Use of Cultivation Data from the Algae Testbed Public Private Partnership as Utilized in NREL's Algae State of Technology Assessments

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Introduction

The Algae Testbed Public Private Partnership (ATP^3) conducted algal growth experiments over the course of 16 months termed the Unified Field Studies (UFS). These experiments were conducted at five different geographic locations in Arizona (ASU), California (CP), Florida (FA), Georgia (GT), and Hawaii (CELL); see http://atp3.org/ for details on these sites. The UFS sought to evaluate different algal biomass harvesting strategies using identical ponds, media, and operational conditions through all four seasons across different geographic regions to isolate the effects on productivity attributed to locational climate and seasonal variability, overlaid by the differing harvest strategies. Set up as the baseline upon which other experiments would build, it must be emphasized that as per the stated, approved experimental goals of the ATP^3 UFS, no attempts at growth or lipid accumulation optimization were made. Rather, the primary focus of the UFS work was to cultivate algal biomass under deliberate, consistent conditions, time periods, and harvesting protocols, to provide public data on year-round outdoor biomass production that could be directly compared between one site and another (with accompanying climate data for each site). Thus the resulting cultivation productivity values in effect represent a conservative baseline of non-optimized algal growth one may expect at these sites. Additionally, weather can vary dramatically from season to season and from year to year, and even within a given "season," where an individual season's data was typically based on 4- to 6-week operating windows. Thus, the cultivation performance numbers also only reflect a short snapshot in time, and must be interpreted carefully in projecting what may be expected over many years or decades (for example, a 30-year facility lifetime as evaluated in techno-economic analysis models).

The UFS work spanned across calendar year 2014 and into summer of 2015. Over different periods across this timeframe, the test-bed sites cultivated *Nannochloropsis maritima* KA32 (saline), *Chlorella vulgaris* LRB-AZ-1201 (freshwater), and *Desmodesmus sp.* C046 (cultivated in saline media). The timeframes for the strains cultivated during the UFS are summarized in Table 1.

Table 1. Operating Days per Season for Each Strain and Site of the UFS

strain	site	2014 Spring	Summer	Fall	Winter	2015 Spring	Summer	total days per strain	total days for UFS per site
N. oceanica KA32	ASU	55	40	40	43	41		219	
	CP	54	40	41	38	40		212	
	CELL	44	36	36	41	33		189	
	FA	49	37	48	42	38		213	
	GT	52	34	38	19	35		177	
C. vulgaris LRB-AZ-1201	ASU		26	42	26	50		143	
	CP		35	38	30	56		158	
	CELL		27	29	28	0		83	
	FA		46	48	30	47		170	
	GT		17	27	32	53		128	
Desmodesmus C046	ASU						40	40	402
	CP						39	39	409
	CELL						38	38	310
	FA						40	40	422
	GT						38	38	343

The data for the complete ATP^3 UFS was deposited on the U. S. Department of Energy's (DOE) Open Energy Information (OpenEI) website (http://en.openei.org/wiki/ATP3) to facilitate open access.

To support DOE's Bioenergy Technologies Office's (BETO) efforts to quantify economic benchmarks attributed to current experimental performance, NREL conducts state of technology (SOT) assessments typically on an annual basis (when data is available), to incorporate experimentally demonstrated parameters for a given pathway into established techno-economic analysis (TEA) models. These SOT models provide a means to establish a baseline for current technical performance and resulting economics (when extrapolated out to a hypothetical "n^{th}-plant" commercial-scale facility), and thus demonstrate (a) economic improvements tied to process and/or R&D improvements moving from one year's SOT to the next; and (b) the necessary improvements that must subsequently occur in the future in order to achieve ultimate cost targets as established in published "design reports" [1, 2]. With the establishment of ATP^3, an SOT case for algal biomass production could be done for the first time in 2015, representing the first time public data was available in sufficient detail for outdoor, year-round algae cultivation (collected throughout 2014). A second SOT was subsequently completed recently in 2016 (utilizing 2015 data). For purposes of running a TEA model, the primary data parameters utilized from ATP^3 for the SOT include cultivation productivity, composition, harvest density, and water balances (i.e., local/seasonal evaporation rates and blowdown requirements), although much more data than these parameters are available from ATP^3. Primarily with respect to biomass costs, the primary TEA cost driver has been shown previously to be cultivation productivity, particularly at low productivity values below 25 g/m^2/day [1]. As such, this document focuses primarily on how productivity was calculated based on available ATP^3 data as utilized for the National Renewable Energy Laboratory's (NREL) SOT assessments and published in BETO's Multi-Year Program Plan (MYPP) reports [3]; however, additional accompanying parameters are also documented here.

Areal (Harvest Yield) Productivity Calculations

Areal productivity was calculated from the amount of harvested algal biomass that was physically removed from the ponds and would thus be available for further processing. Harvest yield productivity was calculated three ways based on a particular portion of interest within the overall growth curve. The three calculations are graphically represented in Figure 1 and are defined as:

1. **Experimental duration:** The total amount of algal biomass harvested over the entire experimental timeframe from the day of inoculation to the final day of complete pond harvest.
2. **Experimental duration – grow out:** The amount of algal biomass harvested starting with the second harvest through to the final day of complete pond harvest. This removes the initial large harvest that typically occurred following pond inoculation, as the density was usually higher than during the rest of the harvests and removes the initial grow out period, which was typically two weeks.

   Experimental duration – grow out – final harvest: The amount of harvested algal biomass only grown during the continuous portion of the experimental production run.

This removes the initial two-week grow out period and the large final harvest that occurs at the end when the entire pond is harvested. This calculation represents the continuous portion of the production run experiment, which is most comparable to typical scale-up models for large hypothetical commercial farms as evaluated through TEA, lifecycle assessment (LCA), and resource assessment (RA). This basis was thus utilized for the SOT models.

