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# Intensive Microalgae Production, Years 1 and 2

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## General Information

*Reporting Period*            September 1, 2005–August 31, 2006; no-cost extension through  
December 31, 2006 (Year 1, final report)  
January 1, 2007–September 30, 2007 (Year 2)

<i>Funding Level</i>	Year	Amount
	<b>1</b>	<b>\$35,000</b>
	<b>2</b>	<b>\$35,000</b>
	TOTAL	\$70,000

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## Objectives

The goal of this project is to evaluate algal photobioreactor technology as an alternative to tank/raceway-based microalgae production systems for use by aquaculture operations in Hawaii and the U.S.-affiliated Pacific Islands (USAPI).

### **Year 1**

1. Complete industry survey on algal production requirements.
2. Generate a current review of available algal bioreactor systems, examining costs of setup and operation for publication in the CTSA newsletter *Regional Notes*.
3. Commission pilot-scale algae production system(s) to evaluate actual cost and ease of setup.
4. On-site operation and testing of algal photobioreactors with several species of marine and freshwater microalgae.

5. Provide education and training to the aquaculture industry through workshops, fact sheets, and continuing education programs at the Oceanic Institute's Oceanic Learning Center (OLC).

### **Year 2**

1. Evaluate and adapt a tubular bioreactor design currently in use in Europe.
2. Review the performance and cost of algal photobioreactor operation.
3. Convene an industry workshop to present findings and discuss live feeds production methods in support of marine finfish production in Hawaii and the USAPI.
4. Establish a permanent demonstration system at the Oceanic Institute (OI) for continued long-term use in training.

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## **Anticipated Benefits**

The advent of photobioreactor systems for algal production provides new opportunities for farmers to reduce the cost of algal production by decreasing labor demands and increasing production output. As an example, current batch production systems for *Nannochloropsis* sp. typically peak at 10–40 million cells/mL, while more modern continuous or semicontinuous algal bioreactors are now achieving algal densities in the range of 0.1 to 1 billion cells/mL. It is becoming increasingly important that U.S. farmers take advantage of such modern advances in available production methods to secure their competitiveness in an ever increasingly competitive worldwide industry. It is especially important with rapid increases in production coming from overseas competitors who have significantly lower land and labor costs. It is also recognized that most small- to medium-sized farms cannot afford the cost of experimentation or the risk associated with implementing new and unproven technologies. Therefore, it is pivotal that research organizations such as OI assist in the early stages of new technology development through research and demonstration projects, such as is being proposed here for algal bioreactors. The successful implementation of this technology clearly has the potential to further strengthen the rapidly growing aquaculture industry in Hawaii and the Pacific islands through increasing production efficiency, allowing commercial operators to focus their efforts on other aspects of their operations.

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## **Work Progress and Principal Accomplishments**

### **Year 1**

#### **Objective 1. Complete industry survey on algal production requirements.**

A comprehensive survey was developed and sent by email to all members of the Hawaii Aquaculture Association to assist in assessing algal culture requirements in Hawaii. The aim of the survey was to

scope current algal production needs by local industry. Only two responses were received, which limited the utility of this objective.

**Objective 2. Generate a current review of available algal bioreactor systems, examining costs of setup and operation for publication in the CTSA newsletter *Regional Notes*.**

A complete review of available bioreactor systems was completed and was used to generate an AquaTips article published in the December 2005 issue of CTSA newsletter *Regional Notes*.

**Objective 3. Commission pilot-scale algal photobioreactors to evaluate actual cost and ease of setup.**

The first phase of project efforts focused on identifying and evaluating available commercial photobioreactor designs. Somewhat surprisingly, we were unable to find readily available commercial products. The cellpharm<sup>®</sup> tubular bioreactors are actively used in a number of European hatcheries and HISTAR<sup>™</sup> system (Rusch, pers. comm.) currently under final design by AST, but these systems were not available for project activities. Therefore, efforts were focused on building two simple prototype systems: the first, a plexiglass plate bioreactor; and the second, a columnar air-lift bioreactor (Figure 1). We were not able to gain access to the commercial tubular bioreactor during the project period, but we are in the process of constructing a prototype system using off-the-shelf parts for future testing under both indoor and outdoor conditions.

