High Frequency Algal Pond Harvest Demonstration: A Proof-of-Principal in Culture Viability under Applied Algal Farm Management and Operations with Methodology Comparisons in the Evaluation and Prediction of Crop Productivity and Yield

Center of Excellence for Hazardous Materials Management (CEHMM)
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Abstract

A 25,000 gallon (94,625 liter) capacity open raceway pond (demonstration pond), maintained at 12,000 gallons (45,420 liters) of algal culture, and a 300 gallon (1,135.25 liter) test pond (circular tank) at the CEHMM algae pond site south of Artesia, New Mexico were subjected to an intensive harvest over a two-week period to assess impact on culture viability and product yield. Fifteen hundred gallons or one-eighth the content of the raceway pond was harvested each day for four days followed by three days of rest. Sampling for ash-free dry weight (AFDW), from which algal concentration (g/l) was calculated, occurred daily. The harvest regimen was repeated during a second week with three measurements of AFDW taken daily. Productivity of an algae growth system in open ponds is commonly assessed by areal yield rather than volumetric yield. CEHMM has adopted an agricultural metric in pounds of product per acre-foot of water per time. Therefore, product density was computed by a conversion of pounds per gallon or grams per liter (g/l) to grams per meter squared (g/m^2) per unit of time. To provide a metric consistent with areal yield, a depth of 12" was used to provide the deficient third dimension to the standard.

Maximum pond productivity during the demonstration was 39.6 g/m^2 (25 April 2011) with 29 g/m^2 minimum (29 April 2011). The average daily pond productivity during the demonstration was 33.08 g/m^2. Daily average for cellular recruitment or growth rate required to reinstate harvested inventory for the entire two week demonstration period, including rest days, was 20 g/m^2 based on pond morphology and culture dynamics. This aligns with recruitment rates based on the acre-foot model averages. Crop projections based on current economic models using resident inventory in concert with growth rates provide evidence that a sustainable inventory in support of economic viability is reasonable (>100 acre-foot of culture).

The pond was fed with a nutrient mix twice a week and makeup water consisted of the supernatant produced during flocculation of the harvested sample along with freshwater to maintain proper salinity. The culture tolerated the high rate of harvest and showed no degradation in viability or productivity.

The circular tank, which was 10 inches deep and 93 inches wide with a holding capacity of 300 gallons or 1,136 liters, had $1/8^{th}$ of its volume or precisely 37.5 gallons (141.94 liters) removed each day for five days, followed by two days of rest, and a repeat of the harvest protocol the second week. A mix of 37.5 gallons of fresh and brine water was added to make up the harvested amount and achieve the proper chemistry and water level. The circular tank was fed every day during harvest. Maximum productivity during the test was 31.51 g/m^2, with a minimum of 26.17 g/m^2. Average productivity for the week was 28.7 g/m^2. No significant change in AFDW was observed.

Although the circular tank was only a fraction of the size of the raceway, CEHMM was able to manage the demonstration more precisely with exact quantities of harvest and make up water. CEHMM collated and validated our data from the larger raceway demonstration. Moreover, many "industry leaders" have indicated that 1,000 liters provides data indicative of "commercial' operations (Oilgae, June 07, 2011).

Introduction

The Center of Excellence for Hazardous Materials Management (CEHMM) has operated an outdoor algal pond facility south of Artesia, New Mexico since June 2006. The current facility has five ponds. Two of these are configured to accommodate culture capacities of 25,000 gallons and three designed with maximum capacities of 100,000 gallons (378,500 liters) each. Actual pond volume is currently maintained at approximately half of the full pond capacity.

CEHMM has repeatedly demonstrated proficiency in algal cultivation and crop management. Culture densities have intentionally been driven as high as 1.2 g/l or 46.6 g/m^2 (e.g., 29 March 2011, 13 June 2011). Pond carrying capacities, however, will not maintain this resident density for more than 24 hours before ponds begin responding negatively (e.g, chlorosis) to overcapacity. Protocols require immediate and aggressive harvesting when densities reach >1g/l (39.5 g/m^2).

