DOE-EE000600; Desert Research Institute; **Algal-Based Renewable Energy for Nevada**; PI-Christian H. Fritsen- teaming members: University of Nevada Reno- Jeff Angermann; AG-Energy Corporation-Claude Sapp,

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Executive Summary

Environmental and biological fluctuations make growing of unialgal strains as a feedstock for fuels/biofuels challenging [1, 2]. During the Aquatic Species Program (ASP) that conducted a myriad of activities aimed at producing algae for oil [1], biomass recycling was an example of a method employed for species maintenance. Even when such measures were employed shifts in the dominant taxa often occurred [1, 3]. Some have henceforth concluded that for successful cultivation for fuels the best approach would be to allow a contaminant native to the area to invade the production ponds [1]. However, these native contaminants would have to be maintained/manipulated to be oleaginous for fuels production. Growing algal biomass in association with geothermal resources has the potential to mitigate negative impacts of low temperatures on biomass production systems- and thus, has the potential for maintaining production in areas where low seasonal temperatures might otherwise preclude high production. Moreover, cultivating microalgae in high temperature environments has the potential to increase intrinsic growth rates and productivity. Throughout much of the arid west/southwest geothermal resources are abundant [4] and are being further developed as a source of energy [5]. Moreover, the arid west/southwest is a location where irradiances are favorable for algal growth throughout much of the year [6]. If water downstream from these power plants- or even directly from hot springs or wells- were to be used to heat algal productions systems (either directly or indirectly), the likelihood that such systems might become viable, for use in some fashion in the algal production industry, would be expanded.

To help in the overall evaluation of the potential for growing algal biomass in high productivity systems, we conducted a study that evaluated water from geothermal sources and cultivated mixed consortia from hot springs in Nevada, we evaluated their growth at moderately high varying temperatures and then evaluated potential manipulations that could possibly increase their biomass and oleaginous production. Studies were conducted at scales ranging from the laboratory benchtop to raceways in field settings. Mixed consortia were readily grown at all scales and growth could be maintained in Nevada year round. Moderate productivities were attained even during the shoulder seasons- where temperature control was maintained by hotwater and seasonally cold temperatures when there was still plentiful solar radiation. The results enhance the prospects for economic feasibility of developing algal based industries in areas with geothermal energy or even other large alternative sources of heat that are not being used for other purposes. The public may benefit from such development as a means for economic development as well as development of industries for alternative energy and products that do not rely on fossil fuels.

Accomplishments:

The overall program consisted of a range of activities aimed at taking water and algae from geothermal sources, screening and culturing algae at different scales and reporting on approaches that were effective or posed challenges. Additional activities were initially proposed, including investigations of potential by products of algal biomass processing. Due to challenges encountered in the implementation of the field more time was devoted to some tasks than others through the process of requesting variances and some tasks were not completed as originally identified (specifically, the task of evaluating by product usage for dust suppression). Despite the challenges the overall efforts for taking consortia to larger scale production facilities at higher temperatures were realized. Reporting on these efforts were documented through quarterly reporting processes as well as in public settings (national meetings and publications). Reporting and publication efforts are still being pursued and we anticipate will continue. Accomplishments associated with the activities of the varied program elements are contained below.

Surveyed Hot Springs in the State:

Geothermal waters were sampled and characterized throughout the state. Although, more of the effort was conducted in proximity to Reno, the spatial coverage of the project included areas of eastern as well as southern Nevada (Table 1 and Figure 1). Most all of surface geothermal waters tested had characteristics of brackish alkaline waters. The pH of these waters mostly ranged between 7 to 9- the exceptions being Stillwater with a near-neutral pH of 6.86 and the Magma power plant water with an uncharacteristically high pH of 10.05 (the Magma power plant water originated from a geothermal well and was collected downstream from use in a geothermal electricity production plant). The chloride content of the waters indicated moderately freshwaters in only the Soldier Meadow, Monitor, Spencer and Diana/s punch bowel locations. All of the remaining waters had chloride concentrations spanning the range from 1,301 to 81,733 µM which are indicative of brackish waters not readily suitable for consumptive use or even sustained land-based agricultural practices.

Sulphate was the relatively more abundant anion in the geothermal waters of the eastern and southern locations sampled in the state (Figure 1). These waters had ionic compositions more similar to those reported as SERI type II waters whereas the remainder had anionic compositions more similar to SERI type I waters [1].

Table 1. Geothermal waters sampled in Nevada and locations (latitude and longitude). Average temperatures, pH and specific conductivities (spC) at time of sampling. Note: with the exception of the Magma power plant waters sampling of surface waters (i.e. hot springs) was primarily guided and directed at sampling waters where microalgae consortia were found and thus this information is not necessarily representative of source waters at the hot springs where source waters were well in mostly excess of 73^{o}C- the upper limit for photosynthesis.

Name/designation	Latitude	Longitude	Temp (^{o}C)	pH	spC (us/cm)
Hazen	39.59994	-119.111	40.09	7.41	3789
Alkali	37.82493	-117.338	--	8.61	--
Beatty	36.97696	-116.722	--	--	--
Lee	39.12302	-118.432	41.73	8.06	2870
Bonham	40.18564	-119.475	36.83	8.39	4460
Soldier Meadows	41.21234	-119.132	44.18	9.03	399
Double Hot	40.88693	-119.008	48.65	8.41	1959
Corral Hot Springs	39.2123	-118.395	53.98	7.79	--
Magma Power Plant	39.55519	-118.838	>73	10.05	NR
Steamboat Hot Springs	39.22459	-119.443	60.3	--	--
Dixie	39.79199	-118.071	51.48	8.39	1320.00
Monitor	39.07981	-116.64	41.35	7.09	627.75
Spencer	39.32722	-116.856	43.9	7.46	1339.67
Diana's Punchbowl	39.03028	-116.666	49.1	7.12	758.00
Bartine	39.55778	-116.36	35.35	7.59	627.00
Salt Wells	39.29442	-118.571	45.5	7.14	4940.00
Stillwater	39.54799	-118.557	42.20	6.86	8800.00

Table 2. Average anion and ammonium concentrations in geothermal waters at time of sampling. All values are reported in micromolar units (uM). OrthoP, Ammonium and SiOx concentrations determined via colorimetric methods (Latchat autonalysis) whereas other anions were determined via ion chromatography.

