Demonstrated large-scale production of marine microalgae for fuels and feed

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ABSTRACT

We present the results from sustained tonne-quantity production of two novel strains of marine microalgae, the diatom *Staurosira* and the chlorophyte *Desmodesmus*, cultivated in a hybrid system of 25-m³ photobioreactors and 400-m² open ponds at a large-scale demonstration facility, and then apply those results to evaluate the performance of a 100-ha Base Case commercial facility assuming it were built today. Nitrogen fertilization of 2-d batch cultures in open ponds led to the greatest yields - from both species - of ~75 MT ha⁻¹ yr⁻¹ biomass, and ~30 MT ha⁻¹ yr⁻¹ lipid, which are unprecedented in large scale open pond systems. The process described here uses only seawater, discharges no nitrogen or phosphorus in any form, and consumes CO₂ at 78% efficiency. We estimate the capital cost of a 111-ha Base Case facility at \$67 million in Hawaii, where actual production was performed, and \$59 million on the Gulf Coast of Texas. We find that large-diameter, large-volume PBRs are an economical means to maintain a continuous supply of consistent inoculum for very short-period batch cultures in open ponds, and thus avoid biological system crashes that otherwise arise in longer-term pond cultures. We recommend certain improvements in cultivation methods that could realistically lead to yields of 100 MT ha⁻¹ yr⁻¹ biomass and >50,000 L ha⁻¹ yr⁻¹ algal oil. Comprehensive techno-economics and life cycle assessment of 20 end-to-end production lineups, based on the cultivation results in this paper, are presented in a companion paper by Beal et al [1].

KEYWORDS: Algae; Biofuel; Animal feed; Cultivation; Nitrogen; Economics

1 Introduction

2 The potential for fuel and feed production from microalgae has been recognized for decades.
3 However, despite significant progress, reliable and cost-effective production of lipid- and protein-rich
4 algal biomass have not been demonstrated at scales $>10\text{ m}^2$. Productivity and cost remain the two
5 fundamental barriers to commercialization.

6 Prior evaluations of the economic and environmental viability of algal biofuels rely on one of the
7 following methods to estimate biomass and lipid productivity: (1) assumed values, (2) modeled values
8 based on climatology, or (3) measured values based on long-term outdoor production in a scalable system
9 [1]. Studies that use assumed values for biomass and lipid productivity project yields ranging from 50 to
10 $>100\text{ MT ha}^{-1}\text{ yr}^{-1}$ biomass and from 5 to $>50\text{ MT ha}^{-1}\text{ yr}^{-1}$ lipid, whether in open ponds [2-17] or PBRs
11 [7, 14], clearly revealing the wide uncertainties of this approach. Modeling studies may incorporate site-
12 specific climatology, but they predict a similar wide range of biomass and lipid yields from both ponds
13 and PBRs [18-23]. To date the few reports of directly measured yields from open ponds at a scale $>10\text{ m}^2$
14 are an order of magnitude lower than assumed or modeled yields [10, 12, 24-26]. By comparison, small
15 diameter PBRs can actually produce sustained yields at assumed or modeled rates, but their high cost [27,
16 28] has led to the conclusion that PBRs are too costly to be employed for biofuel production [4, 6, 22].
17 Furthermore, several recent economic analyses or life cycle assessments presume that lipid yields can be
18 significantly increased by reducing available nitrogen, but this has not been well demonstrated.

19 The prime goals of this study are to (1) report results from dozens of demonstration-scale
20 experiments that evaluate the critical cultivation conditions affecting biomass and lipid yield and then (2)
21 extend those results to inform techno-economic and life cycle assessments of microalgae cultivation and
22 harvesting. We analyze the results of realized, sustained production of two strains of marine microalgae,
23 the diatom *Staurosira* sp. and the chlorophyte *Desmodesmus* sp., cultivated in a hybrid PBR-pond system
24 [29] at the 0.5-ha demonstration scale Kona Demonstration Facility (KDF) in Kona, Hawaii. These strains
25 were selected from a comprehensive screening study of >500 novel isolates, and the tons of biomass

harvested from both species were processed for fuel and feed trials. Further, we apply the KDF results to design a 100-hectare “Base Case” facility were it to be built today.

In addition to the direct quantification of the yields of biomass and lipid at large scale, this study tests at a commercially relevant scale some of the prevailing notions in the field of algal biofuel production, namely that (1) less nitrogen (N) yields more lipid [9, 15, 30], (2) that realized lipid yields from conventional open ponds are too low for economical production [6, 31, 32], and (3) that the cost of PBRs is too high [7, 20, 33]. First, we quantify the effect of N fertilization on productivity, lipid yield, and biomass composition in long-term, large-scale demonstration trials. Second, we compare lipid yields from open ponds in a hybrid system to those from conventional open ponds. Third, we evaluate the overall cost of the hybrid cultivation system, including the actual large-volume PBRs used in this study, and compare those to the cost of a conventional open pond system occupying the same area.

The functional unit of this study is 1 hectare of cultivation area, which facilitates comparison with more conventional biomass production systems based on terrestrial plants. The cultivation and harvesting process analyzed here delivers the biomass of marine microalgae as a viscous slurry, 20% total suspended solids (TSS), amenable to further separation into 2 co-products: (1) a “biocrude” oil fraction, and (2) a residual protein-rich fraction, each suitable as a feedstock for refined fuels and animal feeds, respectively. The biomass production process chain at KDF consists of a hybrid cultivation system of PBRs and open raceway ponds, a harvesting system of natural settling and centrifugation, followed by drying and hexane extraction. The Base Case cultivation process is based directly on yields from the sustained KDF production experiments, while the processing chain includes natural settling and a filter press, followed by Valicor biomass conversion technology [12], which latter processes were demonstrated in smaller scale trials. Comprehensive techno-economics and LCA for the entire production process, incorporating the results of this paper, are provided by Beal et al. [1]. This paper addresses the techno-economic feasibility of the cultivation process in particular, based on measurements of long-term, sustained production at demonstration scale, thus providing a firm foundation from which to advance the

understanding of large-scale production and narrowing the gap between validated results and optimistic models and assumptions.

1. Materials and Methods

1.1. Strain selection

The purpose of strain selection is to rapidly identify algae that are highly productive under large-scale production conditions. Species characteristics of primary value for cultivation and harvesting are rapid growth, and high yields of both lipid and protein in batch culture using a low-cost source of nitrogen, which also has low environmental impact in its manufacture. The ideal strain is both temperature- and salt-tolerant; upon nutrient exhaustion it is negatively buoyant, enhancing gravitational separations in the harvesting process. The ash content is low, as it has little value.

For sustained large scale production runs at KDF we selected two strains, the diatom *Staurosira* sp. and the Chlorophyte *Desmodesmus* sp., by screening hundreds, as follows. We selected strains from the local marine environment throughout the Hawaiian Islands. In less than a year, >500 novel strains were isolated from local waters and pre-screened in 30-ml test tube cultures for temperature tolerance (20°, 25°, and 30°C) and high growth rate, estimated from daily measurements of *in vivo* fluorescence [34].

The High-Throughput Screening (HTS) program evaluated 171 pre-screened strains in duplicate trials at three separate laboratories (JJ Cullen, Dalhousie University; DR Redalje, The University of Southern Mississippi; Z Johnson, Duke University). In the first stage of HTS, strains were first acclimated for >10 generations to 25°, 30°, and 35°C, then grown in triplicate under both nutrient-replete and nutrient-starved conditions in 50-ml cultures for two weeks, and each measured daily as shown in Table 1; a fluorescence-based sinking index was measured at the end of each experiment. Strain performance was ranked using a combination of 17 directly measured and derived variables to identify strains for further study. The second stage of HTS investigated the impact of nitrogen source, offered as either NH₄ or NO₃, and lipid biochemistry was characterized in more detail. The top 24 strains were grown at 30°C under

Table 1 Measured variables at each screening level: Pre-Screening (PS), High-Throughput (HTS), Mid-Scale (MSS), and Large-Scale Production (LSP), showing continuous measurements (⊙), the number of daily measurements in all cultures (①,②,③) and those made only in ponds (**①**,**②**,**③**), for the total number of strains at each screening level. Multiple daily measurements in MSS and LSP included morning (06.00 to 08.00 LT) and evening (16.00 to 18.00 LT) sample times. M.O. = microscope observations; OD₄₄₀ = optical density at 440 nm; POC = particulate organic carbon; PON = particulate organic nitrogen.

Measurement	Screening Level				References
	PS	HTS	MSS	LSP	
PAR		①	⊙	⊙	[35]
Temperature	①	①	⊙	⊙	[36]
pH			⊙	⊙	[36]
M.O.	①	①	①	①	[35]
NH ₄ , NO ₂ , NO ₃		①	③	②	[37]
PO ₄ , SiO ₄		①	③	②	[37]
OD ₄₄₀		①	③	②	[34]
<i>in vivo</i> fluorescence	①	①	③	②	[35]
F _v /F _m	①	①	③	②	[35]
Dry weight		①	③	②	[38]
Ash-free dry weight		①	③	②	[38]
Chlorophyll <i>a</i>		①	③	②	[35]
POC, PON		①	③	②	[35]
Nile Red		①	③	②	[35]
Total lipid		①	③	②	[39]
Triacylglycerols		①	③	②	[40]
Sinking rate		①	①	①	[36]
Number of strains	>500	171	16	2	

nutrient-replete and nutrient-starved conditions using both NH₄ and NO₃. The same variables were measured as in first stage HTS, plus total lipids, total triacylglycerols, and other lipid classes.