This exercise was a demonstration, a proof-of-principle that an intense growth and harvest of an outdoor raceway pond would produce sufficient product to support an algae-based commercial scale operation and the demonstration pond would remain viable. For the purpose of this demonstration, a 25,000 gallon capacity pond with 12,000 gallons of active culture in residence was chosen. This pond has a surface footprint of approximately 1/8th acre. CEHMM routinely harvests these ponds year round with resultant biomass used as feedstock for an experimental oil extraction unit designed and built by SRS, Inc. of Dexter, Michigan.

Objective

The purpose of this demonstration project was to assess the resilience and productivity of a pond which was subjected to intense harvesting techniques over a predetermined period in late April and early May 2011.

Methods

A 25,000 gallon capacity demonstration pond, a harvest control pond of identical size and a 300 gallon circular fiberglass holding tank were used to conduct this demonstration. The harvest control pond, which is the other CEHMM 25,000 gallon capacity pond, served as a control. Ash-free dry weights were measured from grab samples collected from this pond. There was no harvest conducted on this pond during the demonstration period (Table 2). The CEHMM ponds are mixed using paddles and air (not CO_2) sparged into the culture twenty-four hours a day, seven days a week.

As shown in Figure 1, the demonstration pond was maintained at about a 12" depth (approximately 12,000 gallons

Figure 1. Twenty-five thousand gallon capacity raceway pond used during the demonstration. A 100,000 gallon capacity raceway can be seen in the background.

of culture). One eighth of the content of the pond was harvested each day for four days followed by three days of rest. The harvested material was pumped into two cone-bottom tanks where thirty-minute mixing period was required after the addition of the flocculant, as shown in Figures 2 through 4.

Figure 2. Cone-bottom tank into which pond culture was harvested.

Figure 3. Addition of flocculation agent.

Figure 4. Flocculation drops algae out of suspension to the bottom and leaves supernatant at the top.

Make-up water consisted of supernatant recovered from harvested material together with fresh water to reestablish pond chemical parameters and maintain consistent water level.

Figure 5. Supernatant returned to source pond.

Figure 6. Fresh water from a ground water source added to attain proper salinity.

CEHMM pond water volumes are monitored and managed by way of dedicated four inch diameter, inline McPropeller® flow meters. Permanent flow meters are calibrated to manufacturers' and local water conservancy district specifications.

Ash-free dry weight (AFDW) concentrations were measured from each harvest sample. AFDW was determined using prescriptive methodology. Personnel who supervise the collection of pond samples are trained and certified in accordance with federal (e.g., EPA, DOE) and state (e.g., NM Safe Drinking Water Act) edicts which provide prescriptive, procedural sampling protocols thus ensuring objectivity and true representative sample selection. Aliquots of 500 ml were collected at predetermined time intervals from the pond. Samples were collected in triplicate and subsequently weighed in increments of 5 ml each. These were then recorded as initial sample weight (g). Each triplicate is filtered through a laboratory grade, sterile Whatman® 47 mm glass microfiber filter and placed into a VWR® 1300 series drying oven for 1 hour at 105^{o}C. Samples were then weighed and recorded as before ash dry weight (BADW). After recording BADW, samples were placed in a Thermolyne® F4800 ashing furnace for 1 hour at 500^{o}C. Upon removal from the furnace, samples were weighed for a final time as after ash dry weight (AADW).

Algal concentration data, measured in grams per liter, was a calculation based on AFDW and sample weights.

Productivity, expressed in grams per meter squared per unit of time, was derived directly from algal concentration (g/l). Daily lipid percentage was determined using a modified Bligh and Dyer solvent extraction method.

The circular tank, which is 10 inches deep and 93 inches wide with a holding capacity of 300 gallons, had 37.5 gallons removed each day for five days, followed by two days of rest, and a repeat of the harvest protocol the second week. A final removal of 37.5 gallons and water replacement took place after the second two-day rest period. A mix of 37.5 gallons of fresh and brine water was added to make up the harvested amount and restore to a proper level. The circular tank was fed daily during harvest. No significant change in AFDW or algae concentration was observed. Figure 7 shows the shape and size of the circular tank.

Figure 7. Circular tank.