Name/designation	orthoP	NH4	SiOx	Fl	Cl	NO2	Br	NO3	SO4
Hazen	0.75	18.19	942.19	201.38	19,122.73	0.00	16.40	0.50	2,856.37
Alkali	1.25	1.23	881.15	415.86	1,301.62	0.00	0.00	3.75	4,331.72
Beatty	1.18	0.00	969.16	305.46	1,479.42	0.00	4.62	10.21	1,360.21
Lee	0.91	4.24	1,501.30	457.33	11,041.85	0.00	7.71	0.00	5,090.37
Bonham	0.95	37.61	1,242.67	131.12	22,553.13	0.00	27.06	0.00	4,652.99
Soldier Meadows	0.41	0.08	890.91	464.46	532.45	0.00	0.00	18.97	382.14
Double Hot	0.48	23.73	1,082.51	409.13	3,381.35	0.00	8.20	0.00	1,290.44
Corral Hot Springs	0.64	258.12	1,600.22	60.10	49,651.08	0.00	137.58	0.00	654.49
Magma Power Plant	1.07	142.54	1,808.63	49.90	81,773.72	0.00	221.97	11.84	1,020.42
Steamboat Hot Springs	1.14	1.81	1,670.26	83.66	23,024.70	0.00	38.59	0.00	1,696.62
Dixie	0.85	2.96	959.51	496.45	5,820.52	0.00	5.59	3.47	1,286.92
Monitor	0.35	8.44	521.36	89.10	321.70	0.00	0.00	0.00	574.00
Spencer	0.63	141.93	878.21	236.96	661.62	34.30	0.00	12.04	482.72
Diana's Punchbowl	0.49	14.39	687.05	121.67	271.49	26.96	0.00	0.00	595.53
Bartine	0.34	4.58	483.76	178.38	19,029.30	0.00	48.10	0.00	1,752.78
Salt Wells	0.63	24.23	1,000.66	294.83	33,584.16	0.00	26.75	0.00	2,823.10
Stillwater	0.51	326.50	723.05	138.93	71,573.59	0.00	111.35	0.00	1,960.79

Table 3. Average cation concentrations in geothermal waters at time of sampling. All values are reported in micromolar units (uM). Concentrations determined via ion chromatography.

Name/designation	Li	Na	K	Mg	Ca
Hazen	172.2	21,053.8	780.1	7.3	1,470.8
Alkali	238.2	15,091.6	611.1	66.7	1,471.3
Beatty	30.9	7,597.8	193.4	0.0	570.9
Lee	101.0	22,452.3	724.2	0.0	1,337.5
Bonham	24.3	35,810.8	1,110.4	12.8	1,536.1
Soldier Meadows	23.2	3,312.6	25.6	0.0	248.8
Double Hot	15.9	15,719.1	245.2	45.7	344.2
Corral Hot Springs	329.6	66,079.9	906.8	47.1	1,810.6
Magma Power Plant	398.3	62,468.5	3,765.1	0.0	2,894.5
Steamboat Hot Springs	954.0	27,826.3	1,487.0	0.0	129.7
Dixie	54.7	9,640.9	128.0	0.0	593.3
Monitor	31.1	2,097.7	330.5	107.8	1,314.6
Spencer	230.2	9,015.6	907.2	93.4	1,066.9
Diana's Punchbowl	50.5	2,610.2	388.5	125.4	1,299.8
Bartine	11.4	1,714.4	297.6	244.8	1,513.8
Salt Wells	221.0	36,662.3	1,628.4	0.0	1,026.2
Stillwater	307.2	64,561.3	2,527.5	0.0	1,916.2

Phosphate concentrations were submicromolar in most all waters and no area was readily identified as a potential major source for cultivation. Nitrogen as nitrate and nitrite was present on occasion at concentrations in the 1-10 µM range. Ammonium concentrations were variable and often present at moderately high concentrations (greater than 10-100µM). All geothermal waters had Si present at values ranging between 400-2000 µM. Although the concentrations of N- as NH_4^+ are potentially high in natural settings, the amounts needed for high-biomass production systems is 3-10 times higher. The locations where these high concentrations occurred also corresponded to locals where chloride content was similarly high. As expected, all geothermal waters appear to be a moderate source of Si that could potentially sustain some level of diatom frustule development.

The Hazen hotspring pools were sampled the most throughout the study (as its waters were used in multiple laboratory- based culturing efforts and experiments over the span of 2 years) and its chloride content varied up to 30% from the average (data not shown). Although the exact source and nature of this variability was not investigated- some of this variation was likely due to the locations of sampling as well as surface pool and local groundwater recharges that could have changed the chemistry on varying time scales. The variability of the source water as a base for media preparations is likely a factor to consider when assessing suitability for biomass production purposes in the future. From the perspective of limiting variations in basal water chemistry and gaining nutrients in the basal waters- Amending source waters that have not been exposed to surface conditions (i.e. Ground water that have not been exposed to microbial mats, sunlight and oxygen) may need further evaluation/exploration.