The Mid-Scale Screening (MSS) program evaluated the top 16 strains from HTS for productivity, lipid composition, and harvestability. Cultures were grown under outdoor conditions at KDF using a

system of twelve 200-L, pre-sterilized scale-up reactors (see Section 1.2: *KDF system description*) to simulate PBRs, and twelve 200-L paddlewheel-driven “mini-ponds” to simulate ponds; the first several trials used 50-L plexiglass cylinders to simulate the operation of both PBRs and ponds until mini-ponds were fully operational. All strains were grown in f/2 medium [41], with NO₃ as the nitrogen source and Si(OH)₄ included only for diatoms. During each 15-d MSS trial, four strains were screened in triplicate reactors, operated to simulate both PBRs and ponds. Six MSS trials were conducted, all using the best-performing of the first four strains, *Staurosira* sp., as an internal control, for which representative results (Fig 1) illustrate the trial process. For each trial, there were three modes of culture management, representing primary operations at large scale. The “Full Growth Curve” is the scale-up process used in the Base Case, wherein cultures are grown with sufficient dissolved inorganic nitrogen (DIN) to inoculate PBRs. The “PBR” mode simulates the repeated daily dilution of commercial-scale PBRs for 5 days; cultures were grown semi-continuously, harvested at the end of each day when nutrients were depleted, then replenished with nutrients. The final “Pond” mode simulates the operation of final batch cultures, inoculated with exponentially-growing PBR culture and grown beyond DIN depletion for up to 5 days.

Measurements were made daily during scale-up and three times daily during PBR and pond simulations (Table 1), except that total lipids and lipid classes were measured only during the pond mode. Sinking rates were measured at 12.00 h each day of the pond run, and at 16.00 h on day 4, the final “harvest day,” using an 8-ml test tube method, which measures settling over a distance of 7.4 cm in 1 h or, at harvest time, using a 1-L graduated cylinder method, which measures settling at three depths (0, 15, 30 cm) over 3 h.

1.2. *KDF system description*

The 2.5-hectare Kona Demonstration Facility (KDF), designed and built in 2009 for Cellana LLC by the co-authors of this paper Huntley, Machesky, and Archibald, is located on a coastal site, 5 m above sea level, at the Hawaii Ocean Science and Technology Park on the island of Hawaii. The hybrid cultivation and harvesting system consists of 0.5 hectare of PBRs and ponds, facilities for additional dewatering by centrifugation and propane-fueled drying using a ring dryer, and is scaled to cultivate and

process ~3 tons dry weight biomass per month. Fresh seawater from a nearshore intake depth of 30 m is supplied to both PBRs and ponds; the supply to PBRs is pretreated by two-stage filtration to 0.2 μm nominal pore size, followed by UV treatment. Commercial sources supply a regular 1-month supply of carbon dioxide, at >99% purity, stored in liquid form on site, and distributed as a gas to PBRs and ponds; fertilizers are supplied in solid form and pre-mixed on site to highly concentrated stock solutions, which are then delivered as concentrate (~1000 $\times$) to ponds, or pre-diluted in the seawater supply to PBRs. Dispensing of CO₂ and nutrients from storage facilities to individual reactors is managed by a central process-control system. Energy for all activities is provided by on-site generators.

The scale-up reactor is a 200-L vertical cylindrical bag, 0.38 m in diameter, made of clear polyethylene, and structurally supported by a wire mesh cage. Customized bags were fabricated with integrated fittings to our specifications, and shipped, pre-sterilized and ready for deployment. Bag cultures are filled with fresh filtered and UV-sterilized medium, inoculated with a standard 20-L laboratory culture, then, after a week or less can be harvested to inoculate PBRs. Bag cultures are circulated by turbulent convection generated by continuous low-pressure air supplied to a diffuser at the base of the reactor. Light exposure is comparable to that in horizontal tubular PBRs, which are the same diameter (Table 2). A process control system monitors and controls pH at 8.1 ± 0.1 , supplying CO₂ on demand by injection into the air supply. Temperature is monitored but not controlled.

PBRs are of the horizontal tubular, serpentine, airlift-driven type [42], which we have developed for commercial application over the past two decades [29, 43]. These comparatively large reactors - 0.38 m diameter and 25 m³ culture volume *cf* [27, 44, 45] – provide, in our experience, a highly reliable, easily maintained, and continuous supply of high quality, cost-efficient inoculum for pond inoculation (Table 2).

The PBR consists of two lengths of flexible polyethylene tubing, 10-15 mil thickness, each connected at one end by a simple 180° “U”-tube, and at the other by a 180° “End Assembly” that incorporates a 2.5-m vertical airlift located at the edge of a terrace of the same height. The End Assembly

is made of roto-molded polyethylene to accommodate valves and other specified PBR fittings and, like all other PBR components, is entirely recyclable. PBRs are deployed on a reflective geotextile surface

Table 2 Physical design parameters of recirculating PBRs, comparing Base Case design to Actual demonstration system. Numbers in bold emphasize key design differences. Seawater input power of 79 kWh ha⁻¹ d⁻¹ is for Hawaii, increasing to 352 kWh ha⁻¹ d⁻¹ in Texas.

Design Parameter	Units	Actual	Base Case
<i>Unit dimensions</i>			
Tube diameter	m	0.38	0.38
Tube length	m	245	490
Riser height	m	2.5	2.5
Land area	m ²	186	372
Fluid fullness		0.90	0.90
Culture volume	m ³	25	50
Hydraulic radius	m	0.115	0.115
Fluid velocity	m s ⁻¹	0.40	0.40
Cycle time	min	10	20
Reynolds number - night, Re _N		41,700	41,700
Reynolds number - day, Re _D		54,900	54,900
Hydraulic power	W	62	77
Airlift efficiency	%	25	25
Unit Recirculation power	kWh d ⁻¹	5.96	7.42
Dilution rate	% d ⁻¹	50	50
Inoculum output/Seawater input period	h	2	4
<i>System water and power</i>			
Inoculum output	m ³ ha ⁻¹ d ⁻¹	672	647
Seawater input	m ³ ha ⁻¹ d ⁻¹	672	696
Total Recirculation power	kWh ha ⁻¹ d ⁻¹	229	199
Total Seawater input power	kWh ha ⁻¹ d ⁻¹	-	79
Total Inoculum output power	kWh ha ⁻¹ d ⁻¹	-	-
Total Power	kWh ha ⁻¹ d ⁻¹	229	278

designed to provide additional reflected light from space between the tubes, which are one diameter apart, so that cultures are illuminated from below as well as from above, substantially enhancing the direct light that is captured [44, 46]. In typical deployment, the PBR is dismantled, re-installed, and

readied for inoculation by two persons in a day. Allowing one week for the culture to scale up after inoculation, PBRs are designed to be in full production for 31 of every 32 weeks, an operating capacity of 97%.

Algae culture occupies 90% of the PBR volume; the remaining 10% is an air-space continuously filled by the airlift and maintained by back-pressure sufficient to provide turgidity to the reactor. Evaporation from closed photobioreactors is generally negligible [47]; in this design, with only a single vent, most water vapor condenses on the exposed inner tube surface and rejoins the culture. The hydrodynamics are best approximated by open channel flow in a partially filled pipe [48]. The PBR is designed to be operated at $Re \cong 50,000$, which corresponds to the bulk turbulence necessary to maintain homogeneous suspensions of most algal strains. Under ambient conditions at KDF, where temperature in the PBR typically ranges from 18°C at night to >30°C in the day, the kinematic viscosity decreases significantly, directly increasing Re by >30% during daytime (Table 2). Oxygen concentration, which can reach >500% saturation at moderately high productivity in open raceway ponds, significantly inhibiting photosynthesis [49, 50], does not rise to >150% saturation in these PBRs under comparable conditions [43]. A process control system monitors and controls pH at 8.1, supplying CO₂ on demand by injection into the airlift “down-comer,” which provides a counter-current dissolution system with 90% efficiency. At a fluid velocity of 0.40 m s⁻¹, the cycle time between successive injections of CO₂ to the culture is about 10 min (Table 2); at a typical productivity of 25 g m⁻² d⁻¹, the pH deviates by ±0.05 between injections. Temperature is monitored but not controlled.

PBRs are operated as a semi-continuous culture, each yielding 12.5 m³ of inoculum daily at a doubling rate of 1 d⁻¹. Cultures are harvested from PBRs at sunset, with volumes transferred by gravity to ponds on a terrace below. After harvest, the PBRs are refilled with dilute medium made on demand from freshly filtered, UV-treated seawater via the central process control system, which monitors flows and controls actuated valves. Energy use by PBRs is primarily for continuous recirculation, driven by a low-pressure, high volume airlift with an efficiency of 25% (as determined by Beal et al.[1]), which is lower than the estimate of 39-45% for other air-lift tubular PBRs [14, 44]. Energy for recirculation is 4.25 kWh

d⁻¹ for each KDF PBR, or, for each hectare dedicated to PBRs, 229 kWh ha⁻¹ d⁻¹, not including pumping and pre-treatment of seawater (Table 2).

Ponds are of the recirculating open raceway type (Table 3), with berms constructed of native soil and a hardening additive, and covered with a smooth, easily-cleaned, 20-year warranted liner (Hypalon®) overlaid on geotextile (Manning's n=0.010). The six ponds at KDF are each 400 m² in lighted surface area, and 60 m³ in volume. The paddlewheel in KDF ponds is partially submerged - to improve efficiency - in a 40-cm deep sump at one end of the pond that is also used for carbonation and harvesting. To conserve water and reduce fluid transfer volumes, ponds are designed to operate at an average culture depth of 0.15 m.