The demonstration pond was subjected to the following over a two week period:

The first four days the following protocol was used: A grab sample was taken to assess AFDW from which algal concentration and productivity data were calculated. Freshwater was then added to restore chemical parameters and desired water level. Fifteen hundred gallons were then removed from the pond and pumped to cone bottom tanks. Flocculation agents were added to the harvest and the algae settled out over a four hour period. Subsequently the supernatant was pumped back to the source pond. The pond was fed twice a week.

The second four days a modified protocol was used: The first day a grab sample from which AFDW was determined was taken immediately and then fresh water added to restore the pond to proper chemical parameters and water level. A thirty minute interval allowed the culture to fully mix. Two additional samples were taken, one immediately after harvest and one at the end of the day. Sampling for AFDW occurred with the same pattern the remainder of the week. The first day was the only one with a fresh water adjustment before harvest. The penultimate samples for this demonstration were taken on the day after the second three day rest (May 9, 2011). Make up water consisted of the supernatant from the harvested flocculant and well water to ensure culture capacity and proper level. The pond was fed twice during the week. Concentrated algae produced from the harvest was not relevant to this demonstration and was stored for use as a feedstock for algae oil extraction.

Meteorological data were collected with a Dynamet® weather station, equipped with a 2 m station with model DNX® 1000 datalogger, automatic power up EPROM PC400® software and sensors that included a pyranometer, and devices that will measure wind speed and direction, relative humidity, a tipping bucket rain gauge, and air and soil temperature. Optional devices included a sunshine pyranometer with a heater for direct and diffuse radiation and sunshine duration. The Dynamet® weather station was hardwired to a computer on which control software is located. Hourly measurements were recorded on the datalogger and these data downloaded into an Excel spreadsheet authored by CEHMM personnel.

Discussion

Spring and summer are optimal times for outdoor algal cultures because of an increase in temperature and photoperiod in southeastern New Mexico. Weather during the demonstration included high winds (>50 mph gusts) which contributed to evaporative loss, and several nights of atypical low temperatures. This exercise was designed to demonstrate the capability and productivity of CEHMM technology as a potential paradigm for commercial scale harvest of microalgae.

Productivity has been reported as grams per liter (g/l) per unit of time or grams per meter squared (g/m^2) per unit of time. CEHMM prefers grams per liter over time because this measurement reflects change in a volume or inventory of culture while grams per meter squared over time is a two-dimensional measurement with no reference to water depth. Nevertheless, based on requests from some industry professionals, for the sake of this report, CEHMM has chosen to adapt our pond morphology to an acre-foot model with derived values in grams/meter squared.

Results

An acre-foot of culture is a foot of water in an acre surface area. The graphic example below (Figure 8) illustrates an eighth of an acre-foot (orange) within a surface area: volume ratio of 1:1.

Figure 8. The green three-dimensional rectangle is a representation of an acre-foot; the orange volume is 1/8th of the acre-foot.

In order to calculate productivity at commercial scale, it is necessary to consider the following calculations extrapolated out to one acre-foot or 325,846 gallons of culture growing at one gram per liter.

Demonstration Pond (1/8th acre)

1 g/l = .00834540 pounds per gallon
1 acre-foot = 325,846 gallons
.00834540 lb./gal * 325846 gallons = 2719.32 lb.
2719.32 pounds*.125 = 340 lb.
1 pound = 454 grams

Therefore:

340 lb. * 454 g/lb. = 154,360 grams
1 acre = 4,047 square meters

Therefore:

$154360 \text{ g} \div 4047 \text{ m}^2 = 38.14 \text{ g/m}^2$

Tables 1 and 2 summarize productivity results for the demonstration pond and harvest control pond, respectively. These data, based on standard deviations and a 95% confidence interval, support the contention that half of CEHMM algal cultures can be harvested weekly for at least two successive weeks with no significant difference from the harvest control pond from which nothing was harvested for the two week period. The gallons of supernatant returned to the pond plus fresh water do not equal the 1,500 gallons harvested each day. This difference is a function of evaporative loss restoration to desired chemical parameters and reestablishment of initial pond water level. The grams per liter data were developed from the first grab samples in the morning (a.m.). Data validation and verification were performed on sampling protocols as part of programmatic quality assurance and quality control.

An algorithm was written to convert grams per liter data to grams per meter squared using the logic described above for both the demonstration pond and the circular tank. The charts show one standard deviation error bar for each data point.