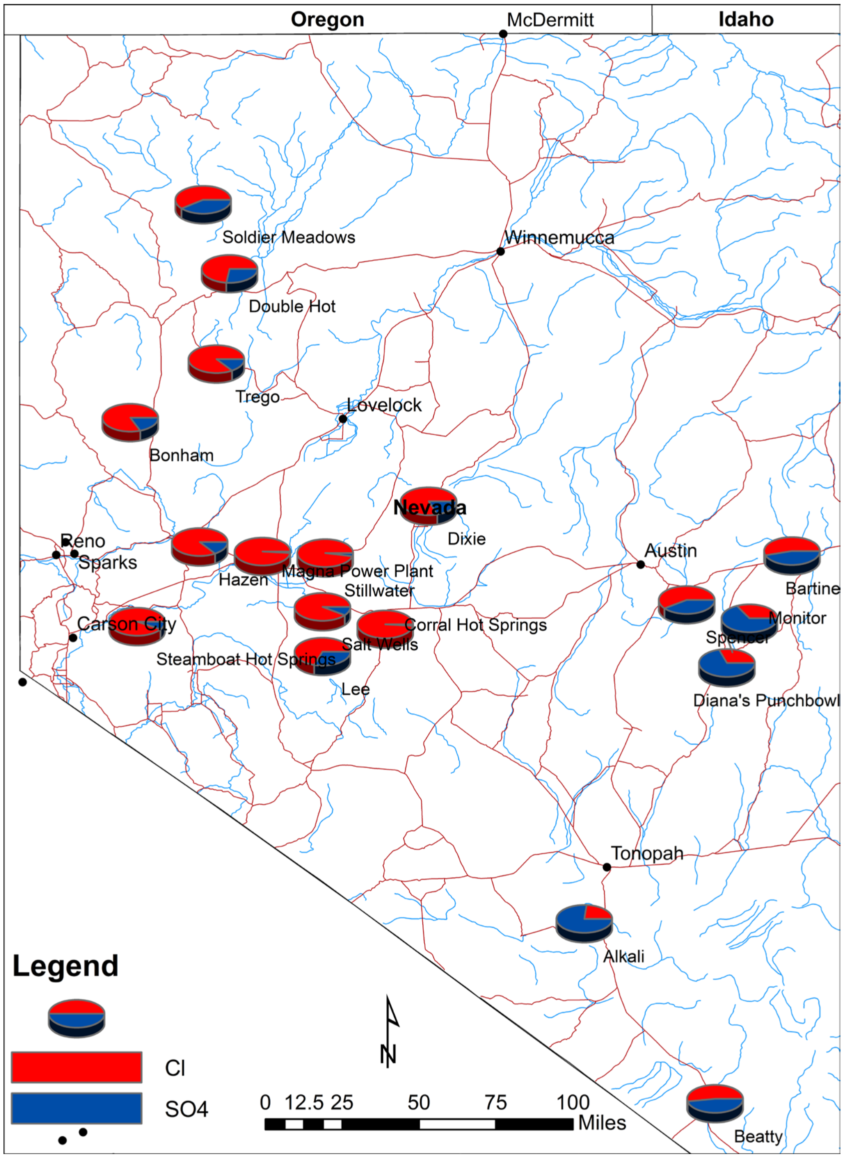

Figure 1. A map of geothermal sites visited with pie charts representing the micromolar concentrations of chloride (Cl) and sulfate (SO_4). SERI type II waters have Cl:SO_4 ratios most similar to Alkali HS, Diana's Punchbowl HS and Monitor HS. All other locations have anionic compositions more similar to SERI type I media.

Tested isolates

Screening of the taxonomic composition of algae collected in conjunction with sampling at the various hot springs was accomplished. Samples were taken from the varied geothermal waters for microscopy, identifications and potential culturing. Taxonomic identifications were accomplished using authoritative keys (e.g. freshwater Algae of North America: Ecology and Classification) [10].

Dominate taxa varied by location. For instance, Monitor hot springs samples were dominated by naviculoid diatoms, *Tribonema*-like filamentous yellow-green algae and *Spirogyra* (green filaments), but also contained a diverse collection of other potential lipid producers such as

Denticula, Epithemia, Rhopalodia and *Achnanthidium*; Spencer HS samples were dominated by *Denticula, Pseudoanabaena*-like (filamentous), *Synechococcus* (coccoid) or *Leptolyngbya*-like (filamentous), but also contained naviculoid diatoms and *Achnanthidium*; Bartine HS samples were dominated by *Synechococcus* (coccoid) cyanobacteria and *Mougeotia*-like filamentous algae, but also contained *Cymbella pusilla*, *Achnanthidium*, *Denticula* and naviculoid diatoms.

Samples of algal material from hot springs water, tufts and mats were used to inoculate media and agar plates to obtain isolates and to enrich mixed consortia cultivations. Amended-geothermal-water liquid media was made at the hot springs locations using sterile stock solution concentrates mixed with 0.2 µm filtered sample water. These enriched samples were transported to the laboratory while being maintained at the moderate to room temperature to those at high in-situ collection temperatures (35^{o}C to 45^{o}C). Agar plates were made back in the lab and streaks from waters were used in efforts to gain isolates using standard sub-culturing practices. Initial incubations were conducted within incubators at in-situ temperatures of 35-45 ^{o}C at irradiances of 200 µmol photons m^{-1} s^{-1} from daylight-white halogen bulbs.

Initial screening of resultant consortia identified several with enhanced potential for biofuel/oligneous product production. Several growing consortia from Hazen, Monitor, Spencer, Bartine, and Brady hotsprings contained a high abundance of taxa known for lipid production. These taxa included both diatoms (*Achnanthidium*, *Denticula*, *Halamphora*, *Navicula*, *Epithemia*) and filamentous green algae (*Tribonema*-like and *Spirogyra*). Several of these consortia (specifically from Hazen, Brady and Monitor) were subsequently targeted for further experimentation that ranged from bench-top manipulations in culture flasks, enclosed photobioreactors (with artificial and natural lighting) to open raceways in enclosed settings and in the field (see sections below).

Plucking and streaking colonies resulted in several isolates in liquid media that were further cultured and evaluated for growth rates at varying temperatures. This screening indicated several isolates/strains that had capacities for growth at moderate to high temperatures. Notably- isolates of Apatoccocus, Acnanthoid diatoms, Ananomies and Halamphora all grew at temperatures greater than 30^{o}C. Several Cyanobacteria genera also grew well at higher temperatures (e.g. *Synechocystis*, *Chroococus*, *Oscillatoria*, and *Leptolyngbya* sp.).