Though open ponds are often operated at greater depths [11], which conserves energy for paddlewheels [51], we found in KDF trials that overall performance at 0.15 m depth compared favorably with that at 0.20 and 0.30 m, with no significant change in areal productivity. The preferred turbulence regime is species-dependent, and can be determined empirically. A variable-speed motor on the paddlewheel delivers a dynamic range of turbulence, up to Re >250,000; lead candidate strains we have tested in ponds so far perform best at Re >50,000 (Table 3). The process control system supplies CO₂ on demand to a diffuser system immersed in the sump so that, at a fluid velocity of 0.40 m s⁻¹ and a cycle time of 7 min (Table 3), pH can be maintained at 8.1±0.05 given a pond productivity of 25 g m⁻² d⁻¹. Temperature is monitored but not controlled. Annually averaged pan evaporation of 4.2±1.3 mm d⁻¹, equivalent to a loss of 2.8% d⁻¹ in a 15-cm deep pond, was estimated using the Penman-Monteith equation [52] based on 474 continuous days of hourly meteorological measurements at KDF [53] just preceding the demonstration period described here (Fig A-1; SI). Energy requirements depend strongly on the efficiency of the pond recirculation device. Overall paddlewheel efficiency in open raceway ponds has been estimated in the range from 10% to 64% [11, 54-56]; we assume a conservative value of ~10% [56].

Table 3 Physical design parameters of open raceway ponds. Seawater process power of 91 and 11 kWh ha⁻¹ d⁻¹ for 1-d and 2-d ponds is for Hawaii, increasing to 406 and 47 kWh ha⁻¹ d⁻¹ in Texas.

Design Parameter	Units	Actual	Base Case: 1-day ponds	Base Case: 2-day ponds
<i>Unit Dimensions</i>				
Length	m	80	347	
Depth	m	0.15	0.15	
Width	m	5.3	31.0	
Land area	m ²	421	10,634	
Culture surface area	m ²	400	10,000	
Culture volume	m ³	60	1500	
Carbonation sump depth	m	-	1.8	
Hydraulic radius	m	0.134	0.147	
Fluid velocity	m s ⁻¹	0.40	0.40	
Cycle time	min	6.7	28.3	
Reynolds number @ 18°C		48,500	53,200	
Reynolds number @ 30°C		63,900	70,100	
Hydraulic power	W	105	1,822	
Paddlewheel/Airlift efficiency		0.10	0.25	
Unit Recirculation power	kWh d ⁻¹	25	175	
Residence time	d	1 to 4	1	2
Seawater:inoculum	m ³ m ⁻³	1	1	1
Seawater input or Harvest period	h	2	4	4
<i>System water and power</i>				
Seawater input	m ³ ha ⁻¹ d ⁻¹		777	212
Evaporation	m ³ ha ⁻¹ d ⁻¹	42	42	42
Inoculum output	m ³ ha ⁻¹ d ⁻¹		1,458	-
Process water output	m ³ ha ⁻¹ d ⁻¹		-	713
Total Recirculation power	kWh ha ⁻¹ d ⁻¹	937	163	163
Total Seawater process power	kWh ha ⁻¹ d ⁻¹	-	91	11
Total Power	kWh ha ⁻¹ d ⁻¹	937	254	174

Ponds are operated as batch cultures. After sunset the empty pond is partially filled with fresh, unfiltered seawater. Concentrated nutrients are added, and fresh medium is allowed several hours to equilibrate with atmospheric temperature. Ponds are inoculated with PBR culture, cultivated for periods of 1 to 3 days, and harvested after nutrients have been depleted to undetectable levels from the medium.

The first stage of harvesting takes advantage of the natural process of aggregation and sinking that is well known among marine diatoms and many other marine microalgae [57]. Such aggregates typically form after post-bloom nutrient exhaustion; observed sinking rates can exceed 1 m h^{-1} [58, 59]. The two strains selected for this study exhibit high sinking rates, order 1 m h^{-1} , after nutrient exhaustion under production conditions. The harvesting process uses the open pond as a first-stage settling tank. On the day of harvest, just before sunset, paddlewheels are turned off, and algal aggregates settle to the bottom within $<1 \text{ h}$. Supernatant is then removed through a standpipe, located in the sump, with its intake at the level of the raceway bottom, making it possible to gently remove $\sim 95\%$ of the spent culture medium while leaving the algal biomass concentrated in a thin film of water over the entire raceway. Spent medium is returned to the ocean via a percolation field. The standpipe is removed from the sump, which is drained to a secondary settling tank while harvesting is completed. The concentrated algal biomass remaining on the raceway bottom is harvested by a team of two persons using power washers to move it along each raceway toward the sump, which requires 10-12 min. When harvest is complete, the pond is clean and ready to be re-inoculated. Several hours later, the secondary settling tank yields a biomass concentrate of $>1\%$ total suspended solids (TSS), a feedstock requirement for the Westphalia disk-stack centrifuge used for subsequent processing to yield a dewatered end product of 20%-30% TSS.

1.3. Large-scale production

The goal of both *Staurosira* and *Desmodesmus* production trials was to evaluate the impact of two independent variables – (i) initial total N, or the sum of particulate organic nitrogen (PON_i) and dissolved inorganic nitrogen (DIN_i), at pond inoculation and (ii) the initial “Fertilizer Ratio” at inoculation, $\text{DIN}_i/\text{PON}_i$ - on pond yield. We also compared yield over 2-day vs. 3-day production runs. The initial total N (TN_i) at pond inoculation was varied from 500 to 2,100 μmol for *Staurosira*, and up to 2,600 μmol for *Desmodesmus*. The initial Fertilizer Ratio was varied from 0 to ~ 3.8 . Another goal of the *Desmodesmus* trials was to evaluate the impact of removing silicate as a required nutrient.

Staurosira and *Desmodesmus* were first scaled up in 200-L reactors (~5 d) and 25-m³ PBRs (~10 d) until three PBR cultures reached maximum productivity, when they began to be harvested routinely at 50% per day to inoculate one pond per day. Ponds were harvested at sunset of day 3, so three ponds were continuously in operation. We conducted 110 production runs with *Staurosira* (Apr 4 to Jul 28, 2010) and >60 with *Desmodesmus* (Apr 28 to Jul 26, 2011), using silicate for the diatom and NO₃ as the nitrogen source. PBR cultures were grown in f/2. Comprehensive data sets were not available for all production runs; *Desmodesmus* production run data are supplemented with results from 31 simulations at mid-scale.

Meteorological data, including photosynthetically active radiation (PAR), were collected using a Davis VantagePro2 weather station on site and archived as hourly averages (Figs A2-4; SI). In addition to continuous in-pond temperature and pH, other measurements (Table 1) were made for PBRs sampled daily, before and after harvest, and ponds sampled at least twice daily, either at 08.00 and 16.00 or at sunrise and sunset. All measured concentrations of nutrients and biochemical content in ponds (Table 1) were expressed in units of [g m⁻²] of pond culture surface area. Protein plus nucleic acid content was calculated from PON using the Kjeldahl ratio of 6.25 and assuming a nucleic acid content of 5% ash-free dry weight (AFW) [60], and carbohydrate content was calculated by difference. Sinking index was measured daily in late afternoon samples using the graduated cylinder method. All ponds were harvested after 3 days of batch culture by gravitational settling, and were required to meet dual standards of <5% loss of biomass and undetectable DIN concentrations in supernatant process water.

1.4. Base Case facility description

The 100-ha Base Case facility design presented in detail by Beal et al [1] is based on the results of this cultivation study. Specifically, the Base Case is Case 4 in the Beal study. Some of the critical differences between the KDF and the Base Case facility are as follows:

Terraced site: The 100-ha Base Case is designed in terraces on a natural 1% slope, which enables volume transfers by gravity and reuse of culture medium for a period of about two weeks. This residence time regulates salinity and effectively avoids contamination by invasive species.

CO₂ and nutrient supply: A 15-km pipeline delivers a waste stream 94% pure CO₂ from a point source. Nitrogen and phosphorus are provided as ammonia and di-ammonium phosphate (DAP).

PBR size: Unit PBRs are doubled in volume, to 50 m³, by doubling the length of flexible polyethylene tubing to 490 m, which adds little to the cost and delivers the same recirculating turbulent régime at a relatively lower energy demand (Table 2). Gas exchange is facilitated through the 10% head space in each PBR, as described for the KDF design above. PBRs provide a total culture volume of 1,344 m³ ha⁻¹ land area.

Pond size: Open ponds are increased to 1.0 ha in area (Table 3), equal to the largest ponds in sustained commercial operation [54]; otherwise, the hydrodynamics are very similar. Ponds provide a total culture volume of 1,411 m³ ha⁻¹ land area.

Pond liner and recirculation: Pond liner is reinforced polypropylene, chosen based on our assessment of cost, durability, and effects on live algal cultures of more than a dozen materials [61]. Ponds are driven by a single airlift in a countercurrent carbonation sump, similar in all respects to the baffle design of Lundquist [11], except that air is injected at the base of the riser to provide bulk flow, replacing the need for a paddlewheel. Airlift-driven raceways have reported power efficiencies of >40% [14, 44, 62]; however, in their Appendix A, Beal et al. [1] determined an efficiency for this design to be 25% (Table 3). The airlift has no moving parts, is easier to install and, when combined with the carbonation sump, provides a more effective process for gas exchange than the less efficient paddlewheel.