Table 1. Demonstration Pond Harvest and Productivity Data

Date	Harvest (gal)	Added Supernatant (gal)	H_2O Replacement (gal)	a.m. g/l	g/m^2
4/25/2011	1500	1050	3600	1.0200	38.8948
4/26/2011	1500	1100	300	0.8200	31.2684
4/27/2011	1500	1250	2400	0.9067	34.5745
4/28/2011	1500	1250	1500	0.7733	29.4876
4/29/2011				0.7467	28.4733
Three day rest					
5/2/2011	1500	1200	2000	0.9133	34.8261
5/3/2011	1500	1400	800	0.8467	32.2865
5/4/2011	1500	1200	800	0.8067	30.7612
5/5/2011	1500	1200	800	0.8400	32.0310
5/6/2011				0.8000	30.5057
Three day rest					
5/9/2011				0.8744	34.3190
			Average Std. Dev. 95% Confidence Level	0.8900 0.9193 0.0637	32.4935 02.8282 01.6713

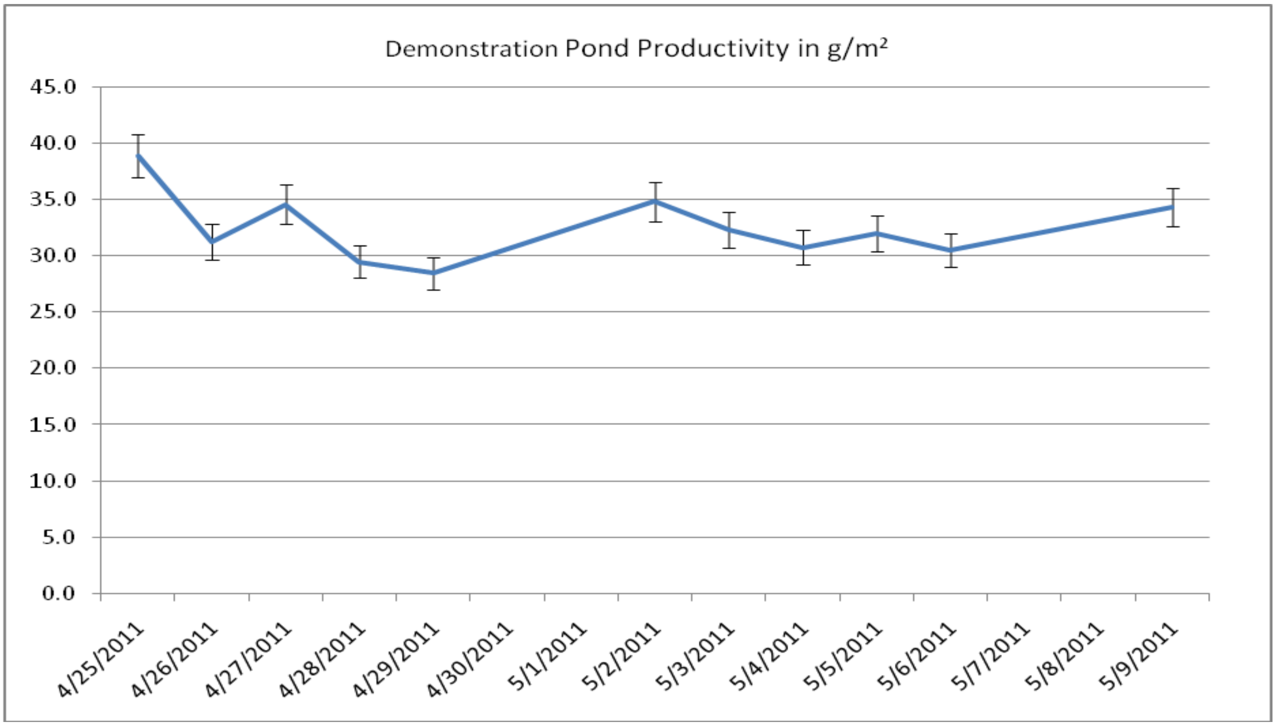

Chart 1. Demonstration Pond Productivity

An unpaired Student t-test between grams per meter squared for the Demonstration Pond and Harvest Control Pond resulted in a t value of 1.55 with a mean difference between samples of

1.99. The probability of this result, assuming the null hypothesis, is 0.138. By conventional criteria, the difference between the data sets was not statistically significant at a 95% confidence interval with nineteen degrees of freedom.