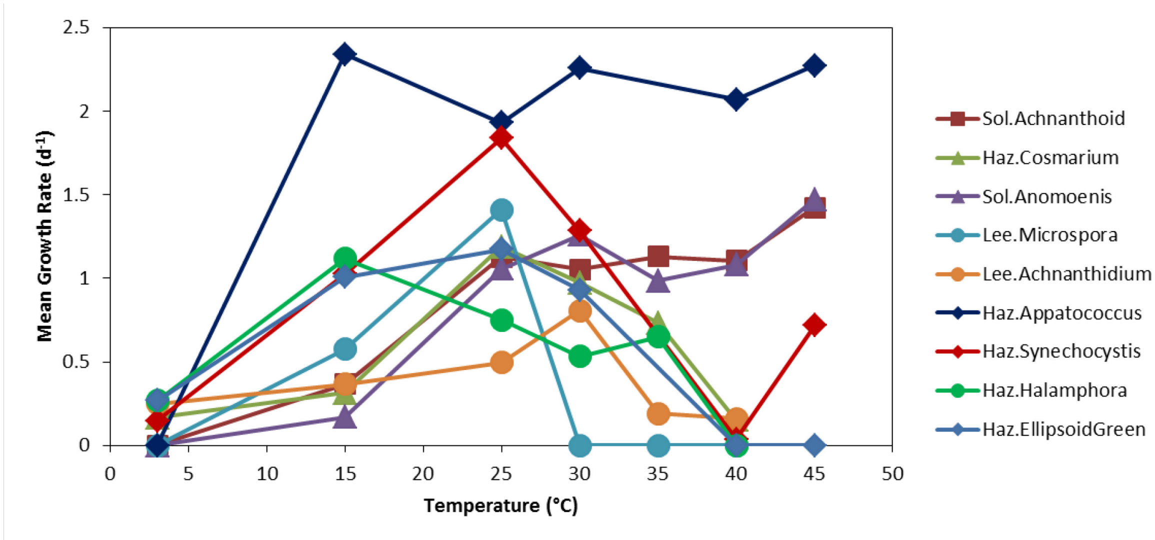

Figure 2. Growth rate vs. temperature for isolates grown in geothermal-water-based media under continuous light (~200 µE m^{-2} s^{-1}) from different geothermal waters (Sol= Soldier Meadows, Haz = Hazen, Lee= Lee Hot springs). *Provisional data. Sol

Strains and Consortia for Experimentation

Experimentation on isolates was accomplished and initially targeted culturing and manipulations of Apatoccocus and Halamphora strains in photobioreactors (Figure X). Consortia that contained a mixture of known lipid producing strains (e.g. diatoms and Apatoccocus) also were initially targeted in subsequent consortia-based experimentation (as

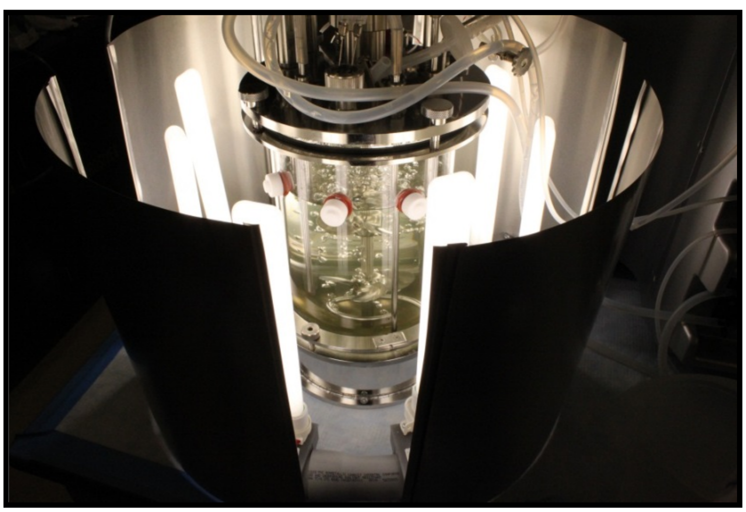

Figure 3. Photobioreactor image. Three liter glass culture vessel with agitation and gas delivery controls provided by a Bio-flo310 system. Light banks constructed with daylight white halogen bulbs produced irradiances up to 700 µmol m^{-2} s^{-1}.

described below). Although initial efforts in the study targeted those consortia and strains that would have a moderate to high lipid production capacities- rapid advances in the field influenced subsequent study aims. Specifically, during the implementation of the study, advances in the processes for gaining fuels and energy from biomass with a range of lipid contents (e.g. through Hydrothermal liquification processes; and making of biochar) led the study to not entirely discount or discontinue experimentation that had high biomass production potential- regardless of the algae's lipid content.

Efforts were undertaken to grow the isolates of Halamaphora and Appatococus in nutrient amended geothermal waters in continual/semi continual modes of cultivation in bench-top photobioreactors. Initial trials were conducted on Halamphora at 30^oC as this was a moderately higher temperature and it was shown to grow at this temperature in our previous work (e.g. see results above). Initial growth in the bioreactors was favorable with growth rates of 0.5 d^{-1} in the initial growth period and high biomass was attained- (> 3,000 µg Chla l^{-1}). During the semi-continual mode of cultivation (that had sustained growth rates varying close to 0.5 d^{-1}) the culture acquired a contaminant (Synechocystis) that grew and became the dominant photoautoroph within the system.

Care was taken in the steps of preparing the nutrient amended geothermal waters that was used to dilute the semi-continual culture. Efforts included autoclaving tubing and glassware used for media delivery and filter sterilization of media; autoclaving the media/geothermal waters was not possible due to precipitation). However, despite the operations being in a laboratory setting, the omni-present contamination issue, that accompanies efforts to grow and cultivate unialgal strains was not avoided.

In addition to contamination being a challenge in culturing *Halamphora* in the bioreactors, the formation of biofilms on surfaces in the vessel was evident (sidewalls, gas delivery tubing, probes etc.). The formation and presence of biofilms in such vessels complicated the assessments of biomass growth and accumulation and the construction of geochemical budgets.

Despite these challenges, results were encouraging in that a local geothermal diatom isolate was cultivated in the laboratory setting for over two weeks and its lipid profile during cultivation as measured. Moreover, the diatom growth could be comparted to that of the cyanobacteria dominated state. Triaceyl glycerols (TAG) and free fatty acids during semi-continual cultivation remained low- averaging 3% of the total dry weight when dominated by *Halamphora* and decreased even further when dominated by the cyanobacteria.

Additional efforts to cultivate strains in photobioreactors yielded similar results. Specifically *Apatococcus* cultivation efforts yielded high standing stocks of 4,000 to 12,000 µg Chl a l^{-1} with estimates of growth rates ranging from 0.7 to 2 per day during the initial two to three weeks. Biofilm formation in these cultivations also proved challenging in regards to measurements and sustaining the culture. Despite these challenges, cultivation and maintenance was accomplished in the photobioreactor at both 30^{o}C and 40^{o}C in excess of 30 days. TAGs and FFAs never comprised more than 2% of the dry mass during any point during these cultivations.