Pond types: Ponds are designed for batch cultures of two types: 1-day ponds are inoculated from PBRs and emptied daily to inoculate 2-day ponds. Both pond types are of the same design and construction, except that 2-day ponds also serve as settling basins during final harvesting.

Harvesting and dewatering: Gravitational settling combined with the filter press produces an end product of 20% TSS. This process compares favorably with other harvesting and dewatering routes we evaluated in large-scale trials, including, for primary harvesting, flocculation by chemical- or auto-induced pH increase [63], induced air flotation [64], and the use of chemical flocculants; and, for

dewatering, solar drying or disk-stack centrifugation using a Westfalia separator (model MSD 300-96-777).

1.5. Base Case capital cost

The Base Case capital cost estimate is for a 111-ha turn-key facility, built to a cost accuracy of $\pm 20\%$ using estimation methods described in Beal et al. [1]. In this paper we present the same costs, but categorized differently to assist readers evaluating cultivation-specific results. Major categories in this analysis include, site preparation, systems for harvesting and cultivation (scale-up, PBRs, and open ponds), and subsystems for onsite delivery of power, CO₂, air, seawater pre-treatment, storage and delivery of nutrients, and process control. Offsite delivery systems for CO₂ and seawater, and all onsite production systems, which occupy 95% of the facility, have been designed and specified in sufficient detail to proceed to the first set of construction drawings. We include the costs of processing facilities for Case 4 in Beal et al. [1] to enable comprehensive comparison to published cost estimates for other end-to-end pathways.

2. Results

2.1. Actual system performance

Here we quantify, for both *Staurosira* and *Desmodesmus*, the effects of initial total N and the Fertilizer Ratio on the yield of all key biochemical fractions and, at the same time compare the performance of 2-day vs 3-day ponds. The overarching measure of performance is the yield of biomass, lipid, and other products, with the additional requirement that DIN is absent from the culture medium at the time of harvest. These results inform key operational aspects of the Base Case system design.

2.1.1. Effects of initial total nitrogen (TN_i) on yield

Yield was greater at higher nitrogen levels. For both *Staurosira* and *Desmodesmus* the yields of lipid, protein, and dry weight increased substantially as a function of TN_i over the range tested, with the most favorable results achieved in 2-day ponds (Fig. 2). At a given level of TN_i both species yielded the same amount of protein, and similar amounts of lipid, but *Staurosira* produced significantly more dry

weight, reflecting its greater ash content. In the range tested, from 0.8 to 4.2 g m⁻² TN_i, lipid yield from *Staurosira* almost doubled while protein yield increased five-fold. *Desmodesmus* showed comparable increases in lipid and protein as a function of TN_i.

2.1.2. Nitrogen uptake and conversion

In PBR cultures cultivated in f/2 medium [41], NO₃ was reduced to undetectable levels every day before harvest, as observed in mid-scale bag cultures (Fig 1). Likewise, concentrations of PO₄ and, for diatoms, Si(OH)₄, were also reduced to undetectable levels. Thus, inoculum from PBR cultures contained N, P and Si only in particulate form.

The Base Case design requires that DIN concentrations in final production ponds are reduced to zero in discharged culture medium at the time of harvest. Furthermore, it makes the assumption that nitrogen is conserved. In other words, all DIN is converted to PON, and no nitrogen is discharged as N₂O. Our results validate these assumptions.

In open pond cultures dissolved nitrogen was entirely converted to particulate form in <2 d at all initial total N concentrations of up to 4.2 g m⁻², or 2.0 mmol N, for both *Staurosira* (Fig 3 A-F) and *Desmodesmus* (Fig 4 A-F) for all ponds with a Fertilizer Ratio of <2.0 (Fig 5). At the same time, concentrations of DIN were undetectable in ponds after 2 d (Figs 2D, 3D), as were those of HPO₄ and Si(OH)₄, indicating that all DIN was fully incorporated into PON.

Independent studies of *Staurosira* pond cultures at KDF showed that the net flux of N₂O was into the ponds [65], not into the atmosphere [66], so after 2 d all initial N should have been present at PON. We found this to be the case for *Desmodesmus* (Fig 4). However, the apparent conversion of NO₃-N to PON in *Staurosira* cultures was only 0.95 in 2-d ponds and 0.87 in 3-d ponds (Fig 3 D, F). We attribute this apparent loss of N to a sampling artifact caused by settling and attachment to the pond liner. The measured slopes thus indicate settling errors of 5% on Day 2 and 13% on Day 3. Subsequent studies at KDF to quantify the “settling error” in *Staurosira* observed it rising up to 50% by Day 4. We conclude that in a 2-day pond culture period all nutrients are converted to particulate form, leaving none dissolved in the spent medium.

2.1.3. Effect of the Fertilizer Ratio on nitrogen uptake and productivity

If all initial dissolved nitrogen, DIN_i , is converted to particulate form at harvest we have:

$$PON_f = PON_i + DIN_i \quad (1)$$

where PON_i and PON_f are the initial and final concentrations of particulate nitrogen, respectively.

Dividing by PON_i , the absolute rate of increase in PON over a given period can be expressed as a function of the Fertilizer Ratio:

$$\frac{PON_f}{PON_i} = \frac{DIN_i}{PON_i} + 1 \quad (2)$$

and thus the doubling rate of PON, μ_N , can be expressed as

$$\mu_N = \left[\ln \left(\frac{DIN_i}{PON_i} + 1 \right) \right] / t \quad (3)$$

where t is the open pond culture period in days.

Actual performance of 2-day pond cultures shows that the doubling rate of PON increased according to equation (3) until the Fertilizer Ratio attained a value of ~2.0, with no apparent difference between species (Fig. 5). The *Desmodesmus* cultures inoculated at Fertilizer Ratios >2.0 did not attain the expected doubling rate of PON, reflecting the fact that after 2 days these were the only cultures that still contained significant DIN (Fig. 4). These results quantitatively inform the Base Case design: at TN_i values up to 4.2 g m^{-2} , a Fertilizer Ratio of 2.0 yields a PON doubling rate of 0.55 d^{-1} ; importantly, these values also dictate the amount of particulate inoculum required and thus affect the ratio of PBRs to ponds.

2.1.4. Effects of initial total nitrogen and Fertilizer Ratio on the C:N ratio

To illustrate the effects of nitrogen fertilization in the present data set, we consider two cases for total nitrogen: (i) a “Low N” case of $1.85 \text{ g m}^{-2} TN_i$, roughly equivalent to $f/2$ medium, and (ii) a “High N” case of $4.2 \text{ g m}^{-2} TN_i$, the apparent upper limit of full conversion of DIN to PON in 2-day ponds (Figs 3 & 4). Full conversion to PON avoids the discharge of DIN and other dissolved nutrients to the environment.

For both *Staurosira* and *Desmodesmus* cultures the C:N ratio [g g^{-1}] was virtually uniform at the time of inoculation, measured at 6.28 ($r^2=0.96$; SI: Fig B-1) and 7.20 ($r^2=0.94$; Fig SI: Fig B-2),

respectively. This initial uniformity, which attests to the high biochemical consistency of inoculum produced by PBRs, rapidly changed as a result of both total TN_i and the Fertilizer Ratio. By the time of harvest on Day 2, for the Low N case, the C:N ratio rose to 11.5 and 11.0 for *Staurosira* and *Desmodesmus*, respectively. By comparison, for the High N case, the C:N ratio rose to 8.5 and 7.7 (Figs 6A, 7A), respectively. It is clear that cultures deprived of N continued to fix C. Consequently, cultures with less initial N were deprived sooner, fixed only C for a longer period, and thus their C:N ratios were greater. These same trends continued on Day 3, with further increases in the C:N ratio for both strains in both cases.

A low Fertilizer Ratio also led to a high C:N ratio at harvest for both strains, again showing that cultures relatively deprived of N developed the greatest C:N ratios (Figs 6B, 7B). At a Fertilizer Ratio >2 there was no apparent effect on the C:N ratio. The combination of low total nitrogen and a low Fertilizer Ratio led to the highest C:N ratios.

2.1.5. Constant mass relationships

Relationships of POC and DW to AFW, and POC to DW, were constant within species on the day of harvest (Figs 8 & 9). There were, however, significant differences between strains. *Staurosira* had a C:AFW ratio of 0.56 ($r^2=0.94$), with a relatively high DW:AFW ratio of 1.46 ($r^2=0.94$; Fig 8A), corresponding to 32% ash content. *Desmodesmus* had a lower C:AFW ratio of 0.50 ($r^2=0.99$), coupled with a lower DW:AFW ratio of 1.028 ($r^2=0.99$; Fig 9A), corresponding to only 2.7% ash; measurements of AFW were not made on Day 2. The relationship of C:DW was effectively constant on Day 2 and Day 3 for both strains, ranging from 0.36 to 0.39 for *Staurosira* ($r^2=0.82, 0.85$; Fig 8B) and constant at 0.49 for *Desmodesmus* ($r^2=0.87, 0.90$; Fig 9B).

2.1.6. Effect of pond duration on lipid and protein yield

At the time of inoculation, for both *Staurosira* and *Desmodesmus* cultures, the lipid content [$g\ g^{-1}$ AFW] was virtually uniform at 38% AFW ($r^2=0.82$; Fig B-3: SI) and 47% AFW ($r^2=0.0.78$; Fig B-4: SI), respectively. The protein content was not only uniform but also similar for both species, at 47% ($r^2=0.91$; SI: Fig B-5) and 46% ($r^2=0.93$; SI: Fig B-6), respectively.