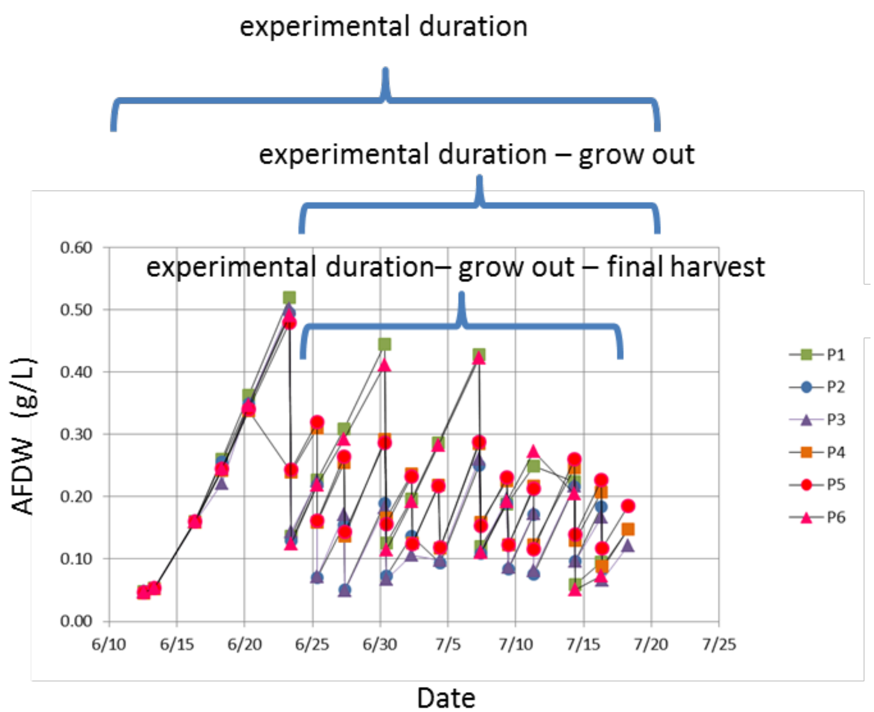
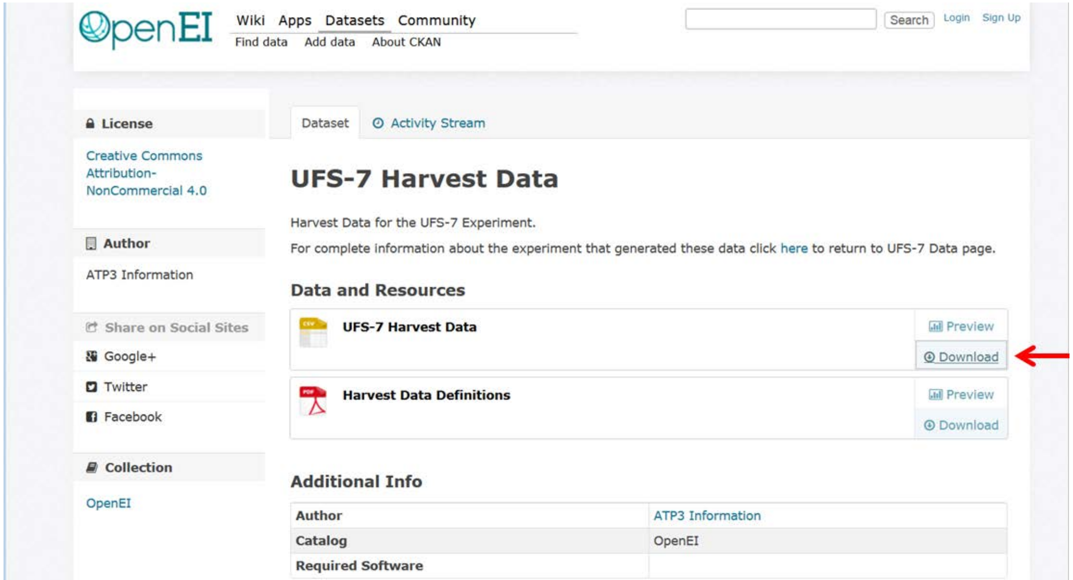


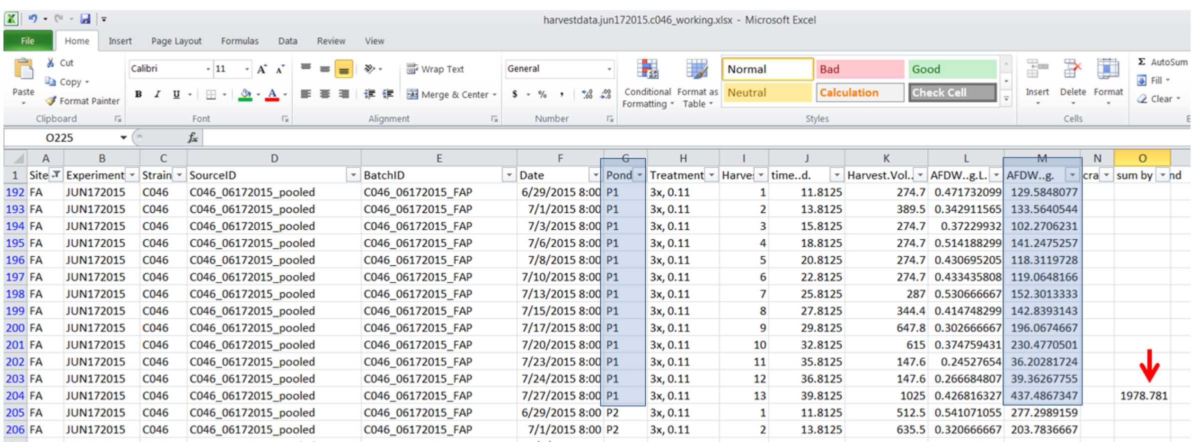
Figure 1. Example of the time periods over which harvest yield productivity was calculated for each of the three calculations