**Plate bioreactor design.** Our first design was a 500-L plexiglass plate bioreactor with a 20 cm light path (Figure 1) based on the designs of Amos Richmond (pers. comm.). The OI system was fitted with UV-treated water, 1  $\mu\text{m}$  filtered air supply, and equipped with pH control to help maintain system sterility. Although not achieving anywhere close to the 400–800 M cells/mL densities described in the literature (Zhang et al. 2001), it did attain relatively high algal densities ( $> 70$  M cells/mL) in several runs (Figure 4), although average densities (26.5 M cells/mL) were not substantially different from those seen in outdoor tank systems. The lower than expected system performance was attributed to a lack of sufficient temperature control, longer light path (20 vs. 10 cm system), and challenges in maintaining necessary system sterility.

**Cylinder air-lift bioreactor.** Our second design was a 12-cylinder air-lift bioreactor system using readily available sunlight tubes (Figure 1). Each of the cylinders were 18 inches in diameter and maintained separate 400-L batches of algae under natural outdoor lighting. Each unit was provided with UV sterilized water mechanically filtered down to 0.2  $\mu\text{m}$  and supplemented with algal nutrients using an automatic dosing system. Air to each unit is also filtered down to 0.2  $\mu\text{m}$  and automatically supplemented with CO<sub>2</sub> as a carbon source and to maintain system pH within the range of 8.0 to 8.4 using individual pH controller units for each cylinder. Temperature is controlled using a chiller with individual titanium coils in each of the 12 reactor cylinders. Only the overflow, created by continuous (or semicontinuous) addition of nutrient enriched water to each cylinder, is harvested.

**Objective 4. On-site operation and testing of algal photobioreactors with several species of marine and freshwater algae.**

Having commissioned two types of photobioreactors, a series of evaluations was completed to provide data on culture performance in terms of algal density, culture duration, operation costs, and critical systems issues.

**Batch vs. Continuous culture.** The first evaluation compared algal output under batch production with that of a continuous production method. To make the comparison direct, the trial was conducted in the air-lift cylinder bioreactor system with six tubes operated under batch culture protocols (0% flow) and six cylinders operated under continuous flow (25%) exchange per day. This setup provided more advanced system control than typically utilized for batch production systems but provided a direct comparison between protocols. Both treatments received the benefits of using filtered air and water, temperature control, and CO<sub>2</sub> supplementation. The advantages of continuous culture over batch culture were two-fold: continuous cultures lasted significantly longer than batch cultures and produced nearly six times the algae (Figure 3).

**System comparisons.** The next series of trials compared culture performance under semicontinuous modes of operation in indoor tanks under 1,000-W metal halide lighting (28,000 lux @ 24 h/d) and depth of 20 cm and in outdoor tanks under natural lighting (average daily peak of 44,000 lux) and depth of 40 cm, as well as in the 46-cm-diameter cylinder and 20-cm-thick plate bioreactor systems. Moving cultures outdoors to utilize natural sunlight clearly provided significant increases in culture density and algal health over indoor cultures; however, culture density tended to vary widely in the outdoor systems (data not shown), dependent upon time of year and responses to changes in light intensity associated with local weather conditions.

The plate photobioreactor, which had the greatest surface area to volume ratio (i.e., shortest light path), attained the highest peak algal densities (> 70 M cells/mL) of the tested systems and demonstrated the highest harvest yield per unit system volume. However, the relative high cost (~\$5,000) of a single 500-L reaction chamber and relatively large system footprint compared with a cylinder-based air-lift system (~\$300 per cylinder) led us to work primarily with the cylinder-based reactor despite higher unit productivity in the plate-based system. Both the cylinder- and plate-based photobioreactor systems generally experienced shorter culture durations on average than either the indoor or outdoor tank-based cultures, indicating the need for considerable improvement in temperature dispersion, gas exchange, and system sterility. The plate bioreactor (without temperature control) proved quite unstable over time, resulting in the highest frequency of crashes and, thus, the shortest mean culture duration (10 days). The air-lift photobioreactor (with temperature control added) was generally more stable, yielding an average culture duration of 14 days, while both the indoor and outdoor cultures maintained under semicontinuous modes of operation lasted for 18 to 19 days on average.

**Algal species comparisons.** Finally, OI researchers compared performances of four species of marine microalgae commonly used in marine finfish and shellfish hatcheries using the same cylinder-based air-lift bioreactor system (Figure 6). As expected, the smaller *Nannochloropsis* sp. attained the highest mean cell densities (mean of 28 million cells/mL) compared with larger-sized *Isochrysis* and *Chaetoceros* species (mean of 6 million cells/mL) and even larger *Tetraselmis* species (1 million cells/mL). *Isochrysis*

and *Chaetoceros* also demonstrated somewhat lower culture durations (17 days) compared with *Nannochloropsis* (27 days) and especially *Tetraselmis* at 50 days.