Culturing and maintaining mixed algal consortia also was examined. Consortia from Hazen and Monitor hot springs were initially cultivated in the native hot springs waters (with media additions), screened in 250 ml volumes and then sequentially increased to 750 ml volumes in the laboratory. These consortia were readily maintained in the laboratory setting for several months in a semi-continual mode of cultivation at temperatures of 35-40^{o}C. Growth rates were on the order of 0.3-0.5 d^{-1} in continual light (200 uE m^{-2} s^{-1}) and reduced by ~50% if maintained in a 12:12 light:dark cycle (indicative of strict light limitation). Mixed consortia/cultures maintained under continual light become predominantly unialgal over time. The Hazen cultures became a dominated by *Leptolyngbya*- a cyanobacterium. Those cultures maintained on a L:D cycle remained more diverse with by *Aphanocapsa* sp, *Synechocystis* sp., *Leptolyngbya* sp. and *Achnanthidium* sp. all comprising the consortia. Therefore, although the

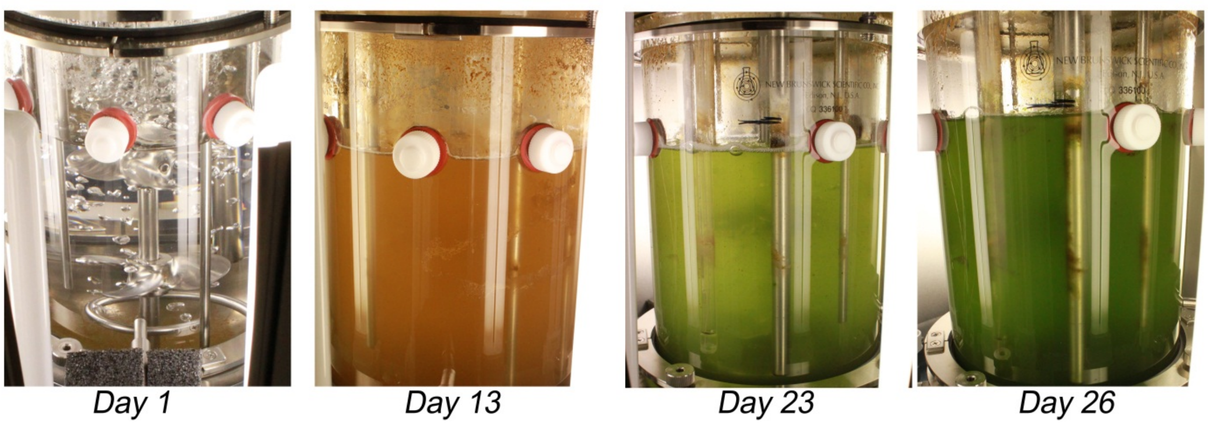

Figure 4. BF310-042711 culture on days 1, 13, 23, and 26 of the experiment. The images on day 1 shows algal material settled on the bottom of the vessel. The culture on day 13 was still dominated by *Halamphora*. Cyanobacteria, primarily *Synechocystis*, grew soon after dilutions around day 20 and their presence can be seen on days 23 and 26 as they became the dominant photoautotroph in the vessel.

light:dark cycle resulted in a lower biomass production rate. This treatment yielded a consortia that contained a range of cyanobacteria as well as a diatom species. The latter culture therefore contained more of a propensity for lipid production. This lipid production potential was documented in both assays for nile red fluorescence (an assay for neutral lipids) as well as in measures of free fatty acids (FFAs) and triaceyl glycerol profiles (TAGs).

Secondary cultivation and treatment efforts also were explored. Specifically, portions of the mixed cultures grown at the higher temperatures were transferred to separate experimental flasks and differently treated to determine if environmental conditions could be manipulated to enhance lipid production once the algal biomass was rapidly produced. Treatments examined included differential additions of bicarbonate, trace metals, nutrients and shifts to lower temperatures. Results pf these efforts illustrated potential to increase lipid content – but only to moderate values of ~8% of the total mass. Lowering temperatures and additions of bi-carbonate were the more effective means of increasing lipid content as well as changing the composition to shorter chain lengths and greater desaturation.

These combined efforts illustrated consortia growth could be accomplished for extended periods, production and growth rates could be accomplished that are comparable to those previously reported as being desirable for industry development and environmental manipulations of consortia does offer the possibility of altering biochemical compositions that could generally be favorable for energy, fuels or natural product uses.

Evaluation of growth on different sources of Nutrients:

Efforts were undertaken to evaluate potential of growing algae on spent media (water already used for culturing) and in waters modified with waste water centrate as potential sources of media for growth. Additional efforts were undertaken to evaluate the nominal amendments needed for some geothermal waters and potential cost effectiveness of additions amendments.

Experimentation with centrate additions for growing isolates were yielded results indicating growth inhibition for geothermal isolates (e.g. *Anonomeis* sp.) unless additions were to full additions of standard media. Thus, the utility of using centrate additions for the geothermal isolates did not appear to be a promising pursuit. Given this specific centrate had nitrogen present in the forms of ammonia and nitrate present in millimolar concentrations and phosphate available in excess of 100 uM, a means to utilize these nutrients seems desirable. However, the initial tests on algae from the geothermal sources that were not obviously eutrophic were not promising. Additional experiments were conducted on some isolates obtained from more eutrophic settings in Nevada and included tests with a Chlorella sp. that had moderately high temperature optima for growth (30C). Centrate additions did offer a means to stimulate this isolate's growth in the absence of other nutrients (Sun et al. in prep).

Additional work was undertaken to evaluate biomass and cost benefit of different media preparations and were reported on by Bywaters (2014). This work indicated biomass production returns were optimized and realized differently in the different in waters. In general however, waters amended with just sodium nitrate and potassium phosphate additions without the additions of other common media contents (e.g. vitamins trace metals etc) were generally more cost effective for consortia biomass production. the analyses also indicated that the media used in the original biomass program (SERI type I and Type II) were not the most effective nor the most cost effective means to approach culturing of the geothermal isolates/consortia. An important result was noted in these efforts (as well in results of monitoring water chemistries in bio reactor experiments) in that phosphorus depletions were recorded- even in the absence of substantial biomass production in several experiments. Calcium concentrations were often documented as decreasing during incubations as well. This coincidental depletion of a this particular cation may be indicative of calcium phosphate formation during certain waters and production conditions. Thus, phosphate additions and chemistries will likely need specific considerations and perhaps refined approaches for additions when culturing in the mostly alkaline geothermal waters similar to those studied.