Lipid yield increased significantly by the end of Day 2, and then increased slightly again on Day 3, achieving the highest values for both species at high TN_i . The relative increase in lipid was greater on Day 2 than on Day 3 for both species (Fig 10). By comparison, the protein yield reached a maximum by harvest time on Day 2, and did not increase on Day 3 (Fig 11), reflecting the depletion of DIN to zero.

Taken together, the foregoing results provide a clear illustration of the interwoven dynamics of C, N, protein, and lipid. After all DIN is converted to protein, the continued fixation of C leads, for *Staurosira* and *Desmodesmus*, to a continued increase in lipid synthesis. The sooner a culture has depleted the available DIN, the greater will be its C:N ratio and lipid content after the same amount of time. Under all conditions tested, daily averaged protein yield was clearly greater in 2-d vs 3-d ponds. In the following summary we provide the complete mass balance for *Staurosira* and *Desmodesmus* for both Low N and High N cases, allowing a broader evaluation of 2-d vs 3-d ponds.

2.1.7. Effects of N fertilization: a summary

Here we compare the average daily productivity and annually averaged yield of all key biochemical constituents as a function of N fertilization in both 2-d and 3-d ponds based on the foregoing empirical data. Using a value of $1.8 \text{ g m}^{-2} TN_i$ for the Low N case, equivalent to common *f/2* medium, and a value of $4.2 \text{ g m}^{-2} TN_i$ for the High N case, we calculated the yields of DW, AFW, lipid, and protein on the day of harvest from regression equations shown above (Figs 3-11). In all cases we assumed a Fertilizer Ratio of 2.0, which yielded the highest rate of PON production and converts all DIN to PON after 2 days (Fig 5). Knowing that $1.8 \text{ g m}^{-2} TN_i$ at inoculation will yield $1.8 \text{ g m}^{-2} PON$ at the time of harvest (Figs 3,4), we estimated the yield of POC from the C:N ratio in at the time of harvest in both 2-d and 3-d ponds for *Staurosira* (Fig 6A) and *Desmodesmus* (Fig 7A). So, for example, from regression lines shown in Fig 6A it can be seen that a pond inoculated at $1.8 \text{ g m}^{-2} TN_i$ has a C:N ratio [g g^{-1}] of 11.5 after 2-d and 14.0 after 3-d. Thus, the yield of POC from a 2-d pond for the Low N case is $1.8 \text{ g m}^{-2} N \times 11.5 \text{ gC gN}^{-1} = 20.7 \text{ g m}^{-2} C$, and the yield from a 3-d pond is $25.2 \text{ g m}^{-2} C$. For the High N case of $4.2 \text{ g m}^{-2} N$, regression lines in Fig 6A show a C:N ratio of 8.5 after 2-d and 10.0 after 3-d, so the yields of POC are 35.7 and $42.0 \text{ g m}^{-2} C$, respectively.

The corresponding yields of AFW and DW were calculated from the C:AFW and C:DW ratios for both species (Figs 8A, 9A), and ash was calculated by difference. For example, the C:AFW ratio of 0.562 ($r^2=0.94$; Fig 8A) for *Staurosira* yields $20.7 \text{ g m}^{-2} \text{ C} \div 0.562 \text{ g C g AFW}^{-1} = 36.8 \text{ g m}^{-2} \text{ AFW}$ for the Low N Case in 2-d ponds. Applying the AFW:DW ratio of 1.46 ($r^2=0.94$; Fig 8A) yields $36.8 \text{ g m}^{-2} \text{ AFW} \times 1.46 \text{ g DW g AFW}^{-1} = 53.7 \text{ g m}^{-2} \text{ DW}$, and calculating the ash by difference we obtain $53.7 \text{ g m}^{-2} \text{ DW} - 36.8 \text{ g m}^{-2} \text{ AFW} = 16.9 \text{ g m}^{-2} \text{ ash}$. The corresponding lipid yields were calculated from regressions of yield measurements on TN_i (Fig 10), which, for *Staurosira* in 2-d ponds, is $\text{lipid} = 2.59 \text{ TN}_i + 12.1$. Thus, the Low N case yields $(2.59 \times 1.8 \text{ g m}^{-2} \text{ N}) + 12.1 = 16.8 \text{ g m}^{-2} \text{ lipid}$, and the High N case yields $23.0 \text{ g m}^{-2} \text{ lipid}$. Yields of chlorophyll, which amounted to $\sim 1\%$ AFW, were calculated in the same manner based on regressions provided in the supplementary information (Fig B-7, B-8). Protein yields (Fig 11) were calculated using the ratio of protein:PON = 6.25 [60] assuming complete conversion of TN_i to PON. Carbohydrate content was calculated by difference by rearranging the identity: $\text{AFW} = \text{protein} + \text{lipid} + \text{chlorophyll} + \text{carbohydrate}$. Comprehensive results of this analysis are summarized for both species for the Low N case (Table 4) and the High N case (Table 5); quantitative assumptions are listed separately in table captions for each species in each case. Yield and productivity values are referenced to pond surface area in m^2 or ha.

The effect of nitrogen fertilization on productivity clearly shows that both species are significantly less productive in the Low N Case (Table 4) than in the High N Case (Table 5). At a given level of N, both species yield equal amounts of protein and similar amounts of lipid, but *Staurosira* produces substantially more ash.

For *Staurosira* in the Low N case, lipid yield ranges from 26 to $31 \text{ MT ha}^{-1} \text{ yr}^{-1}$ for 3-d and 2-d ponds, respectively (Table 4A); in the High N case, lipid yield increases significantly, from 34 to $42 \text{ MT ha}^{-1} \text{ yr}^{-1}$, with the greatest yield again from 2-d ponds (Table 5A). Lipid yield from *Desmodesmus* is slightly greater. In the Low N case, lipid yield from *Desmodesmus* is $32 \text{ MT ha}^{-1} \text{ yr}^{-1}$ in both 2-d and 3-d ponds (Table 4B). In the High N case, lipid yields increase to $34 \text{ MT ha}^{-1} \text{ yr}^{-1}$ in 3-d ponds and

Table 4A *Staurastrum* sp., Low N fertilization – $1.8 \text{ g m}^{-2} \text{ TN (f/2)}$: Productivity, yield, and composition in 2-day ponds vs 3-day ponds. Key assumptions are that (i) all DIN is converted to PON (Fig 3); (ii) the C:N ratio changes from 6.28 on Day 1 (Fig B-1: SD) to 11.5 on Day 2, 14.0 on Day 3 (Fig 6A); (iii) the C:AFW ratio is 0.562 and the DW:AFW is 1.46 (Fig 8A); (iv) P and Si are consumed in the ratio provided, i.e. the N:P:Si ratio of *f/2* medium (N:P=11.01 g g^{-1} and Si:P=2.965 g g^{-1}), (v) protein and nucleic acids are 16% N [60]; (vi) lipid is 0.38 AFW on Day 1 (Fig B-3: SD); 2.59 TN_i +12.1 on Day 2, and 2.83 TN_i + 16.1 on Day 3 (Fig 10); (vi) the chlorophyll:PON ratio is 0.24 (Fig B-7: SD); (vii) carbohydrate was estimated by difference; and (viii) the fertilizer ratio, $\text{DIN}_i/\text{PON}_i$, is 2.0 (Fig 5).

Material	Input		Output		Productivity		Yield		Composition	
			g m^{-2}		$\text{g m}^{-2} \text{ d}^{-1}$		$\text{MT ha}^{-1} \text{ yr}^{-1}$		% AFW	
	Day 1	Day 2	Day 3		2-d Pond	3-d Pond	2-d Pond ^a	3-d Pond ^b	2-d Pond	3-d Pond
<i>Elemental Composition</i>										
POC	3.77	20.7	25.2		8.5	7.1	37.8	30.7	56.2%	56.2%
N	1.80	1.80	1.80				3.29	2.19		
PON	0.60	1.80	1.80		0.60	0.40	3.29	2.19	4.89%	4.01%
DIN	1.20	0	0				0	0		
P	0.05	0.16	0.16				0.30	0.20	0.44%	0.36%
Si	0.16	0.48	0.48				0.89	0.59	1.32%	1.08%
<i>Bulk Composition</i>										
DW	9.8	53.8	65.5		22.0	18.6	98.3	79.8		
Ash	3.08	16.9	20.6		6.9	5.85	31.0	25.1		
AFW	6.7	36.8	44.8		15.1	12.7	67.3	54.7	100%	100%
Protein	3.8	11.3	11.3		3.75	2.50	20.6	13.7	30.5%	25.1%
Lipid	1.2	16.8	21.2		7.79	6.67	30.6	25.8	45.5%	47.2%
Carbohydrate	1.6	8.4	12.0		3.38	3.45	15.4	14.6	22.8%	26.8%
Chlorophyll	0.1	0.4	0.4		0.14	0.09	0.8	0.5	1.2%	0.9%

Table 4B *Desmodesmus* sp., Low N fertilization – $1.8 \text{ g m}^{-2} \text{ TN (f/2)}$: Productivity, yield, and composition in 2-day ponds vs 3-day ponds. Key assumptions are that (i) all DIN is converted to PON (Fig 4); (ii) the C:N ratio changes from 7.20 on Day 1 (Fig B-2: SI) to 9.0 on Day 2 and 13.0 on Day 3 (Fig 7A); (iii) the C:AFW ratio is 0.498 and the DW:AFW is 1.028 (Fig 9A); (iv) P and Si are consumed in the ratio provided, i.e. the N:P:Si ratio of *f/2* medium (N:P=11.01 g g^{-1} and Si:P=2.965 g g^{-1}), (v) protein and nucleic acids are 16% N [60]; (vi) lipid is 6.6 PON – 0.7 on Day 1; 2.65 TN_i +12 on Day 2, and 0.77 TN_i + 25 on Day 3 (Fig 10); (vi) the chlorophyll:PON ratio is 0.05 (Fig B-8: SI); (vii) carbohydrate was estimated by difference; and (viii) the optimal fertilizer ratio, $\text{DIN}_i/\text{PON}_i$, is 2.0 (Fig 5).

