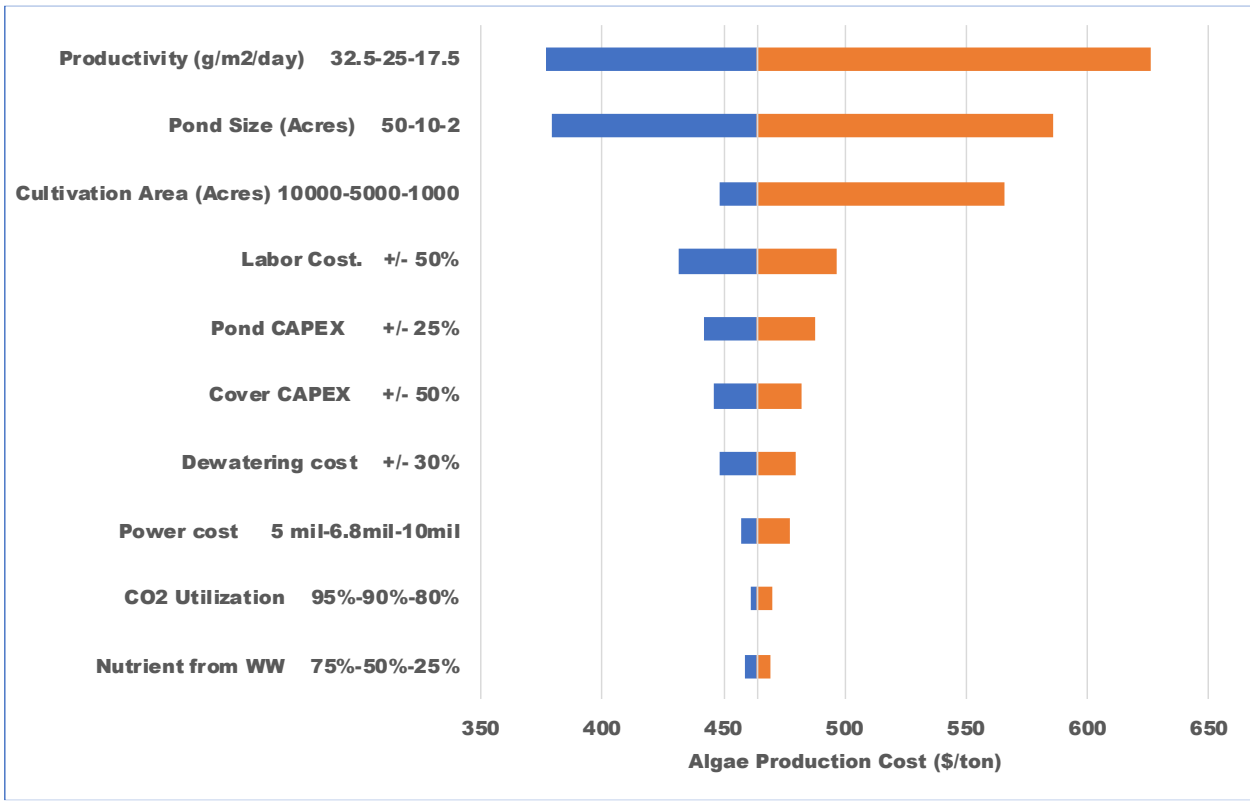


Figure 43: Tornado Diagram: Sensitivity of Algae Production Cost (Base = $464/dry ton-algae)

Figure 43 depicts the sensitivity of algae production cost to several key parameters. The most important factor by far is algae productivity. Increasing the average annual algae productivity by 30% to 32.5 g/m2/day lowers the production cost by ~$90/ton. Conversely a 30% lower productivity than the base case increases the production cost significantly to ~$625/ton. The size of the pond is another important variable. A wetted area of 2 acres, typical of current state of the art, would increase the algae production cost to ~580/ton while using 50-acre ponds, which represents new learning, would reduce production cost by ~$85/ton.

The use of a much smaller farm (1000 acres) increases production cost substantially to ~$565/ton because many of the labor and infrastructure costs are not proportionally reduced in a smaller facility. Conversely going to a 10,000-acre farm only reduces the algae cost modestly by ~$16/ton. This indicates that the 5000-acre base case provides useful perspective on the economics of a much larger facility as would be required for instance for a 500MW power plant. The product cost sensitivity to the cost of the top cover, a new feature introduced in this project, is indicated to be modest. Similar is the case for dewatering cost, degree of CO_2 utilization and the fraction of nutrients obtained from waste water.