CO2 Capture/exchange by geothermal consortia growing in geothermal fluids:

Growing algae for biomass and energy use while also being used as a means of carbon capture has been proposed on many an occasion. The overall concept is simple in principle and is reliant on the transfer of carbon dioxide to reaction centers in photosynthesis such that the carbon dioxide is converted to simple sugars in the dark reactions of photosynthesis that is fueled by energy gained in the light reactions. Despite the simple concept of carbon fixation being a potential means to capture carbon, there are many factors (physical, chemical and biological) that can release CO2 and make the net capture of CO2 into biomass in algal growing systems not as simple as the concept might imply. Among the processes that might short circuit/mitigate/ or lessen the carbon capture potential of algal culturing systems and lessen

Figure 5. Incubation vessels instrumented with gas lines for air delivery and analyses as well as water quality (e.g. dissolved oxygen, pH) sensors

their CO2 capture efficiencies are changes in CO2 solubility in systems due to factors such as changes in temperature, pH, and alkalinity. Photorespiration, microalgal respiration, bacterial respiration (community respiration), precipitation and dissolution of carbon bearing minerals also are among biogeochemical processes that influence the net C02 capture dynamics.

To measure the net CO2 capture potential of geothermal consortia growing in geothermal waters, we assessed net carbon dioxide exchange within enclosed photobioreactors. Specifically, we grew consortia from hotsprings (Hazen and Olam) in 19 Liter drinking water containers/jugs (Figure 5) modified and instrumented with air delivery and recovery systems and LiCoR infrared gas analyses systems such that differential CO2 could be used to determine the net carbon capture of the entire culturing system. In some experiments containers also were outfitted with airtight ports that allowed water quality sondes to be inserted, such that DO, pH and electrical conductivity could also be measured. A distinct advantage of utilizing this system for culturing and assessing carbon capture, as opposed to just measuring biomass change/production, is that the overall net dynamics of all the physical, chemical and biological reactions involving CO2 are integrated and the overall net exchange of CO2 is determined (regardless of the rates of light-dark reactions, bacterial respiration and inorganic carbon dissolution/ precipitation). Additionally, the desirable aspects of photo-bioreactors that have been extensively reviewed and evaluated are applicable to the system employed herein (including low evaporation losses of water, safeguards against macrograzer colonization etc..). The non-desirable aspects are also applicable (e.g. higher equipment/capital costs, scaling challenges and maintenance costs).

Geothermal consortia's carbon capturing potential were assessed as several times throughout the annual cycle. Because temperature was held constant at 35-40^{o}C and nutrients were provided to augment the geothermal waters, it was an expected result that production and algal growth would be strongly tied to solar irradiance and day length. Indeed, the strong ties to solar radiation was demonstrated in both growth rates as well as primary production. Assessments in summer, spring and fall showed algal growth rates of 0.3-0.5 per day and production values up to 25 g AFDM m^{-2} d^{-1} (Bywaters 2014). During these growth processes oxygen dynamics were documented and showed very large diurnal swings with nighttime respiration driving oxygen levels to lows of 5-6 mg l^{-1} (50-60% saturation at these higher temperatures) and photosynthesis driving levels to high over 15 mg l^{-1} (150-200% saturation). Differential carbon dynamics similarly showed diurnal variations with largest differentials being documented during the daylight hours when primary production led to capturing carbon during photosynthetic processes. The net carbon differentials of still demonstrated net carbon utilization\capturing by the entire system during the night time hours, even when net respiration processes consume oxygen and release CO_2 (Figure X). Unlike oxygen gas, the $C0_2$ gas dynamics are also tied to the carbonate buffering in the water such that the net primary production and CO_2 capturing by the overall system led to carbon capture throughout the diurnal cycle regardless of the season due to the net drawdown of CO_2 as averaged throughout the daily cycle.

This primary production and carbon capturing potential was even demonstrated at winter solstice when daylight was limited to ~9.5 hours (Figure 6; incubations were conducted in Reno, Nevada at 39.5^{o}N latitude). During winter experimentation differential carbon capture rates, net primary production, algal growth indicated net primary production at 2-4 g C m-2 d-1. Although these values are below those of the fuel industry targets for sustained economical biomass production they demonstrate the potential utility of utilizing geothermal waters and associated heat as a potential means to relieve constraints imposed by seasonally low temperature environmental conditions to sustain carbon capture or algal growth for products.

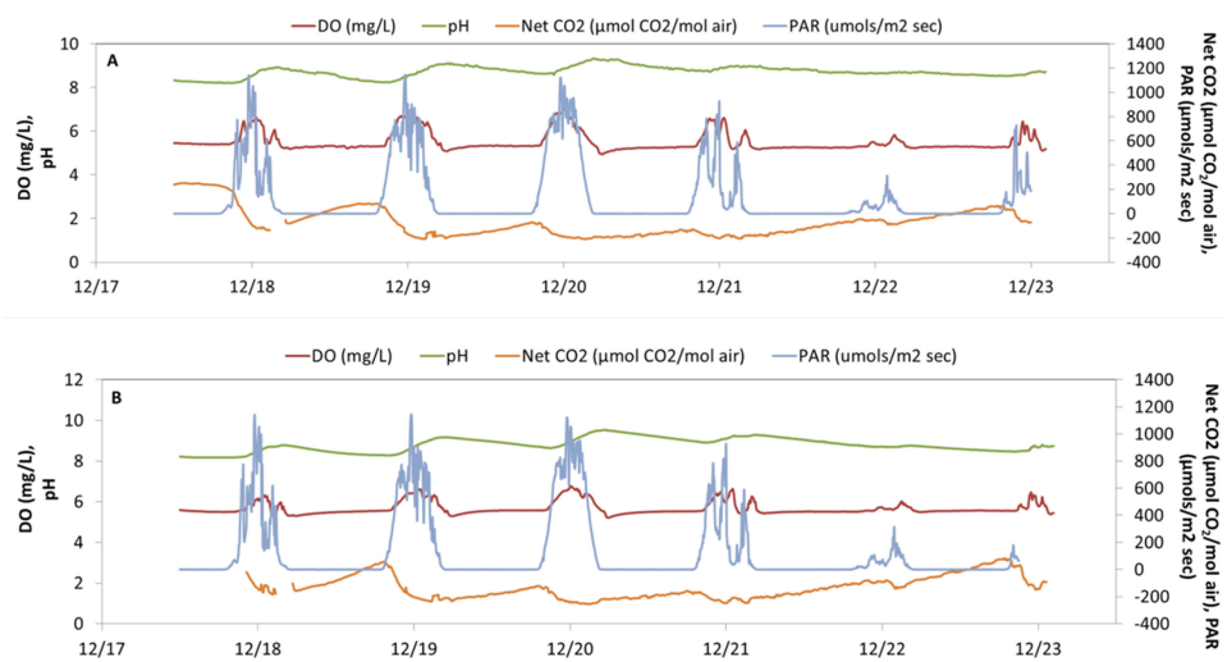

Figure 6. Time series of physical and chemical parameters measured in the photobioreactors (containing algal consortia) equipped with instrumentation for measuring the net CO_2 difference between in air delivered and air leaving the vessel, Photosynthetically Active Radiation (µmols m^{-2} sec^{-1}), Dissoved Oxygen (DO; mg L^{-1}) and pH during December. A) bioreactor 1 and B) bioreactor 2.