Table 2. Harvest Control Productivity

Date	a.m. grams per liter	g/m^2
4/25/2011	0.8333	31.7756
4/26/2011	0.7267	27.7107
4/27/2011	0.9133	34.8261
4/28/2011	0.8000	30.5057
Three day rest		
5/2/2011	0.6667	25.4227
5/3/2011	0.7667	29.2359
5/4/2011	0.8000	30.5057
5/5/2011	0.7933	30.2503
5/6/2011	0.8000	30.5057
Three day rest		
5/9/2011	0.9067	34.574
Average Std. Dev. 95% Confidence Level	0.8700 0.0183 0.0119	33.1750 0.6997 0.4571

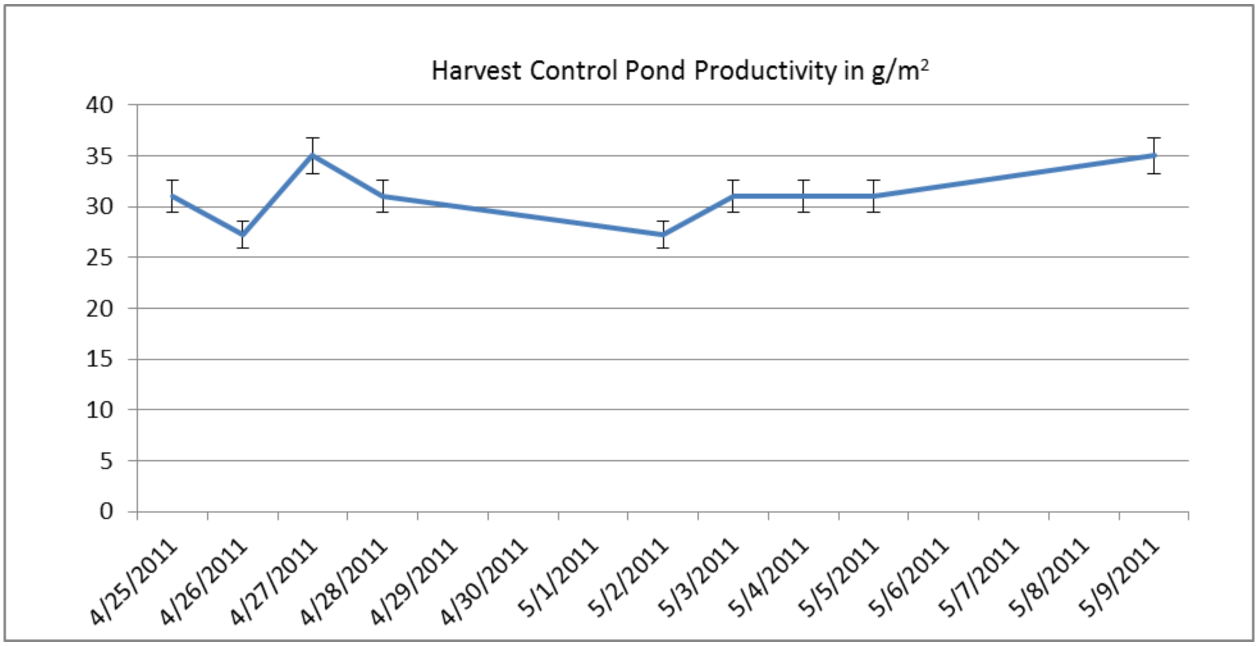

Chart 2. Harvest Control Pond Productivity

Circular Fiberglass Tank

The 8' tank had a diameter of 93 inches. The radius was 3.875 feet. The depth was 10 inches.

Therefore:

Area of the tank = $3.875^2 * \pi$ = 47.1730 sq. ft. which is equivalent to 4.3825 m^2

Volume removed = 37.5 gallons

Therefore:

37.5 gal * 3.785 (metric conversion) = 141.9521 liters harvested every 24 hours.