**Objective 5. To provide education and training to the aquaculture industry through workshops, fact sheets, and continuing education programs at the Oceanic Institute's Oceanic Learning Center (OLC).**

As relayed above, an AquaTips article, titled "Microalgae production for aquaculture in Hawaii and the Pacific Islands" was written and published in CTSA's *Regional Notes*. At project completion, an industry workshop will be convened to review and summarize project findings. Preliminary project findings and demonstration of current air-lift bioreactor operation were provided to industry stakeholders at a workshop covering the current status of marine ornamental fish (and live feeds) culture research held at the Oceanic Institute on April 5, 2006.

**Year 2**

**Objective 1: Evaluate and adapt a tubular bioreactor design currently in use in Europe.**

Kimberly Falinski was hired in June 2007 to replace Aaron Ellis as research assistant and to conduct the majority of this project's remaining work plan. Falinski has a degree in engineering from the Massachusetts Institute of Technology and is presently engaged in a Master's in Engineering program through Cornell University. Over this reporting period for Year 2, Falinski has been training in algae production and working on the design and commissioning of a vertical tubular photobioreactor design based on systems currently in use in Spain. The current design utilizes a submersible horizontal tubular system for the solar collection portion of the system and airlift pump for degassing and movement of algae within the system. Unlike other bioreactor designs, this system can be scaled in a modular fashion and is designed to facilitate daily care and cleaning toward increasing system stability and reducing operational labor requirements. Commissioning of this OI system should be completed in time to have this system ready for preliminary testing in January 2008.

**Objective 2: Review the performance and cost of algal photobioreactor operation.**

Under Year 1 project activities, a detailed review on algal photobioreactor operation (Ellis and Laidley 2007) was generated and published in CTSA newsletter *Regional Notes*. Upon completion of system testing for a third bioreactor design, project researchers will follow up with a second CTSA *Regional Notes* article that reviews system performance and provides a cost analysis for operation of the different systems.

**Objective 3: Convene an industry workshop to present findings and discuss live feeds production methods in support of marine finfish production in Hawaii and the USAPI.**

Toward the end of project activities, project researchers will convene an industry workshop to disseminate results of project activities to interested stakeholders and provide a forum to discuss live feeds production methods in support of marine finfish hatchery operations in Hawaii and the Pacific region.

**Objective 4: Establish permanent demonstration systems at the Oceanic Institute (OI) for continued long-term use in training of students and industry.**

The tubular photobioreactor system developed under this project will continue operation at the Oceanic Institute and be utilized for long-term training of students and industry. This objective is particularly useful in relation to OI's recent affiliation with Hawaii Pacific University and the development of a new graduate program in Marine Sciences, which is providing new opportunities for both undergraduate and graduate training in live feeds production and other aspects of aquaculture.

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## Reference

Zhang, C. W., O. Zmora, R. Kopel, and A. Richmond. 2001. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture* 195:35–49.

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## Work Planned

Project researchers will complete the commissioning of the final (tubular) photobioreactor design, followed by a final article for publication in the CTSA newsletter *Regional Notes* and an industry workshop on microalgae culture.

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## Impacts

New opportunities for farmers to reduce the cost of algal production by decreasing labor demands and increasing production output are provided by the advent of photobioreactor systems for algal production. It is becoming increasingly important that U.S. farmers take advantage of such modern advances in available production methods to secure their competitiveness in an ever increasingly competitive worldwide industry. This is especially important with rapid increases in production coming from overseas competitors who have significantly lower land and labor costs. It is also recognized that most small- to medium-sized farms cannot afford the cost of experimentation or the risk associated with implementing new and unproven technologies. Therefore, it is pivotal that research organizations such as OI assist in the early stages of new technology development through research and demonstration projects, such as is being proposed here for algal bioreactors. The successful implementation of this technology clearly has the potential to further strengthen the rapidly growing aquaculture industry in Hawaii and the Pacific region by increasing production efficiency, allowing commercial operators to focus their efforts on other aspects of their operations.