A mid-size race way was operated inside DRI's Fritz Went facility to evaluate geothermal algal growth potential in open race way conditions as well as during natural irradiance conditions.

Raceway/test bed

Construction and operations of the pilot-scale raceway occurred at Olam Spices and Vegetable Ingredients, Inc. near Fernley, Nevada (Figure 7). This site was a deviation from initial plans to have facilities built and tested near a waste water treatment facility that had a source of hot water. The location near the waste water treatment facility no longer was viable for the study when the facility stopped receiving hot/geothermal waters due to the large temperature fluctuations killing their microbial reactors and effecting their abilities to maintain effective water treatment.

Moving the facility to the Olam/Brady hot springs site required site purposing and evaluations of water uses and modifications such that the intended uses met all the facilities permitting purposes. The Olam site allowed access to hot water that was being discharged to a cooling pond after the water's heat was used in onion and garlic drying processes at Olam's facilities. Olam received their geothermal water downstream of a geothermal electricity production facility (Ormat) after its initial thermal uses were realized.

The construction of the open raceway followed the general design of algal production raceways- with the addition of an outer raceway that was constructed to contain circulating hot water for heating the inner culturing/algal production raceway. The construction materials consisted of an industrial temperature resistant industrial liner (lining all raceways), standard fencing supplies (for framing the thermal exchange wall) and brick for the outermost wall (of the temperature control raceway) and the inner raceway separation wall.

The raceway facility was operated using locally sourced algae and was operated over a range of conditions during spring, summer and fall periods. Challenges occurred during the operations due to the source of the geothermal water being dependent on the plant operations that often interrupted the hot water supply such that hot water at times was not available.

Figure 7. Pilot-scale raceway (with inner production raceway and outer temperature control raceway) that was built and operated at Olam Spices and Vegetable Ingredients, Inc. near Fernley, NV. The geothermal source water was contained in the cooling pond in the fenced area behind the raceway. The two images on the right show two sets of experiments/ manipulations with different microalgal consortia at different temperatures.

Despite the challenges in the sighting, building and operating the raceway facilities the project was able to conduct several tests of the capabilities and potential for cultivations of mixed geothermal algal consortia. Tests in the spring (May) and fall (October) without heating yielded a raceway with temperature fluctuations ranging between 5^{o}C and 22^{o}C with average daily temperatures being on the order of ~15^{o}C. Applying heat during the fall and spring (in the form of circulating geothermal waters in the outer raceway) allowed for increasing the inner raceway temperatures by 10-15^{o}C achieving temperatures of 25-35^{o}C when air temperatures were fluctuating between 4-20^{o}C (Bywaters 2014). Higher temperatures of 30-40^{o}C were realized when environmental temperatures were more moderate (less wind and air temperatures being ranging between the 15-20^{o}C.

Algal growth and production tests were conducted in the raceways during these times and yielded mixed consistorial cultures with high biomass (600-1000 ug Chla l^{-1}). Growth rates and biomass production were measured at 0.96-0.99 d^{-1} and 16-28 g AFDM m^{-2} d^{-1} when heating was applied in the Spring of 2013 (Bywaters 2014). Tests when the raceway was not heated yielded values for growth and biomass production that were approximately 50% of those when heated. Raceway algal consortia was dominated by the diatom *Halamphora* when temperatures were colder whereas they became dominated by a *Chlamydomonas* sp. when temperatures were artificially increased.

Challenges of open raceway operations were encountered as was expected from prior reports and experiences (Bywaters 2014). For instance invasions of invertebrates in the raceway when the pond was not heated was realized during one spring season. Grazer invasions were not observed when raceways were heated. However, evaporation losses of water were noted at all times and losses were very pronounced and increased by about 50% when heating the raceways with geothermal waters. Thus, the operations of the facilities for longer time series required a freshwater delivery system to be developed that was intended to offset the evaporative losses that led to conductivity/salinity increases that were doubling roughly every week in the absence of freshwater additions or harvesting and water replacements.

Training

Four graduate students (3 at UNR and 1 at UNLV) worked extensively on the project's activities (Clint Davis UNR, Eric Wirthlin UNR; Kathryn Bywaters UNR; and Farah Moazeni UNLV). Three used this work in their dissertations and theses (C. Davis worked on the project but his dissertation eventually was solely focused on stream ecology topics). Multiple undergraduates (Bailey Wong; Emily Ulrich; Erica Romero; and Teresa Schwedlen- all at UNR) also worked on the project and were trained in multiple aspects of algal research. Two high school students joined the efforts and used the as part of their Sr. Research Experience internships at their Charter School (Slade Glavish, Elyse Olesinski).

Content of special topics undergraduate courses were augmented with the topics from the project. Notably Teresa Schwedlen utilized temperature growth experiments for special topics course and Erica Romero utilized the thermal outdoor raceway information for a modeling effort as the basis for her senior thesis special topics course. Several graduate student courses at UNR were augmented with content from the project (Graduate Seminar Series and biogeochemical cycles in the Environmental Sciences department being among the courses effected).

Summary

In general the range of efforts demonstrated that a range of natural microalgal species can be obtained from geothermal features across the Great Basin, geothermal waters (both surface and subsurface) can be readily amended for mass culturing efforts and temperature enhanced cultivations can be realized in co-located facilities. Species control during open raceway operations was not targeted nor achieved yet demonstrated the feasibility of operations and highlighted the potential economic benefit of approaching cultivations for biomass production purposes in areas that could be co-located with sources of geothermal waters to be used for heating purposes as well as potential source waters for cultivations. A targeted oleaginous strain production process would require more control than was realized or targeted in the larger scale open raceway facilities operated during these relatively short-term efforts. However, the cultivation of *Halamphora* dominated consortia in the moderate sized raceway inside a greenhouse was effectively conducted for months at a time- thus demonstrating the potential of

This body of work, as a whole, offers insights into the possible methods and mechanisms that can be employed to make algal based production systems more economically feasible. Geothermal, microalgal, consortia production rates have been shown to be competitive with those of unialgal stains. Costs of production can be reduced by tailoring media without impacting production rates. Heating production systems with readily available geothermal water can decrease the losses in production seen in colder months. These concepts help bring algal production systems forward and closer to being a reality. However, algal production systems are capricious, changing with taxa cultured as well as culturing conditions themselves. Future work needs to be done to establish bench marks in production rates for consortia as well as being able to sustain production rates in heated raceways.