0.8 g/l * 141.9521 liters = 113.56 grams harvested

113.55 g ÷ 4.3825 m^2 (surface area of tank) = 26.2570 g/m^2

0.7 g/l * 141.9521 liters = 99.3664 grams harvested

99.3664 g ÷ 4.3825 m^2 = 22.6735 g/m^2

To convert to an acre-foot model at a 12 inch depth, multiply g/m^2 by 1.2.

Hence,

for 0.8 g/l, 26.2570 g/m^2 * 1.2 = 31.5084 g/m^2

for 0.7 g/l, 22.6735 g/m^2 * 1.2 = 27.2082 g/m^2

Table 3. Circular Tank Harvest and Productivity

Date	Harvest (gal)	H_20 Replacement (gal)	a.m. g/l	g/m^2
4/25/2011	37.5	37.5	0.7867	30.5780
4/26/2011	37.5	37.5	0.8000	31.0950
4/27/2011	37.5	37.5	0.7800	31.5084
4/28/2011	37.5	37.5	0.7867	30.3176
4/29/2011	37.5	37.5	0.7200	27.9855
Two day rest				
5/2/2011	37.5	37.5	0.6933	26.9474
5/3/2011	37.5	37.5	0.7333	28.5024
5/4/2011	37.5	37.5	0.6867	26.6912
5/5/2011	37.5	37.5	0.6933	29.9477
5/6/2011	37.5	37.5	0.6733	26.1703
Two day rest				
5/9/2011	37.5	37.5	0.7667	29.80071
		Average	0.7382	28.6922
		Std. Dev.	0.0445	1.7514
		95% Confidence Level	0.0254	1.0350

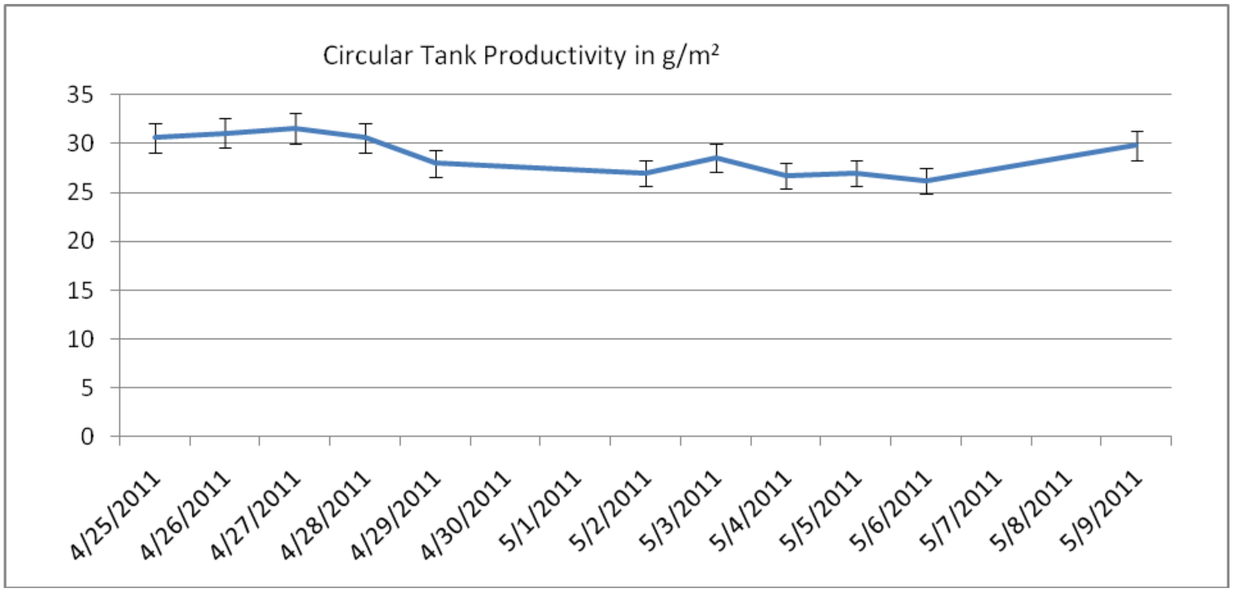

Chart 3. Productivity for the circular tank

Weather conditions during the demonstration are found in Table 4. Data were collected at 9:00 a.m. local time. High winds exacerbated evaporative loss during the demonstration period.