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## Publications in Print, Manuscripts, and Papers Presented

- Ellis, A. A. and C. W. Laidley. 2006. Microalgae production for aquaculture in Hawaii and the Pacific islands. *CTSA Regional Notes* 17(4):3–7.
- Laidley, C. W. 2006. Current status of marine ornamental research at the Oceanic Institute. Presentation given as an industry workshop, Oceanic Institute (OLC), Waimanalo, Hawaii.
- Laidley, C. W. 2006. Current status of marine ornamental research at the Oceanic Institute. Presented at the Hawaii Institute of Marine Biology Seminar Series, University of Hawaii, Kaneohe, Hawaii.
- Laidley, C. W. 2006. Revitalization of Hawaiian fishponds. Presented at the Hawaiian Business Conference & Exposition, Hawaii Conference Center, Honolulu.
- Laidley, C. W. and C. K. Callan. 2007. Development of captive culture technology for marine ornamental fishes: challenges and forward progress. Paper presented at Aquaculture 2007, the annual meeting of the World Aquaculture Society, San Antonio, Texas.

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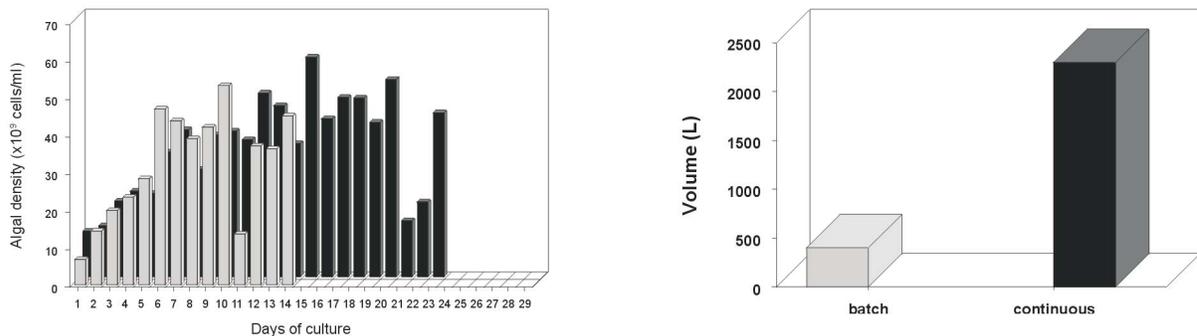
## Appendix



*FIGURE 1. Photos of large-scale photobioreactor systems, including a 500-L flat-panel bioreactor at OI (left), an OI cylinder-based air-lift bioreactor design (center), and a tubular photobioreactor system (right, photo courtesy of Randy Cates).*



**FIGURE 2.** Photographs of the OI prototype columnar air-lift bioreactor system showing (a) the water purification and dosing system, (b) in-line air filters and algal harvest lines, (c) chiller unit, (d) chilled water circulation pump and reservoir, (e) cold-finger inflow and outflow lines, (f) chiller fingers in empty cylinders, (g) water quality monitor and control unit, (h) pH, temperature and dissolved oxygen probes, and (i) algal harvest container.



**FIGURE 3.** Comparison of algal (*Nannochloropsis* sp.) production under batch and continuous (25% exchange-harvest per day) flow protocols.