Identify products developed under the award and technology transfer activities, such as:

Publications (list journal name, volume, issue), conference papers, or other public releases of results. If not provided previously, attach or send copies of any public releases to the DOE Project Officer identified in Block 15 of the Assistance Agreement;

Bywaters, K.F., and C.H. Fritsen. 2014. Biomass and neutral lipid production in geothermal microalgal consortia. *Frontiers in Energy Research: Bioenergy and Biofuels.* doi: 10.3389/fbioe.2014.00082

Bywaters, K.F., and C.H. Fritsen. 2014. Maximizing algal growth rates using consorita from Nevada geothermal hot springs. Presentation/Poster: Algal Biomass Organization (ABO) Annual meeting- San Diego.

Bywaters, K.F., 2014 Geothermal Microalgal Consortia: bench-top to raceway scale cultivation. PhD Dissertation. University of Nevada Reno.

Bywaters, K.F., C.H. Fritsen, P. Melarkey, J. Memmott. In prep. Bench-top to raceway scale cultivation of Geothermal Microalgae consortia. *Algae Research*.

Fritsen, C.H. B. Coulombe, and K.F. Bywaters. In prep. Carbon capture by geothermal microalgal consortia in bioreactors.

Jena, U., C.H. Fritsen, J.R. Kastner, S.K. Hoelman, K.C,. Das. In prep. A Novel Concept of Two-stage Hydrothermal Processing of Algae and Aqueous co-Product (ACP) Recycling.

Sun, H.J., F. Moazeni, G. Zhang, J. Ma, S.A. Shanahan, X. Zhou, C.H. Fritsen. Submitted. New microalgae isolates demonstrate cultivation in municipal wastewater for biodiesel feasible. *J. Applied Phycol*.

Acharya, K., S. Sueki, K.F. Bywaters and C.H. Fritsen. In prep. Recycling water and nutrients for use in algal biomass cultivation.

b. Web site or other Internet sites that reflect the results of this project;

NA

c. Networks or collaborations fostered;

Potential Collaborations fostered with attendees of Algal Biomass Conferences and algal production research facilities (e.g. ATP3, Solas, Clearas, etc..).

d. Technologies/Techniques;

As disclosed and outlined in above report and cited dissertation/works.

e. Inventions/Patent Applications, licensing agreements:

NA

f. Other products, such as data or databases, physical collections, audio or video, software or netware, models, educational aid or curricula, instruments or equipment.

Geothermal water chemistry data has been deposited within the geothermal database maintained by the Nevada Bureau of Mines and Geology (http://www.nbmg.unr.edu/).

All data bases and measures associated with the activities are archived and available for future use and publication purposes. Personnel working on the project are still working to publish results in peer-reviewed journals and data archiving requirements for such journals will be adhered to. Depositing data will also be accomplished in the future as appropriate database repositories are identified.

References:

1. Sheehan, J., et al., *A look back at the U.S. Department of Energy's aquatic species program - Biodiesel from algae.* NREL/TP 580-24190, 1998.
2. Brennan, L. and P. Owende, *Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products.* Renewable and Sustainable Energy Reviews, 2010. **14**(2): p. 557-577.
3. Weissman, J.C. and J.R. Benemann, *Biomass recycling and species competition in continuous cultures.* Biotechnology and Bioengineering, 1979. **21**(4): p. 627-648.
4. Faulds, J.E., et al., *Why is Nevada in hot water? Structural controls and tectonic model of geothermal systems in the northwestern Great Basin.* Geothermal Resources Council Transactions, 2004. **28**: p. 649-654.
5. United States. Congress.Senate.Committee on, E., E. Natural Resources.Subcommittee on, and R. Mineral, *Geothermal energy development in Nevada's Great Basin: hearing before the Subcommittee on Energy and Mineral Resources of the Committee on Energy and Natural Resources, United States Senate, Ninety-eighth Congress, second session, to examine the current status and future needs of Nevada's geothermal energy industry, Sparks, NV, April 17, 1984*. Vol. 98-801. 1984, Washington U6 - ctx_ver=Z39.88-2004&ctx_enc=info%3Aofi%2Fenc%3AUTF-8&rfr_id=info:sid/summon.serialssolutions.com&rft_val_fmt=info:ofi/fmt:kev:mtx:book&rft.genre=book&rft.title=Geothermal+energy+development+in+Nevada%27s+Great+Basin&rft.date=1984-01-01&rft.pub=U.S.+G.P.O&rft.volume=98-801.&rft.externalDocID=b2581915x&paramdict=en-US U7 - eBook: U.S. G.P.O.
6. Davis, C.J., *Periphyton Ecology in Great Basin Rivers: Winter Blooms, Hyporheic Exchange Effects, and Reservoir-tailwater Productivity*, 2012, ProQuest, UMI Dissertations Publishing.
7. Liao, N., *Determination of orthophosphate in waters by flow injection analysis colorimetry.* QuikChem® Method 10-115-01-1-M, 2002: p. 17.
8. Prokopy, W.R., *Determination of ammonia by flow injection analysis.* QuikChem® Method 10-107-06-2-C, 2003: p. 10.
9. Wolters, M., *Determination of silicate in brackish or seawater by flow injection analysis.* QuikChem® Method 31-114-27-1-D, 2002: p. 12.
10. Wehr, J.D. and R.G. Sheath, *Freshwater algae of North America: Ecology and Classification*, ed. J.D. Wehr and R.G. Sheath2003, Amsterdam: Academic Press. 918.

11. Clesceri, L.S., A.E. Greenberg, and A.D. Eaton, eds. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. 1998, American Public Health Association, American Water Works Association, Water Environment Federation: Baltimore, MD.
12. Welschmeyer, N.A., *Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments.* Limnology and Oceanography, 1994. **39**(8): p. 1985-1992.
13. Samburova, V., et al., *Analysis of triacylglycerols and free fatty acids in algae using ultra-performance liquid chromatography mass spectrometry.* Journal of the American Oil Chemists' Society, 2013. **90**(1): p. 53-64.