	Input		Output		Productivity		Yield		Composition	
	Day 1	Day 2	Day 3	g m^{-2}	2-d Pond	3-d Pond	2-d Pond ^a	3-d Pond ^b	2-d Pond	3-d Pond
Material										
<i>Elemental Composition</i>										
POC	4.32	16.2	23.4		5.9	6.4	29.6	28.5	49.8%	49.8%
N	1.80	1.80	1.80				3.29	2.19		
PON	0.60	1.80	1.80		0.60	0.40	3.29	2.19	5.53%	3.83%
DIN	1.20	0	0				0	0		
P	0.05	0.16	0.16				0.30	0.20	0.50%	0.35%
Si	0.16	0.48	0.48				0.89	0.59	1.49%	1.03%
<i>Bulk Composition</i>										
DW	8.9	33.4	48.3		12.3	13.1	61.1	58.9		
Ash	0.24	0.9	1.3		0.3	0.36	1.7	1.6		
AFW	8.7	32.5	47.0		11.9	12.8	59.5	57.3	100%	100%
Protein*	3.8	11.3	11.3		3.75	2.50	20.6	13.7	34.6%	23.9%
Lipid	3.4	17.5	25.9		7.06	7.49	32.1	31.5	53.9%	55.1%
Carbohydrate	1.5	3.7	9.8		1.09	2.76	6.7	11.9	11.2%	20.8%
Chlorophyll	0.03	0.1	0.1		0.03	0.02	0.2	0.1	0.3%	0.2%

Table 5A *Staurosira* sp., High N fertilization – 4.2 g m⁻² TN; Productivity, yield, and composition in 2-day ponds vs 3-day ponds. Key assumptions are as in Table 4 except that the C:N ratio changes from 6.28 on Day 1 to 8.5 on Day 2 and 10.0 on Day 3 (Fig 6A).

Input		Output		Productivity			Yield			Composition	
g m ⁻²		g m ⁻²		g m ⁻² d ⁻¹			MT ha ⁻¹ yr ⁻¹			% AFW	
Day 1	Day 2	Day 3	2-d Pond	3-d Pond	2-d Pond ^a	3-d Pond ^b	2-d Pond	3-d Pond			
Elemental Composition											
POC	8.79	35.7	42.0	13.5	11.1	65.3	51.2	56.2%	56.2%		
N	4.20	4.20	4.20			7.68	5.12				
PON	1.40	4.20	4.20	1.40	0.93	7.68	5.12	6.61%	5.62%		
DIN	2.80	0	0			0	0				
P	0.13	0.38	0.38			0.70	0.46	0.60%	0.51%		
Si	0.38	1.13	1.13			2.07	1.38	1.78%	1.51%		
Bulk Composition											
DW	22.8	92.8	109.1	35.0	28.8	170	133				
Ash	7.20	29.2	34.4	11.0	9.06	53.4	41.9				
AFW	15.6	63.5	74.7	23.9	19.7	116	91.1	100%	100%		
Protein*	8.8	26.3	26.3	8.75	5.83	48.0	32.0	41.3%	35.1%		
Lipid	5.9	23.0	28.0	8.51	7.34	42.0	34.1	36.2%	37.4%		
Carbohydrate	0.6	13.3	19.5	6.35	6.31	24.4	23.8	21.0%	26.1%		
Chlorophyll	0.3	1.0	1.0	0.33	0.22	1.8	1.2	1.56%	1.3%		

Table 5B *Desmodemus* sp., High N fertilization – 4.2 g m⁻² TN: Productivity, yield, and composition in 2-day ponds vs 3-day ponds. Key assumptions are as in Table 4 except that the C:N ratio changes from 7.20 on Day 1 to 7.70 on Day 2 and 9.0 on Day 3 (Fig 7A).

	Input		Output		Productivity		Yield		Composition	
	g m ⁻²		g m ⁻²		g m ⁻² d ⁻¹		MT ha ⁻¹ yr ⁻¹		% AFW	
Material	Day 1	Day 2	Day 3		2-d Pond	3-d Pond	2-d Pond ^a	3-d Pond ^b	2-d Pond	3-d Pond
<i>Elemental Composition</i>										
POC	10.08	32.3	37.8		11.1	9.2	59.1	46.1	49.8%	49.8%
N	4.20	4.20	4.20				7.68	5.12		
PON	1.40	4.20	4.20		1.40	0.93	7.68	5.12	6.47%	5.53%
DIN	2.80	0	0				0	0		
P	0.13	0.38	0.38				0.70	0.46	0.587%	0.50%
Si										
<i>Bulk Composition</i>										
DW	20.8	66.8	78.0		23.0	19.1	122	95.1		
Ash	0.57	1.8	2.1		0.6	0.52	3.3	2.6		
AFW	20.2	64.9	75.9		22.3	18.6	119	92.5	100%	100%
Protein*	8.8	26.3	26.3		8.75	5.83	48.0	32.0	40.4%	34.6%
Lipid	8.9	24.6	27.7		7.86	6.28	45.0	33.8	37.9%	36.5%
Carbohydrate	2.5	13.9	21.7		5.67	6.40	25.3	26.5	21.3%	28.6%
Chlorophyll	0.1	0.2	0.2		0.07	0.05	0.4	0.3	0.3%	0.3%

45 MT ha⁻¹ yr⁻¹ in 2-d ponds (Table 5B).

AFW productivity in the Low N case is comparable for both species: from 12.7 to 15.1 g m⁻² d⁻¹ for *Staurosira* and from 11.9 to 12.8 g m⁻² d⁻¹ for *Desmodesmus*. In the High N case, AFW productivity virtually doubles: from 19.7 to 23.9 g m⁻² d⁻¹ for *Staurosira* and from 18.6 to 22.3 g m⁻² d⁻¹ for *Desmodesmus*. The highest values in both cases are from 2-day ponds, which yield 116 and 119 MT ha⁻¹ yr⁻¹ for *Staurosira* and *Desmodesmus*, respectively.

Both species yield the greatest amount of protein in 2-day ponds, 48 MT ha⁻¹ yr⁻¹ in the High N case, along with carbohydrate yield of ~25 MT ha⁻¹ yr⁻¹. The greatest difference between species is the amount of ash produced at these high yields: 53.4 vs. 3.3 MT ha⁻¹ yr⁻¹ for *Staurosira* and *Desmodesmus*, respectively. The ash not only comes with the added cost and environmental impacts of supplying Si(OH)₄, it also reduces the co-product value. The presence of ash in the lipid-free co-product reduces the protein content from 63% in *Desmodesmus* to 38% in *Staurosira*; for reference, the value of protein-rich feed components increases with protein content, which rises from 28 to 32% in distiller's dried grains (DDGS), to 48% in soymeal, 67% in fishmeal, and 75% in soy protein concentrate [67].

Based on the comparative performance summarized in Tables 4 and 5, a Base Case design that employs 2-d ponds for the production of *Desmodesmus* in the High N case is clearly indicated (Table 5B). This design produces the highest annual yields of valuable organic products combined with the lowest yield of low-value ash.

2.2. Effect of results on Base Case design and cost

2.2.1. Facility configuration based on nitrogen uptake

The facility is comprised of a series of cultivation modules, each consisting of a single 1-day pond, four 2-day ponds, and 30 PBRs, together occupying 6.4 ha; a total of 16 cultivation modules adds up to a total cultivation area of 103 ha (Table 6). There are 8 ha devoted to processing and other facilities, bringing the total facility area to 111 ha [1]. Relative areas of unit operations within the cultivation area are based on the observed doubling rate of nitrogen in 2-day ponds ($\mu_N = 0.55$ d⁻¹; Fig 5) and on the

operational doubling rate of 0.69 d^{-1} in PBRs, assumed to be the same in 1-day ponds. Thus, the daily facility production from PBRs inoculates 1-day ponds, producing 6.9 MT d^{-1} of inoculum for 2-day ponds, which in turn yield 21 MT d^{-1} AFW from the facility.

2.2.2.Economics

Capital costs of the Base Case facility are estimated at $\$534,000\text{ ha}^{-1}$ for the Gulf Coast and $\$599,000\text{ ha}^{-1}$ for Hawaii (Table 7); the difference being driven by regional cost factors for labor and materials [68]. Thus, a 111 ha Base Case facility would cost $\$59$ million on the Gulf Coast, and $\$67$ million in Hawaii.

The cost of land preparation amounts to $\$34,000\text{ ha}^{-1}$ for the Gulf Coast and $\$52,000\text{ ha}^{-1}$ for Hawaii. The seawater system costs more in the Gulf Coast than Hawaii ($\$87,000$ vs $\$5,100\text{ ha}^{-1}$) because seawater is delivered via a 5-km pipeline from the sea, rather than by a system of submersible pumps from a shallow saline aquifer. The CO_2 delivery system, also using a 15-km pipeline, is $<4\%$ of total capex at both locations. All other costs are greater for Hawaii.

