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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-m^3 photobioreactors and 400-m^2 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 CO_2 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].

1. Introduction

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

Prior evaluations of the economic and environmental viability of algal biofuels rely on one of the following methods to estimate biomass and lipid productivity: (1) assumed values, (2) modeled values based on climatology, or (3) measured values based on long-term outdoor production in a scalable system [1]. Studies that use assumed values for biomass and lipid productivity project yields ranging from 50 to >100 MT ha^{-1} yr^{-1} biomass and from 5 to >50 MT ha^{-1} yr^{-1} lipid, whether in open ponds [2–17] or PBRs [7,14], clearly revealing the wide uncertainties of this approach. Modeling studies may incorporate site-specific climatology, but they predict a similar wide range of biomass and lipid yields from both ponds and PBRs [18–23]. To date the few reports of directly measured yields from open ponds at a scale >10 m^2 are an order of magnitude lower than assumed or modeled yields [10,12,24–26]. By comparison, small diameter PBRs can actually produce sustained yields at

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assumed or modeled rates, but their high cost [27,28] has led to the conclusion that PBRs are too costly to be employed for biofuel production [4,6,22]. Furthermore, several recent economic analyses or life cycle assessments presume that lipid yields can be significantly increased by reducing available nitrogen, but this has not been well demonstrated.

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

2. Materials and methods

2.1. 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 NH_4 or NO_3, and lipid biochemistry was characterized in more detail. The top 24 strains were grown at 30 °C under nutrient-replete and nutrient-starved conditions using both NH_4 and NO_3. 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 2.2: *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 NO_3 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

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 (❶,❷,❸), 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; OD_{440} = 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]
In vivo fluorescence	①	①	③	②	[35]
F_v/F_m	①	①	③	②	[35]
Dry weight		①	③	❷	[38]
Ash-free dry weight		①	③	❷	[38]
Chlorophyll *a*		①	③	❷	[35]
POC, PON		①	③	❷	[35]
Nile Red		①	③	❷	[35]
Total lipid		①	❸	❷	[39]
Triacylglycerols		①	❸	❷	[40]
Sinking rate		①	❶	❶	[36]
Number of strains	>500	171	16	2	