To calculate the harvest yield areal productivity, the amount (mass) of algal biomass harvested from each of the six ponds was summed, divided by 4.2 m^2 (the surface area of the ponds including the paddlewheel area), and divided by the timeframe as defined by the start and end days for each of the three calculations described above. The productivity for each pond was then averaged with the other ponds of the same harvest strategy to arrive at the productivity number for that harvest strategy for that experiment. Typically, three ponds were run using an identical harvest strategy where either the dilution rate or the harvest frequency was varied as a different strategy. Using these calculation methods, harvest yield productivity was calculated for each site for each experiment across the UFS. A step-by-step guide to performing these calculations based on an example case for the summer 2015 *Desmodesmus* run is as follows:

1. Download the appropriate Summary Harvest Data from OpenEI. The summer 2015 *Desmodesmus* run corresponds to the "UFS-7 Experiment (Jun-Jul 2015)" data file.

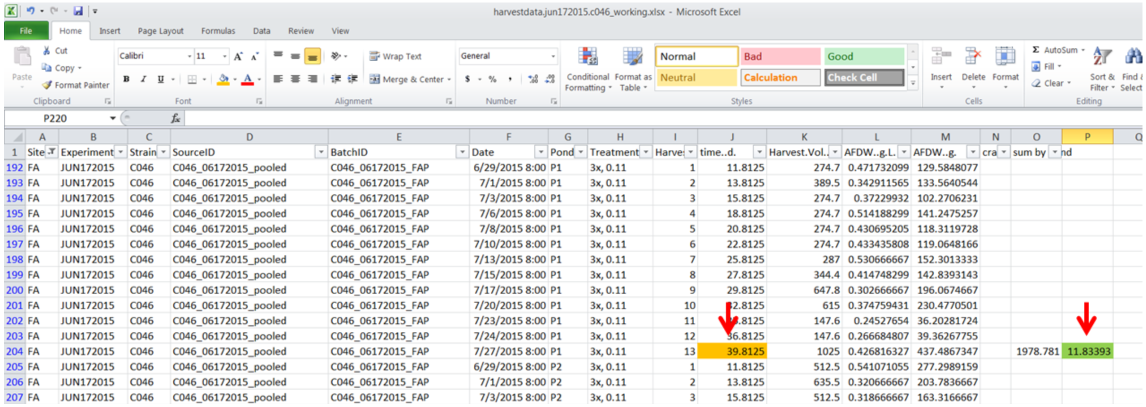


2. The first calculation will provide the average productivity for the entire experimental duration (with initial grow out and final harvest included). This example uses the Florida Algae (FA) pond 1 (P1) data for the 3x, 0.11 harvest treatment. First, sum the algae mass measurements in column "AFDW.g". The result is the total mass of algae harvested from pond 1 for the entire experimental duration.



	Site	Experiment	Strain	SourceID	BatchID	Date	Pond	Treatment	Harve	time..d.	Harvest.Vol..	AFDW..g.L.	AFDW..g.	cra	sum by
192	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	6/29/2015 8:00	P1	3x, 0.11	1	11.8125	274.7	0.471732099	129.5848077		
193	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/1/2015 8:00	P1	3x, 0.11	2	13.8125	389.5	0.342911565	133.5640544		
194	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/3/2015 8:00	P1	3x, 0.11	3	15.8125	274.7	0.37229932	102.2706231		
195	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/6/2015 8:00	P1	3x, 0.11	4	18.8125	274.7	0.514188299	141.2475257		
196	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/8/2015 8:00	P1	3x, 0.11	5	20.8125	274.7	0.430695205	118.3119728		
197	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/10/2015 8:00	P1	3x, 0.11	6	22.8125	274.7	0.433435808	119.0648166		
198	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/13/2015 8:00	P1	3x, 0.11	7	25.8125	287	0.530666667	152.3013333		
199	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/15/2015 8:00	P1	3x, 0.11	8	27.8125	344.4	0.414748299	142.8393143		
200	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/17/2015 8:00	P1	3x, 0.11	9	29.8125	647.8	0.302666667	196.0674667		
201	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/20/2015 8:00	P1	3x, 0.11	10	32.8125	615	0.374759431	230.4770501		
202	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/23/2015 8:00	P1	3x, 0.11	11	35.8125	147.6	0.24527654	36.20281724		
203	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/24/2015 8:00	P1	3x, 0.11	12	36.8125	147.6	0.266684807	39.36267755		
204	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/27/2015 8:00	P1	3x, 0.11	13	39.8125	1025	0.426816327	437.4867347		1978.781
205	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	6/29/2015 8:00	P2	3x, 0.11	1	11.8125	512.5	0.541071055	277.2989159		
206	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/1/2015 8:00	P2	3x, 0.11	2	13.8125	635.5	0.320666667	203.7836667		
207	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/3/2015 8:00	P2	3x, 0.11	3	15.8125	512.5	0.318666667	163.3166667		

3. Next, divide the algae mass sum calculated in step 2 by 4.2 (m^2 pond area), then divide by the total experimental duration. The total experimental duration is located in the cell corresponding to the row of the final harvest of the experimental run, column "time.d." The column time.d is a cumulative running total of the elapsed days of the experimental run, thus it is not necessary to sum the values in column time.d. The resulting value is the productivity in $g/m^2/day$ for the entire experimental duration.