Ponds occupy 76.4% of the facility area and dominate the capital costs, ranging from $\$161,000$ to $\$251,000\text{ ha}^{-1}$ of facility area, or 30% to 42% of the total, for the Gulf Coast and Hawaii, respectively. Normalized to pond surface area only, our estimate for the installed cost of a pond system ranges from $\$21.11$ to $\$32.87\text{ m}^{-2}$, comparable to the value of $\$34.10\text{ m}^{-2}$ for high-rate ponds in California estimated by Lundquist et al [11].

The relatively low cost of PBRs is worth noting. These large-diameter reactors occupy 16% of the cultivation area (Table 6), but account for $<3\%$ of total capital (Table 7). Normalized to the PBR area only (including space between PBRs), our estimate of the installed cost of a PBR system ranges from $\$6.49$ to $\$10.10\text{ m}^{-2}$ (Gulf Coast vs Hawaii), which is less than half the capital cost of ponds and significantly lower than many other estimates. Part of the reason is due to the materials used in this design. In some designs, the entire PBR culture vessel is considered a capital expense. In our design 99% of the PBR culture vessel consists of PVC tubing that is replaced four times annually, and therefore is an operating expense, detailed by Beal et al [1]. However, even when normalized to PBR area only, and

Table 6 Base Case - Cultivation system configuration based on observed performance of *Desmodium* at high N fertilization (Table 5), showing yields of AFW for 2-Day ponds, 1-Day ponds, and PBRs. The yield from 2-day ponds is the facility yield; the yields from PBRs and 1-day ponds are used for inoculation. Areas shown are the total land area, except for yield per unit, which refers to the culture surface area.

Cultivation Unit	Yield per unit			System		Cultivation Module			Facility			
	g m ⁻²	g m ⁻³	kg ha ⁻¹	kg ha ⁻¹ d ⁻¹	Relative area	Units per ha	ha	# Units	kg d ⁻¹	ha	# Units	MT d ⁻¹
Cultivation Pond	64.9	433	611	305	4.00	0.94	4.25	4	1,299	68.1	64	20.8
2-day Pond												
1-day Pond	43.3	289	407	407	1.00	0.94	1.06	1	433	17.0	16	6.9
PBR	43.3	289	388	194	1.06	26.6	1.13	30	219	17.8	480	3.5
Total Area							6.44			103		

Table 7 Capital expenses, in \$000s per hectare of total facility area, for a Base Case microalgae production and processing facility of 111 ha. Land preparation: clearing, grading, trenching, pond berm formation, and all mass earthmoving. Seawater system: all main supply and discharge lines to and from the site, including pumps. CO₂ delivery system: includes capture at the source, compression, and pipeline to the facility gate. PBR system: includes the reactor itself, all internal fittings, and plumbing that connects the PBR to main supply or discharge system; replaceable tubing not included. Pond system: pond, pond liner, water propulsion system, valves and pumps directly connected to ponds, and pipework that connects each pond to main supply or discharge system. Harvest system: clarifiers and filter press equipment. Processing: equipment for Case 4 described by Beal et al. [1]. Instrumentation and control: process control system and laboratory equipment. Buildings: 1 hectare under roof for warehouse storage, nutrient preparation, and laboratory space. Other: nutrient mixing, air supply, scale-up culture system, and other infrastructure. All costs include materials and labor.

Item	\$ per ha (000's)	
	Hawaii	Gulf Coast
Land preparation	52.4	33.7
Seawater system	5.1	86.9
CO ₂ delivery	20.5	13.2
PBR system	16.1	10.4
Pond system	251.0	161.2
Harvest system	25.3	16.3
Processing	77.2	49.6
Instrumentation & control	15.5	10.0
Electrical	27.3	17.5
Buildings	72.9	46.8
Other	35.6	88.3
TOTAL	599	534

considered as a capital cost over the 30-year life of other PBR components, the tubing adds \$11.78 to \$18.33 m⁻² to the effective cost, bringing the total PBR cost into the range of \$18.26 to \$28.43 m⁻², similar to our estimate of pond costs, all detailed in Table C-1 (SI). It is the large diameter and large volume of

this PBR design that sets it apart from other tubular and flat plate reactor designs [21, 27, 69], which by virtue of their much greater surface area:volume ratio are more costly in terms of both area and volume. In the present hybrid system, the total culture volume per hectare is similar for both PBRs and ponds (1,344 and 1,411 m³ ha⁻¹, respectively; Tables 2 and 3), so cost per unit volume is comparable, in the range of \$135 to \$211 per m³ for PBRs and \$150 to \$233 per m³ for ponds in the Gulf Coast and Hawaii, respectively (Table C-1; SI).

Davis et al [7] conclude that PBRs are almost 20 times more costly than open ponds, basing their cost estimate on a small diameter PBR design that accommodates only 200 m³ ha⁻¹, or <15% of the culture volume of our PBRs. The order of magnitude difference in cultivation area required to support the same culture volume in such small-diameter PBR systems explains their much greater capital intensity. Similarly, in their 2002 economic analysis, Hallenbeck and Benemann (p 1190) [70] say that “the photobioreactors were not further specified and were merely assumed to cost \$100/m², which with [other costs] gave a total capital cost of \$135/m².” Acien et al [27] estimated a cost of €15,000 for a total PBR volume of 33 m³, or ~\$6,500 per m³; the high cost of those PBRs is understandable as they are of a very different design, being a vertically suspended array of 9-cm diameter tubes with considerably more permanent infrastructure than required by the design presented here.

Processing facilities and equipment for the Base Case, or Case 4 detailed in Beal et al. [1], account for 9% to 13% of total capital expenses, about equal to the cost of buildings. Harvesting equipment accounts for <5% of capital expenses; electrical and instrumentation equipment costs are similar in magnitude, with all absolute costs greater in Hawaii.

3. Discussion

3.1. High yields compared to other cultivation systems

To enable comparison with other microalgae cultivation systems, we report yields with reference to the entire area required for cultivation. For the hybrid cultivation system described here, 34% of the cultivation area is dedicated to inoculation and the remaining 66% to production ponds (Table 6). Thus,

the areal yield values for production ponds (Tables 3 and 4) are normalized to the total cultivation area so, for example, the lipid yield of 45 MT ha⁻¹ yr⁻¹ from *Desmodesmus* production ponds at High N (Table 5A) corresponds to 30 MT ha⁻¹ yr⁻¹ when normalized. To compare with published values of yield from conventional open pond systems we normalize in the same way, as follows. For conventional open pond cultivation systems, pond productivity and yield are also reported with reference to the lit surface area of the production pond, as in Tables 3 and 4. However, those designs typically require inoculation ponds, which must also be included in the total cultivation area. In the representative Lundquist [11] design, only 1% of the cultivation area is dedicated to inoculation and the remaining 99% to production ponds, but the industry standard described by Benemann et al [4] is 10%, and “achieving 1% will require advances in the ability to prevent pond crashes” [12], which has not been demonstrated to date. We therefore assume open pond systems require 10% of the cultivation area for inoculation, and have normalized reported yields accordingly for the purpose of comparison (Figs 12 and 13).

The yields of algal biomass and lipids achieved in the hybrid cultivation system are greater than reported yields from open ponds at large scale, and greater than many assumed or modeled yields, even after normalizing for the relatively large area dedicated to inoculation in the hybrid system design (Figs 12 and 13). In the High N case, biomass (AFW) and lipid yields are 78 and 30 MT ha⁻¹ yr⁻¹, respectively, for *Desmodesmus* and 77 and 28 MT ha⁻¹ yr⁻¹, respectively, for *Staurorsira*. The biomass yield of ~75 MT ha⁻¹ yr⁻¹ observed for both species is about double that achieved at the Sapphire Energy pilot facility by Liu et al [10], which is among the highest reported based on actual productivity at large scale (Fig 12).

The realized lipid yields for *Staurorsira* and *Desmodesmus* at high N fertilization (Fig 13) are comparable to Chisti’s [31] estimated upper limit from conventional open ponds of ~33 MT ha⁻¹ yr⁻¹, and much more than Benemann’s [4] estimate of the “best that could be expected is 20,000 L ha⁻¹ yr⁻¹,” or ~18 MT ha⁻¹ yr⁻¹ algal oil. Flat plate reactors do not seem to do much better. The modeled biomass productivity from *Nannochloropsis* in flat panel reactors deployed at large scale in Hawaii was 12.8 g m⁻²

d⁻¹ [21] , or 47 MT ha⁻¹ yr⁻¹ , with a corresponding lipid yield of ~20 MT ha⁻¹ yr⁻¹ , equal to yields observed in the Low N case for *Staurosira* and *Desmodesmus*.

We offer several reasons why the lipid yields from open ponds reported here equal or exceed the perceived upper limit for conventional open pond systems [6, 31], and exceed all reports we could find of sustained biomass yields from open ponds at a scale >10 m². First, we attribute much to the process of strain selection, which clearly identified *Staurosira* and *Desmodesmus* as leaders among 500 candidates; both strains had >36% lipid content at large-scale, even with high N fertilization. Many published measurements of large-scale production are based on the cultivation of either unspecified strains or strains that were not selected for lipid production.