Table 4. Weather Data During Demonstration

Date	Air Temp (C) Avg.	Rel. Humidity (%) Avg.	Solar Rad. Avg. KW/sq. m Avg.	Wind Direction degrees	Wind Speed mph	Rain Gauge mm Total	Soil Temp (C) Avg.	Tot. Rad. W/sq. m	Diffuse Rad. W/sq. m	0 = no sun 1 = sun
4/25/11	21.38	19.21	0.628	308.4	39.57	0	20.58	670.4	324.7	1.005
4/26/11	23.59	13.05	0.643	264.8	33.68	0	20.10	684.8	252.0	1.006
4/27/11	15.75	8.97	0.664	317.8	31.96	0	17.82	721.0	260.5	1.005
4/28/11	15.66	35.25	0.638	157.4	39.21	0	17.14	672.5	246.0	1.005
4/29/11	18.69	31.22	0.545	144.2	40.00	0	18.59	588.7	199.3	1.006
4/30/11	21.85	12.44	0.549	1.725	19.73	0	19.72	590.2	212.4	1.005
5/1/11	12.65	27.44	0.462	10.48	17.28	0	18.84	460.2	412.0	0.183
5/2/11	8.78	52.10	0.605	335.9	00.02	0	14.40	640.9	315.9	1.003
5/3/11	10.99	27.61	0.568	85.8	52.30	0	13.44	586.4	208.9	1.006
5/4/11	13.47	21.44	0.596	129.2	11.06	0	14.86	617.7	244.3	1.006
5/5/11	15.84	32.80	0.667	349.5	26.73	0	17.75	689.8	266.1	1.005
5/6/11	18.35	26.27	0.468	163.8	24.05	0	18.08	523.2	233.4	1.005
5/9/11	27.37	6.45	0.331	208.3	24.58	0	21.32	543.5	273.2	1.006

Conclusions and Comments

A pond which held 12,000 gallons of algal culture was intensely harvested with half the content removed in each of two consecutive weeks. There was no significant degradation of the culture based on productivity, measured daily in either grams/liter or grams per meter squared. A test pond was also subjected to intense harvest. No significant impact on the culture was observed over the two-week period using the same evaluation criteria.

This result suggests that open raceway algal ponds can withstand high levels of seasonal harvests in southeastern New Mexico. This would imply that intense harvest rates could be applied to a large number of algal ponds in support of a commercial operation.

Results from this project graphically demonstrate that the combined elements of CEHMM crop management and crop protection provide sustainable cultures commensurate with commercial dimensions. CEHMM conclusively demonstrated that the CEHMM crop management system is capable of meeting or exceeding this performance criterion.

CEHMM maintains, however, to calculate productivity using two dimensional criteria is neither accurate nor meaningful in the conduct of operations of a commercial algal facility. The metric of grams per meter squared is an antiquated density determination with indeterminate genesis and contradicts common sense understanding of what the term productivity actually implies. In summary, current industry and academic trends calculate productivity based on the resident inventory as a "snapshot in time" rather than as a calculation for the reproductive rates of a crop required to compensate for inventory loss or as a prediction for crop production yields.

Agronomy can be defined as the science and technology of cultivating a photosynthetic organism such as plants in the production and use of food, fuel, and feed. Furthermore, agronomy is recognized as the **applied application** of disciplines such as biology, chemistry, engineering, ecology, and genetics. These definitions conclusively demonstrate that the path to commercialization in algae is strictly, by definition, FARMING. Current industry trends should be redirected to commercial viability as agricultural productivity which can be determined by either tons or bushels of product per acre. CEHMM has proven repeatedly that algal crop yields, based on tons per acre-foot of water, in addition to pond recovery can be assessed and predicted. Moreover, CEHMM has repeatedly demonstrated proficiency in the construction, maintenance, and operation of commercial scale production ponds and related infrastructure.

Challenges before the industry remain the consistency of the product quality, quantity and sustainability. CEHMM has developed, implemented, and demonstrated effective crop protection and crop management at commercial scale that provides for a consistent domesticated wild strain monoculture with predictable oil content and intrinsic values for food, fuel and feed markets.