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199	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/15/2015 8:00	P1	3x, 0.11	8	27.8125	344.4	0.414748299	142.8393143			
200	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/17/2015 8:00	P1	3x, 0.11	9	29.8125	647.8	0.302666667	196.0674667			
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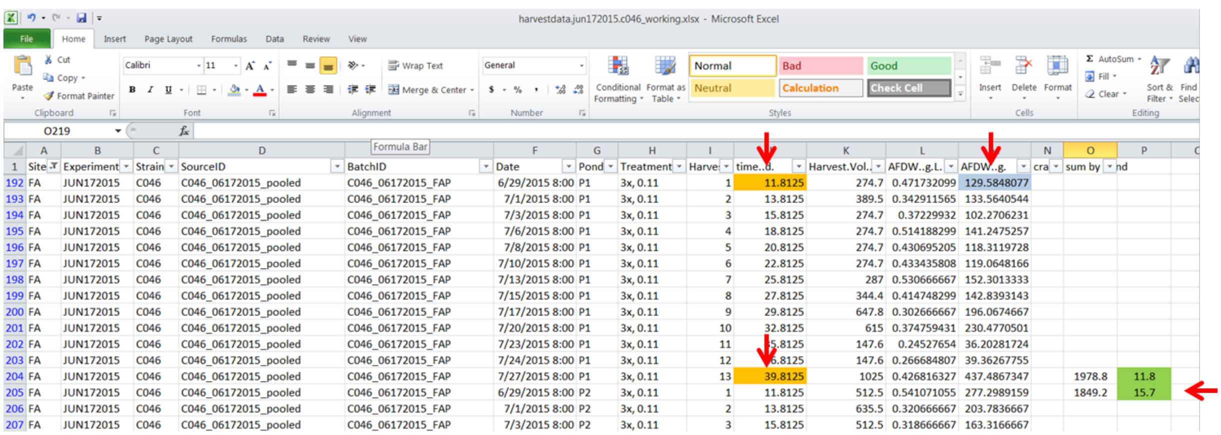
4. To calculate productivity without the initial grow out, repeat the previous calculations, excluding the first harvest. To do this, sum the algal mass measurements in column AFDW.g, excluding the mass measurement for the first harvest. Next, divide by 4.2 and the experimental duration excluding the first harvest. The experimental duration excluding the first harvest is calculated by subtracting the time required to reach the first harvest (column time.d for harvest 1) from the time required for the entire experimental duration (column time.d for the last harvest). The result is the productivity for the experimental duration minus initial grow out.



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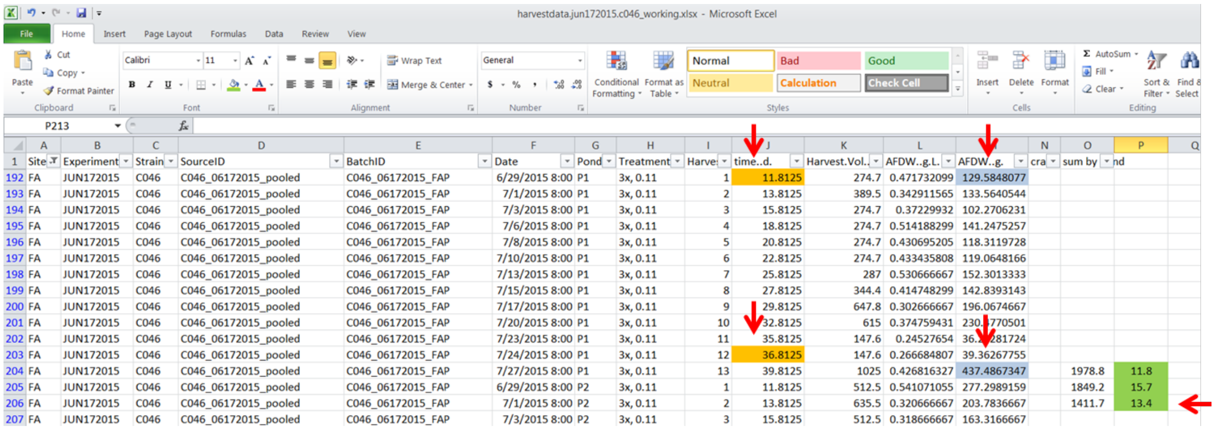

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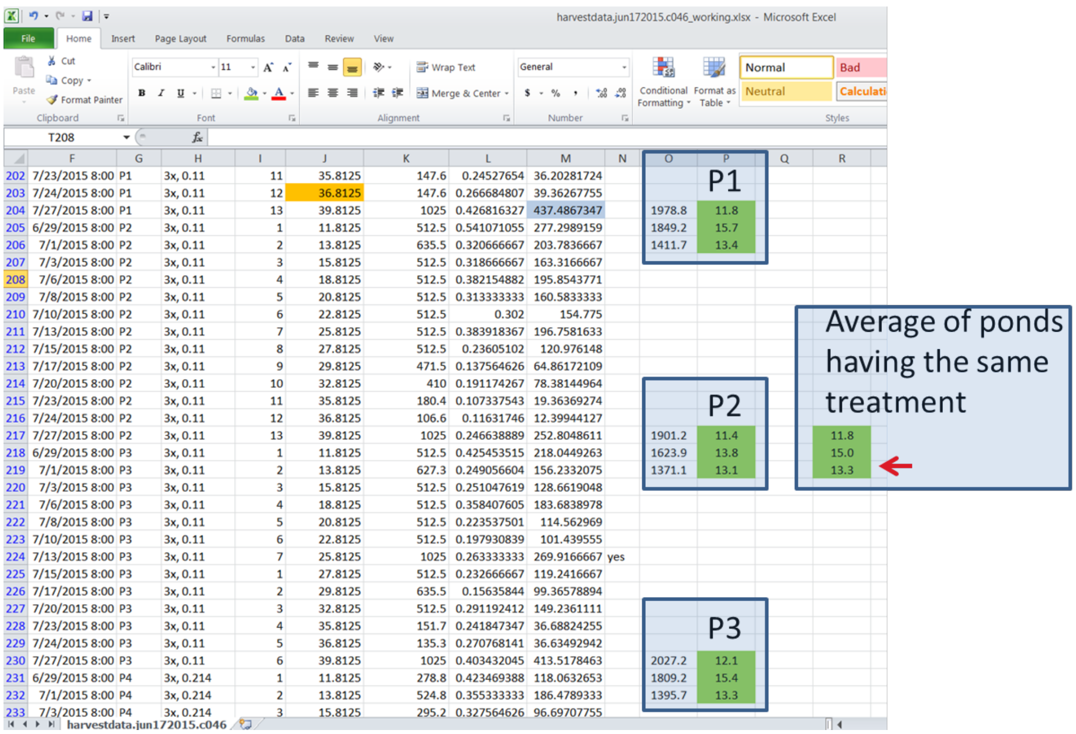
199	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/15/2015 8:00	P1	3x, 0.11	8	27.8125	344.4	0.414748299	142.8393143			
200	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/17/2015 8:00	P1	3x, 0.11	9	29.8125	647.8	0.302666667	196.0674667			
201	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/20/2015 8:00	P1	3x, 0.11	10	32.8125	615	0.374759431	230.[illegible]770501			
202	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/23/2015 8:00	P1	3x, 0.11	11	35.8125	147.6	0.24527654	36.[illegible]281724			
203	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/24/2015 8:00	P1	3x, 0.11	12	36.8125	147.6	0.266684807	39.36267755			
204	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/27/2015 8:00	P1	3x, 0.11	13	39.8125	1025	0.426816327	437.4867347		1978.8	11.8
205	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	6/29/2015 8:00	P2	3x, 0.11	1	11.8125	512.5	0.541071055	277.2989159		1849.2	15.7
206	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/1/2015 8:00	P2	3x, 0.11	2	13.8125	635.5	0.320666667	203.7836667		1411.7	13.4
207	FA	JUN172015	C046	C046_06172015_pooled	C046_06172015_FAP	7/3/2015 8:00	P2	3x, 0.11	3	15.8125	512.5	0.318666667	163.3166667			