Figure 44 shows the sensitivity of the net revenue and credits to key parameters. Waste water credit has the biggest impact and the use of algae for the removal of nitrogen and phosphorous from municipal waste water treatment plan can greatly increase net revenue. While the biofuel price can vary significantly due to market factors, Fig 44 shows that even at a relatively low cost price of $2/GGE, the net revenue from products can be on the order of $400/ton. The ability to make 3 unrelated market products, each contributing significantly to the overall revenue, reduces the risk to revenue potential from disruption in

any one market. In commercial operation this will be an important factor in developing a viable business proposition. For reasons discussed earlier, CO_2 credits are not used in the base case. While high levels of CO_2 credit may be difficult to practically implement, Figure 44 shows that the CO_2 capture credit is not a significant driver at the levels commonly discussed at present.

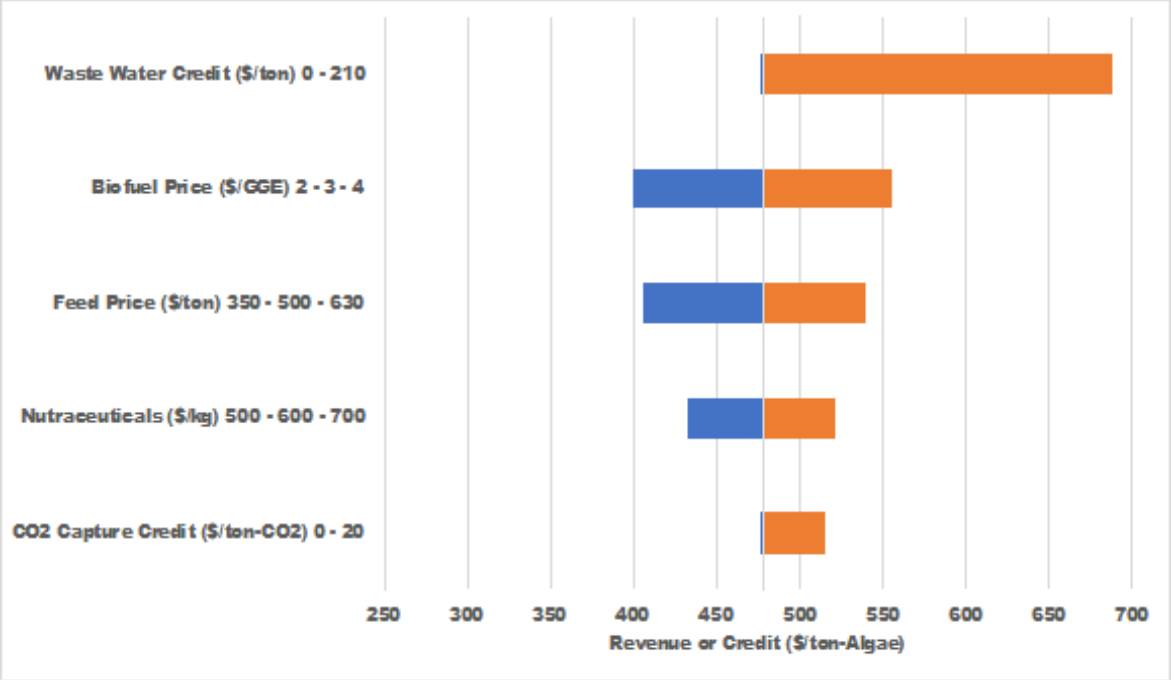


Figure 44: Tornado Diagram: Sensitivity of Algae Revenue and Credits
(Base Case Revenue = $478/dry ton-algae)

Task 6: Project Management (Helios)

This task ensured that all programmatic activities were completed in time and on budget to ensure successful completion of the overall project. Subsequent to signing of the Phase II contract with DOE, Helios-NRG executed contracts and necessary confidentiality agreements with UB. Schedules for task completion, billing and reporting were established. Meetings were held with Dr Haiqing Lin and his team at UB on a bi-weekly basis and other partners as needed. Project expenditures were tracked against progress in tasks. Progress and financial reports were submitted to DOE as required. Final project cost was $ 1,009,588 vs. a budget of $ 1,009,588.

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