Second, we conclude that adding nitrogen is a good thing to do; results clearly show that by increasing total nitrogen by a factor of 2.3, not only does the biomass yield almost double, from ~40 to 75 MT ha⁻¹ yr⁻¹ for both species, but the total lipid yield also increases, by about 50%, to 28 MT ha⁻¹ yr⁻¹ for *Staurosira* and 30 MT ha⁻¹ yr⁻¹ for *Desmodesmus*. This result is entirely opposite to the projection of Lardon et al [9] that an increase in nitrogen would reduce lipid yields of *Chlorella* from 27 MT ha⁻¹ yr⁻¹ to 16 MT ha⁻¹ yr⁻¹ , and that of Vasuvedan et al [15], who expect that lipid yield would be reduced from 63 MT ha⁻¹ yr⁻¹ to 16 MT ha⁻¹ yr⁻¹ (Fig 13). Furthermore, we have demonstrated how one may employ the Fertilizer Ratio to obtain the highest biomass yield possible while ensuring zero discharge of dissolved nitrogen.

Third, we suggest that pond depth may be a factor in limiting yields. From a preliminary study of *Staurosira* production in 400 m² open ponds operated side-by-side at 15, 20, and 30 cm depths, we found that deeper pond cultures became light-limited sooner, and observed that 15-cm deep ponds gave the highest yields. It may not be purely coincidental that observed yields lower than ours were produced in 30 to 35-cm deep ponds (Table 10). Weissman et al [71] estimated relatively high average biomass productivity values of 16 g m⁻² d⁻¹ , or 58 MT ha⁻¹ yr⁻¹ , from fall and winter experiments in 1 m² , 15-cm

deep ponds using strains selected for high growth and lipid characteristics, but rates at larger pond scales of 3 m² or 1,000 m² were substantially lower.

Finally, the open ponds in the hybrid system described here are not operated like conventional open ponds. Conventional open ponds are typically operated as semi-continuous cultures, partially harvested and refilled daily at rates ranging from 10% d⁻¹ [12] to an average of 25% d⁻¹ [4, 11] until some part of the system crashes, at which time it is restarted from inoculum ponds. Open ponds in the hybrid system, by contrast, are harvested and re-inoculated either daily or, for production ponds, every other day, while the inoculum (PBR) system is harvested at a rate of 50% d⁻¹. Simply considering these two systems at facility scale as two semi-continuous culture systems, the conventional open pond system operated at a dilution rate of 25% d⁻¹ should, on average, yield less biomass than a hybrid system diluted at almost twice the rate. Viewed from this perspective, it is perhaps not surprising that a conventional pond harvested at 10% d⁻¹ yields only 11 MT ha⁻¹ yr⁻¹ of *Nannochloropsis* biomass [12].

3.2. Achieving higher yields

We suggest three specific areas where improved processes would lead to substantially greater yields. First, new fertilizers (nutrient medium) should be developed. Fertilizer development is the fundamental basis of improved yields in global agriculture over the past 50 years [72], but algae production still relies on nutrient media developed 50 years ago [41]. As one small example of what might be accomplished, we find the source of N may significantly improve yield. In high throughput screening we compared the doubling rate of 24 lead candidate strains on NH₄ vs NO₃, and found that, on average, doubling rates were 20% greater on NH₄. We used NO₃ for large-scale production at KDF. However, were NH₄ to be used we would expect N uptake rates to be 20% greater, leading to yields of 94 MT ha⁻¹ yr⁻¹ biomass and 38 MT ha⁻¹ yr⁻¹ lipid. We believe that serious research in algae nutrient media, having been ignored for half a century, would lead to rapid advances in demonstrated yield.

Second, methods should be explored to reduce respiratory losses of C. We estimate average nighttime respiratory losses of 12%, which amounts to a comparable loss in yield from production ponds; for High N *Desmodesmus* ponds this equates to a loss of 25 MT ha⁻¹ yr⁻¹, so innovations in reducing

nighttime respiration would improve yield accordingly. It is worth noting that nighttime respiratory losses are compounded so conventional open pond production systems, which typically have longer residence times [4, 11], are inherently less efficient in net C sequestration.

Third, the management of gas exchange in general should have a beneficial effect on yield. Oxygen concentrations often exceeded 400% saturation in our pond cultures, well beyond levels known to inhibit photosynthesis [50, 73], were generally reached before noon, and lasted for several hours [65]. It is difficult to accurately estimate how much yields could be increased by reducing O_2 concentrations to <150%, but we believe the effect could be significant. This would require a method of enhancing the rates of gas exchange during the middle of the day, enabling an enhanced uptake rate of CO_2 and manifested directly as increased productivity. Such gains as might be achieved through the ability to manipulate gas exchange rates could be, we believe, in the range of 10 to 20 $MT\ ha^{-1}\ yr^{-1}$ or more. In summary, we believe it remains realistic to pursue production targets of 100 $MT\ ha^{-1}\ yr^{-1}$ biomass and 45 to 50 $MT\ ha^{-1}\ yr^{-1}$ algal oil.

3.3. Achieving lower costs

The PBR design presented here is demonstrably effective at an order of magnitude less cost, per ha or per m^3 , than other common designs [19, 20, 27], with installed costs comparable to those for conventional open ponds [11]. This finding suggests that PBRs warrant reconsideration in the design of large-scale production facilities. Further cost reductions are explored in detail by Beal et al [1], who consider a range of unit operations that lead to demonstrable improvements in the entire production process.

We highlight one fundamental operation, CO_2 delivery, as a potential constraint for the otherwise tremendous potential of algal biofuels. Our facility model assumes a nearby, nearly pure CO_2 source, located within 15 km. However, in the real world pure CO_2 sources are rare, and even dilute sources may not be near large tracts of land. The facility cost increases at the rate of ~\$1,300 ha^{-1} for each km of CO_2 pipeline, so at a distance of 100 km the cost of the CO_2 delivery system would be substantial. Unless other methods of CO_2 capture and utilization are developed and deployed, the algae biofuels industry will

642 forever be limited by its short tether to stack gases. If yields can be pushed significantly beyond what we
643 have demonstrated here, 75 MT ha⁻¹ yr⁻¹ biomass and 30 MT ha⁻¹ yr⁻¹, or 35,000 L ha⁻¹ yr⁻¹ algal oil, then
644 other sources of CO₂ may well be worth pursuing because the low-hanging fruit of nearby, nearly pure
645 CO₂ sources will quickly be claimed, leaving no alternative.

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Figure 1

Figure 1 Mid-scale screening trial results for *Staurosira* sp., showing changes in total dissolved N (■) and biomass (◆), measured as optical density at 440 nm. Error bars are standard deviation for 3 replicate reactors.

Figure 2

Figure 2 Yield of lipid, protein, and dry weight from 2-day pond cultures as a function of initial total nitrogen (TN_i), all in $g\ m^{-2}$

Figure 3

Figure 3: *Staurosira* sp: Time series of DIN_f (○; final dissolved inorganic nitrogen) and TN_f (●; final total nitrogen) as a function of initial total nitrogen (TN_i), in 3-day pond batch cultures. After inoculation before sunrise on Day 1, ponds were sampled near sunrise (AM: 06:00 to 08:00 LT) and sunset (PM: 16:00 to 18:00 LT) on the days indicated.

Figure 4

Figure 4: *Desmodesmus* sp: Time series of DIN_f (○; final dissolved inorganic nitrogen) and TN_f (●; final total nitrogen) as a function of initial total nitrogen (TN_i), in 3-day pond batch cultures. After inoculation before sunrise on Day 1, ponds were sampled near sunrise (AM: 06:00 to 08:00 LT) and sunset (PM: 16:00 to 18:00 LT) on the days indicated.

Figure 5

Figure 5: Doubling rate of PON, μ_N (d⁻¹), as a function of the fertilizer ratio, DIN_i/PON_i, on Day 2 for *Staurosira* (○) and *Desmodesmus* (○), compared to the expected doubling rate if all DIN is converted to PON, based on equation (3). The dashed lines indicate values used for the Base Case

Figure 6

Figure 6: *Staurosira* sp. C:N ratio as a function of (A) initial total nitrogen (TN_i) and (B) the Fertilizer Ratio on Day 1 AM (●), Day 2 PM (●), and Day 3 PM (○).

Figure 7

Figure 7: *Desmodesmus* sp: C:N ratio as a function of (A) initial total nitrogen (TN_i) and (B) the Fertilizer Ratio on Day 1 AM (●), Day 2 PM (●), and Day 3 PM (○).

Figure 8

Figure 8: *Staurosira* sp: (A) Particulate organic carbon (●) and dry weight (○) as a function of ash-free dry weight at harvest time on Day 3. (B) POC as a function of dry weight at harvest time on Day 2 (●) and Day 3 (○).

Figure 9

Figure 9: *Desmodesmus* sp: (A) and (B) as described in Fig. 8

Figure 11

Figure 11: Protein yield as a function of total initial nitrogen (TN_i) for both species at the time of inoculation on Day 1 (●) and at harvest time on Day 2 (●) and Day 3 (○).

Figure 12

Figure 12 Reported annual yields of biomass (MT ha⁻¹ yr⁻¹) in techno-economic and/or life cycle assessment studies of three types: using (a) assumed, (b) modeled, and (c) realized values of productivity, for pond systems >10 m², compared to values reported here for *Staurosira* and *Desmodesmus*. Low N treatments: hatched bars, data labels in italics. High N treatments: solid bars, data labels in bold italics. References: [2-17, 20, 22-26].

Figure 13

Figure 13 Reported annual yields of algal oil (MT ha⁻¹ yr⁻¹) in techno-economic and/or life cycle assessment studies of three types: using (a) assumed, (b) modeled, and (c) realized values of productivity for pond systems >10 m², compared to values reported here for *Staurosira* and *Desmodesmus*. Low N treatments: hatched bars, data labels in italics. High N treatments: solid bars, data labels in bold italics. References: [3-13, 15, 17, 22]