6. Calculate these numbers for each pond. For the three ponds of the same treatment, average the productivities together. In this case, P1, P2, and P3 are the same treatment. The experimental duration – grow out – final (13.3 $g/m^2/day$ identified by the red arrow in the figure below) is the productivity used in the SOT (Table 2).



	F	G	H	I	J	K	L	M	N	O	P	Q	R
202	7/23/2015 8:00	P1	3x, 0.11	11	35.8125	147.6	0.24527654	36.20281724					
203	7/24/2015 8:00	P1	3x, 0.11	12	36.8125	147.6	0.266684807	39.36267755					
204	7/27/2015 8:00	P1	3x, 0.11	13	39.8125	1025	0.426816327	437.4867347		1978.8	11.8		
205	6/29/2015 8:00	P2	3x, 0.11	1	11.8125	512.5	0.541071055	277.2989159		1849.2	15.7		
206	7/1/2015 8:00	P2	3x, 0.11	2	13.8125	635.5	0.320666667	203.7836667		1411.7	13.4		
207	7/3/2015 8:00	P2	3x, 0.11	3	15.8125	512.5	0.318666667	163.3166667					
208	7/6/2015 8:00	P2	3x, 0.11	4	18.8125	512.5	0.382154882	195.8543771					
209	7/8/2015 8:00	P2	3x, 0.11	5	20.8125	512.5	0.313333333	160.5833333					
210	7/10/2015 8:00	P2	3x, 0.11	6	22.8125	512.5	0.302	154.775					
211	7/13/2015 8:00	P2	3x, 0.11	7	25.8125	512.5	0.383918367	196.7581633					
212	7/15/2015 8:00	P2	3x, 0.11	8	27.8125	512.5	0.23605102	120.976148					
213	7/17/2015 8:00	P2	3x, 0.11	9	29.8125	471.5	0.137564626	64.86172109					
214	7/20/2015 8:00	P2	3x, 0.11	10	32.8125	410	0.191174267	78.38144964					
215	7/23/2015 8:00	P2	3x, 0.11	11	35.8125	180.4	0.107337543	19.36369274					
216	7/24/2015 8:00	P2	3x, 0.11	12	36.8125	106.6	0.11631746	12.39944127					
217	7/27/2015 8:00	P2	3x, 0.11	13	39.8125	1025	0.246638889	252.8048611		1901.2	11.4		11.8
218	6/29/2015 8:00	P3	3x, 0.11	1	11.8125	512.5	0.425453515	218.0449263		1623.9	13.8		15.0
219	7/1/2015 8:00	P3	3x, 0.11	2	13.8125	627.3	0.249056604	156.2332075		1371.1	13.1		13.3
220	7/3/2015 8:00	P3	3x, 0.11	3	15.8125	512.5	0.251047619	128.6619048					
221	7/6/2015 8:00	P3	3x, 0.11	4	18.8125	512.5	0.358407605	183.6838978					
222	7/8/2015 8:00	P3	3x, 0.11	5	20.8125	512.5	0.223537501	114.562969					
223	7/10/2015 8:00	P3	3x, 0.11	6	22.8125	512.5	0.197930839	101.439555					
224	7/13/2015 8:00	P3	3x, 0.11	7	25.8125	1025	0.263333333	269.9166667	yes				
225	7/15/2015 8:00	P3	3x, 0.11	1	27.8125	512.5	0.232666667	119.2416667					
226	7/17/2015 8:00	P3	3x, 0.11	2	29.8125	635.5	0.15635844	99.36578894					
227	7/20/2015 8:00	P3	3x, 0.11	3	32.8125	512.5	0.291192412	149.2361111					
228	7/23/2015 8:00	P3	3x, 0.11	4	35.8125	151.7	0.241847347	36.68824255					
229	7/24/2015 8:00	P3	3x, 0.11	5	36.8125	135.3	0.270768141	36.63492942					
230	7/27/2015 8:00	P3	3x, 0.11	6	39.8125	1025	0.403432045	413.5178463		2027.2	12.1		
231	6/29/2015 8:00	P4	3x, 0.214	1	11.8125	278.8	0.423469388	118.0632653		1809.2	15.4		
232	7/1/2015 8:00	P4	3x, 0.214	2	13.8125	524.8	0.355333333	186.4789333		1395.7	13.3		
233	7/3/2015 8:00	P4	3x, 0.214	3	15.8125	295.2	0.327564626	96.69707755					

Harvest density measurements are provided in the column titled "AFDW..g.L." An average harvest density for each pond can be calculated by simply averaging the measured harvest densities for each pond, including or excluding the first or final harvests as needed.

Based on all of the available ATP[3] data, NREL's SOT assessments selected the best-performing case to establish the SOT benchmark. In both the 2015 SOT (utilizing 2014 data) and the 2016 SOT (utilizing 2015 data), the best year-round performance was observed at Florida Algae (although higher peak seasonal productivities occurred at other sites as well). This site also was relevant for NREL's TEA purposes as it falls within the general region typically within focus for

BETO harmonization modeling activities with other partners, namely Argonne National Laboratory (ANL) and Pacific Northwest National Laboratory (PNNL) (see for example [4, 5]), which have prioritized the U.S. Gulf Coast region to be optimum for high productivity and low net water consumption (with a particular emphasis in Florida). Under the FA site's cultivation data, the 2015 SOT selected productivity values based on the same algal strain and the same operational strategy across the year (Table 2), in an effort to reflect biomass costs as consistently as possible with unchanging cultivation conditions. The best performing case under this constraint was for FA *Nannochloropsis* cultivation. For the 2016 SOT, we brought in the concept of seasonal crop rotation and selected one strain for spring and winter *(Nannochloropsis)* and a different strain for summer and fall *(Desmodesmus)* based on their different performances during these seasons (Table 2), without necessarily constraining selections to maintain the same consistent dilution rates or harvest frequencies (recognizing that, in fact, for optimal production rates, such parameters likely *should* be varied to seasonal optima).

Given the results shown in Table 2, there was some validation that the FA site showed repeatability in cases where the same strain was run in the same season over multiple years (namely *Nannochloropsis* cultivated in spring and fall, in both cases showing close agreement between productivities observed in 2014 and 2015), which in turn validates that the increased productivities observed for *Desmodesmus* during summer and fall 2015 appear to be meaningful results. To capitalize on the higher productivities observed for *Desmodesmus*, the 2016 SOT included those seasonal performance values for the available cases. Because the *Desmodesmus* data are only available for summer and fall 2015, we cannot conclude whether this strain only performs better during those seasons (i.e. strain rotation with *Nannochloropsis* as assumed in the SOT) or whether in fact it would perform better than *Nannochloropsis* over the full year given the opportunity to be cultivated in colder seasons (winter and spring).

Table 2. Cultivation Productivity (AFDW), Harvest Density (AFDW), and Daily Evaporation Rate for Selected 2014 and 2015 Cultivation Trials at ATP3's Florida Algae Site as Input to the SOT

	Productivity, $g/m^2/day$	Harvest density, g/L	Evaporation rate[a], cm/day	Algae strain	Harvests per week	Harvest volume, fraction of pond	Daily dilution rate, fraction of pond
2015 SOT							
Spring 2014	11.4	0.36	0.14	*Nanno*	1x	0.75	0.11
Summer 2014	10.9	0.25	0.02	*Nanno*	1x	0.75	0.11
Fall 2014	6.8	0.22	0.01	*Nanno*	1x	0.75	0.11
Winter 2014	5.0	0.23	0.01	*Nanno*	1x	0.75	0.11
Average	**8.5**	**0.27**	**0.04**				
2016 SOT							
Spring 2015	11.1	0.28	0.14	*Nanno*	3x	0.25	0.11
Summer 2015	13.3	0.32	0.02	*Desmo*	3x	0.50	0.11
Fall 2015[b]	7.0	0.20	0.01	*Desmo*	3x	0.50	0.214
Fall 2015[c]	*6.7*	*0.17*	*0.01*	*Nanno*	*3x*	*0.50*	*0.214*
Winter 2014[d]	5.0	0.23	0.01	*Nanno*	1x	0.75	0.11
Average	**9.1**	**0.26**	**0.04**				

[a] Evaporation rate estimates from 2015 SOT were maintained for the 2016 SOT.
[b] Fall 2015 data from Advanced Field Studies; not yet available on OpenEI outside of UFS data.
[c] Fall 2015 *Nanno* case was not selected for the 2016 SOT, but is shown here to demonstrate repeatability from fall 2014 *Nanno* case and to highlight better results demonstrated with *Desmo* (the basis selected for the 2016 SOT).
[d] No new winter data is available, therefore winter data from the 2015 SOT (winter 2014) is used for the 2016 SOT.

As shown in Table 2, the calculated seasonal productivities for the 2015 SOT (2014 data) were 11.4, 10.9, 6.8, and 5.0 $g/m^2/day$ respectively, translating to an annual average productivity of 8.5 $g/m^2/day$ (ash-free dry weight [AFDW] basis). These values were input to the 2015 SOT model including considerations for seasonal variability (not based on a single year-average case). Corresponding harvest densities were on average 0.27 g/L (AFDW), which represent roughly half of the targeted harvest densities as documented in NREL's 2016 algae farm design case of 0.5 g/L [1]; this translates to increased dewatering costs, namely for the primary dewatering step, to accommodate the increased throughputs at lower densities. The pond evaporation rates were measured in 2014, averaging 0.04 cm/day over the course of the year (the majority of evaporation taking place during the spring); in some cases, net evaporation was calculated to be negative, implying more precipitation than evaporation over the given trial period, in which case a marginal 0.01 cm/day rate was conservatively assigned.

The 2016 SOT productivities improved by roughly 7% relative to the 2015 SOT basis, largely due to the switch to strain rotation with *Desmodesmus* as well as relaxing the stringency on consistent harvest frequencies/dilution rates (the most notable improvement was during the summer, resulting in roughly 22% higher productivity with *Desmodesmus* than with *Nannochloropsis* previously). The resulting 2015 seasonal productivity rates were 11.1, 13.3, 7.0, and 5.0 $g/m^2/day$, translating to an annual average of 9.1 $g/m^2/day$. As noted in Table 2, ATP^3 did not run new cultivation experiments in winter 2015, thus the prior winter 2014 basis was maintained for that case. The measured harvest densities were nearly identical to 2014, at 0.26 g/L average over the year. 2015 pond depth measurements frequently indicated net evaporation rates below measurement limits for most of the year, which may have been due to poor granularity in measurement ability for this parameter; thus, the same water evaporation estimates were maintained as 2014.

Algae Compositional Analysis

Compositional analysis of algal biomass produced as part of the ATP^3 UFS experiments described above was carried out according to procedures established and implemented at different testbed sites after initial harmonization of the procedures. One biomass sample, *Nannochloropsis* sp., was selected as a reference material because of its availability in large quantities of freeze-dried biomass. A total of 200 g of homogenized, freeze-dried biomass was distributed to each of five sites and stored at -20 ºC. This material was included with each set of analyses and serves as a check to ascertain whether the analytical procedures were performed correctly and the data falls within previously determined acceptability limits.

The compositional analysis procedures are available as open access procedures online (www.nrel.gov/bioenergy/microalgae-analysis.html). In brief, samples (1–4 L) for biochemical analysis during the production runs were collected from the ponds within 60 minutes after sunrise and concentrated to a pellet by centrifugation, freeze dried, and stored until the conclusion of the experiment and compositional analysis could begin. The biomass samples were split into experimental sets of 15–20 samples and each set included triplicates of the quality control (QC) biomass. All data were collected in a standardized spreadsheet, distributed, optimized, and continuously updated during the round robin experiments. Unique identifiers were included to link the biochemical data to the production data.

The methods applied include the following: moisture (either under vacuum at 40 ºC or not under vacuum at 60 ºC) and ash content, through combustion at 575 ºC, after which the residue is weighed and included as the basis of normalization to an ash-free dry weight basis. Lipids are quantified via an *in situ* transesterification procedure, which has been validated to provide a robust measure of the fuel fraction (fatty acid) portion of the lipids in the biomass. Carbohydrates are measured as monosaccharides after an inorganic acid hydrolysis and subsequent derivatization of the monomeric sugars. The derivatization with methyl-benzo-thiazolinone-hydrazone provides a means to detect the monosaccharide content by spectrophotometry and is not interfered with by some of the more prominent contaminants present in the hydrolyzed liquors. Protein was derived from elemental nitrogen (N) composition and multiplied by a factor (4.78) based on literature and in-house validation of nitrogen-to-protein conversion. In addition to the chemical compositional analysis procedures outlined here, routine CHN elemental composition was carried out and for a subset of the samples, phosphorus was determined to validate the applicability of the assumed constant ratios of CHNP composition. For samples where no C or P data were available from a primary measurement, these values were estimated from an assumed ratio. All data were collected in a summative analysis spreadsheet, where each harvest sample was analyzed in duplicate, alongside a triplicate analysis of the QC *Nannochloropsis* material. If a significant deviation from the consensus composition of the QC material was observed for any of the measurements, the entire analysis set was repeated, for samples where enough biomass material was available. If no material was available and the dataset was suspect because of the lack of adequate performance of the QC measurement, the respective data was eliminated and not included in the overall reported averages.

The compositional data corresponding with the productivity data shown in Table 2 for the ATP^3 cultivation experiments (as were utilized for the SOT) are presented in Tables 3–5. The composition data are averaged over the respective season and include CNP data for

Nannochloropsis KA32 and *Desmodesmus* (estimated C concentration based on measured N). The data were averaged by pond over the experimental duration with the exclusion of the first and last two harvest points, to avoid interference with the grow out batch culture composition and to remove impact of contamination on the harvested biomass composition. Because no significant difference between the different ponds was observed, ultimately, the data was summarized to a single set reflecting the seasonal composition. The mass balance of the sum of the primary measurements accounted to ~70% of the biomass. This is an indication that we are missing components and is an inherent challenge with the methods currently employed. The methods were chosen to represent an unambiguous determination of biomass composition, rather than a comprehensive description of the component closure. In order to close the mass balance, additional measurements are needed, but not always possible within the framework of the rapid and routine fingerprinting of the (often small amounts of) biomass across such a large quantity of samples. For the measured dataset described here, the components were differentially adjusted to meet 100% mass balance closure. This is not an ideal approach, but in the context of meeting the needs for TEA modeling based on the data at hand, we made the following adjustments: all components, protein, carbohydrate, and lipids were adjusted with carbohydrate and lipid content subjected to a proportionally larger adjustment (1.5-fold) than protein (25 %), given our current understanding that a large portion of the unaccounted for fraction are lipids and carbohydrates. In addition to the adjustments, 4% of the cell mass was allocated to components not typically measured, such as nucleic acids, pigments, algaenan, and other complex cell constituents. This was carried through the calculation and the normalization and is shown in the mass closure adjusted Table 4.

The elemental composition data indicates that the elemental composition does not change dramatically between seasons (Table 5). The elemental composition data sets are not complete, because routinely N composition is measured as part of the workflow, but C, H, and P are not routinely included. On average, we have observed a higher C:N and lower N:P ratio in *Desmodesmus* (C:N = 6.8 and N:P = 7.4) than in *Nannochloropsis* (C:N = 5.9 and N:P = 8.3), but again, this would need to be more carefully integrated over the productivity and different testbed locations. The proximate composition of *Nannochloropsis* and *Desmodesmus* is quite different, with the majority of the differences attributed to the lower ash and protein content and the higher carbohydrate content in *Desmodesmus* relative to *Nannochloropsis*. The lipid content does not seem to be significantly different on average for the summarized data over the seasons represented here. It is worth noting that relative to the 2015 SOT case (2014 cultivation data), which relied on exclusive use of *Nannochloropsis*, the composition of the *Desmodesmus* strain used in the summer and fall 2015 ATP[3] cultivation trials begins to match more closely with the assumed composition of high-carbohydrate *Scenedesmus* as projected in NREL's 2016 algae farm design case, namely 27% lipids (as fatty acid methyl esters [FAME]), 48% fermentable carbohydrates, 13% protein, and 2% ash [1].

Table 3. Summary of Component Compositions as Measured for ATP^3 Biomass Associated with Seasonally Cultivated Strain Cases Shown in Table 2. The data is shown as the mean ± standard deviation and includes the number of points across the production season that were averaged (*N*).

	Algae strain	Ash (wt%)	Protein (wt%)	FAME lipid (wt%)	Total carbohydrates (wt%)	Mass Balance (%)	*N*
2015 SOT							
Spring 2014	*Nanno*	20.3 ± 1.3	30.7 ± 0.8	9.6 ± 0.7	9.5 ± 0.7	70.1	6
Summer 2014	*Nanno*	17.5 ± 5.7	31.5 ± 3.2	6.9 ± 2.2	12 ± 2.4	67.9	6
Fall 2014	*Nanno*	20 ± 7.4	32.1 ± 3.2	7.5 ± 1.7	6.4 ± 1.2	66.1	19
Winter 2014	*Nanno*	15.8 ± 1.9	34.8 ± 2.2	11.2 ± 1.5	7.3 ± 1.1	69.1	18
2016 SOT							
Spring 2015	*Nanno*	18 ± 4.8	29.9 ± 6.2	11.3 ± 4.2	10.5 ± 2.4	69.8	47
Summer 2015	*Desmo*	19.4 ± 4.2	28.5 ± 3.9	5.8 ± 1.3	16.4 ± 4.9	70.1	72
Fall 2015	*Desmo*	19.6 ± 3.1	30.7 ± 3.9	7.8 ± 1.5	9.9 ± 3.5	68.0	37
Winter 2014[a]	*Nanno*	15.8 ± 1.9	34.8 ± 2.2	11.2 ± 1.5	7.3 ± 1.1	69.1	18

[a] No new winter data is available, therefore winter data from the 2015 SOT (winter 2014) is used for the 2016 SOT.

Table 4. Summary of Component Compositions for ATP^3 Biomass Associated with Seasonally Cultivated Strain Cases Shown in Table 2. All data proportionally adjusted to account for missing components (as described in the text) and normalized to 100%. A small proportion of the cell biomass was included to account for nucleic acids, pigments, and other components not typically measured.

	Algae strain	Ash (wt%)	Protein (wt%)	FAME lipid (wt%)	Total carbohydrates (wt%)	Cell mass (wt%)	Mass Balance (%)
2015 SOT							
Spring 2014	*Nanno*	22.2	42.1	15.7	15.6	4.4	100.0
Summer 2014	*Nanno*	19.6	44.1	11.6	20.2	4.5	100.0
Fall 2014	*Nanno*	23.5	47.1	13.3	11.4	4.7	100.0
Winter 2014	*Nanno*	17.4	47.8	18.4	12.0	4.4	100.0
2016 SOT							
Spring 2015	*Nanno*	19.6	40.6	18.4	17.1	4.3	100.0
Summer 2015	*Desmo*	21.1	38.6	9.4	26.7	4.3	100.0
Fall 2015	*Desmo*	22.1	43.3	13.3	16.8	4.5	100.0
Winter 2014[a]	*Nanno*	17.4	47.8	18.4	12.0	4.4	100.0

[a] No new winter data is available, therefore winter data from the 2015 SOT (winter 2014) is used for the 2016 SOT.

Table 5. Summary of Elemental Compositions (wt% AFDW) for ATP3 Measured Biomass Associated with Seasonally Cultivated Strain Cases Shown in Table 2. N was measured, with C estimated based on N; O, S, P were estimated based on the elemental Redfield ratio, which was confirmed on a subset of samples. Elemental data are incomplete across all four seasonal cases but generally do not exhibit notable variations between one season and another.

	Algae strain	C	H	O	N	S	P
2015 SOT							
Spring 2014	*Nanno*	49.3%	7.8%	33.2%	8.1%	0.6%	1.0%
Winter 2014	*Nanno*	49.5%	7.7%	32.9%	8.4%	0.6%	1.0%
2016 SOT							
Summer 2015	*Desmo*	49.7%	7.7%	33.2%	7.4%	0.6%	1.0%
Fall 2015	*Desmo*	49.7%	7.8%	33.2%	7.4%	0.6%	1.0%

Summary

The ATP^3 consortium has produced a large amount of algae cultivation data over the past two years (far in excess of the specific cases presented here as were utilized for NREL's SOT modeling) and is available publicly at http://en.openei.org/wiki/ATP3. While those data for the UFS trials were not based on efforts to optimize cultivation productivity or compositional quality (thus do not indicate the "best possible" values for such parameters), they represent an important utility to provide transparent, comprehensive datasets as required for modeling (e.g. TEA, LCA, RA, and predictive growth modeling) and general understanding for initial performance benchmarks, which had not previously been available at this level of detail or timeframe. The scope of this document is focused on providing additional details than are currently documented in the public domain [3] behind the cultivation/harvesting methods, data, and use of that data as incorporated into SOT benchmark TEA models; primarily with respect to translating the available raw data into averaged productivities and harvest densities, as well as the corresponding harvested biomass compositions. The intended scope is *not* focused on the TEA models themselves (details of which may be found in NREL's 2016 algae farm design report [1] or the TEA results of the SOT cases (details of which may be found in BETO's MYPP reports [3])). Moving forward, ATP^3 plans to publish results of newer work conducted under the Advanced Field Studies, which shifts the focus from consistent cultivation practices and strains across all test-bed sites toward affording each site more flexibility in evaluating different options and opportunities to improve upon benchmark UFS performance.

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