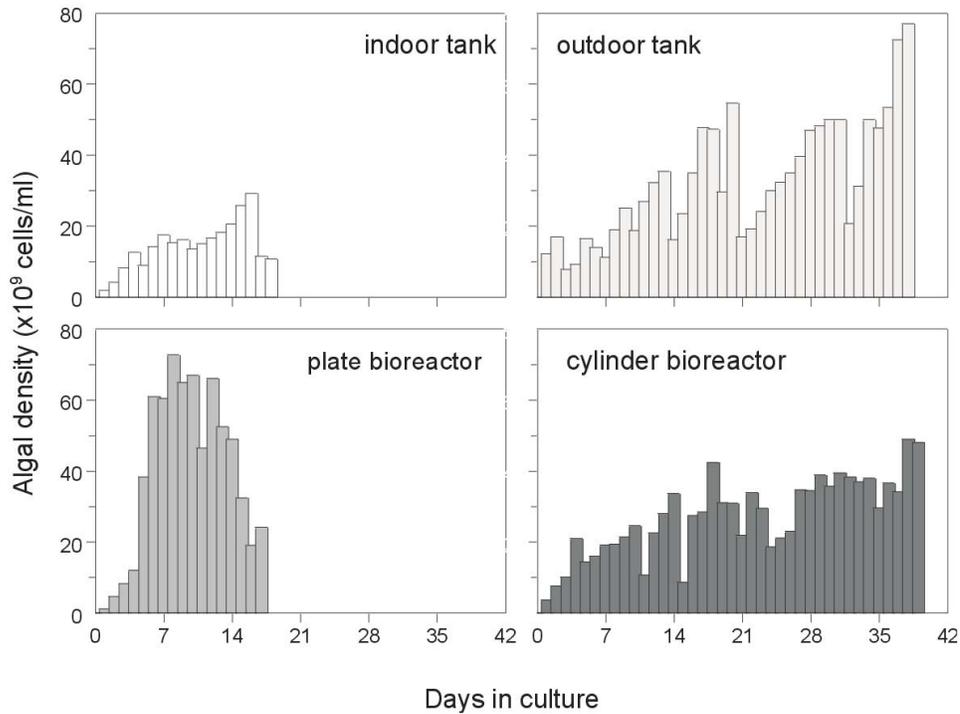


FIGURE 4. Average cell harvest (cells/L/day) of *Nannochloropsis sp.* from indoor tank, outdoor tank, air-lift cylinder, and plate algal culture systems.

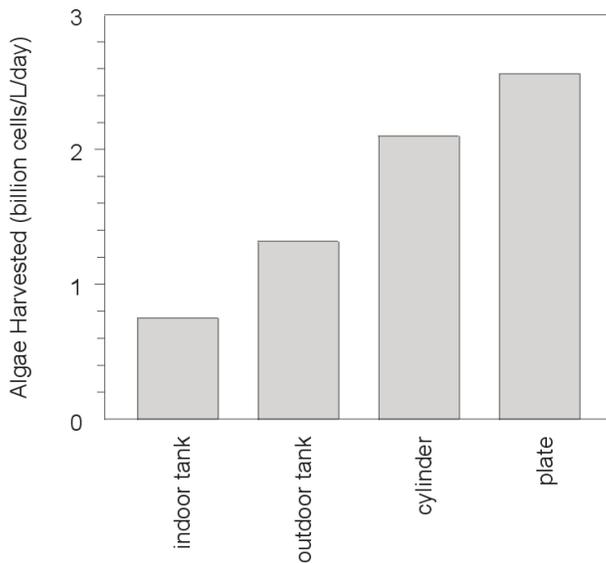
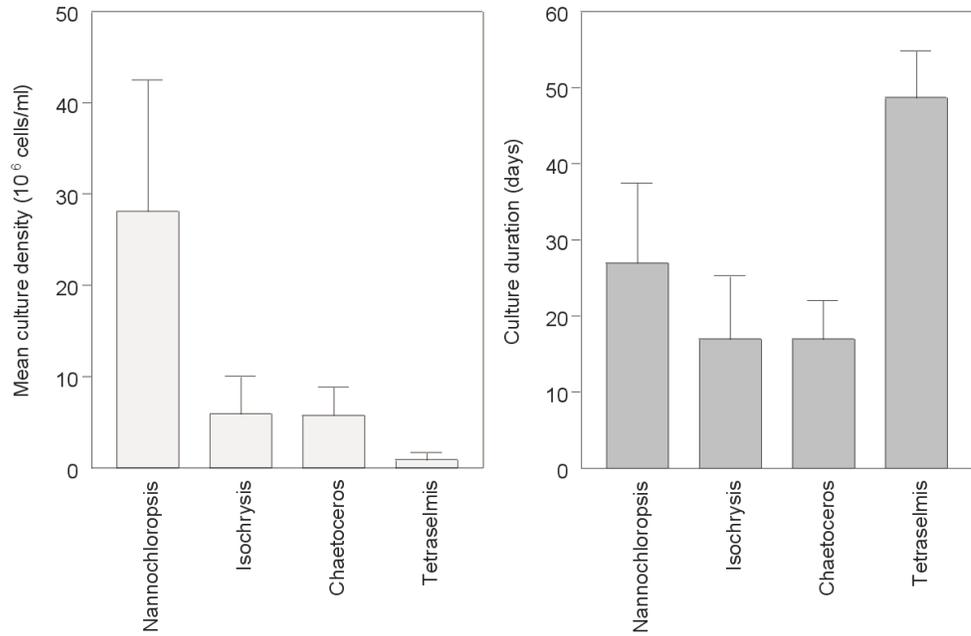


FIGURE 5. Average cell harvest (cells/L/day) of *Nannochloropsis sp.* from indoor tank, outdoor tank, air-lift cylinder, and plate algal culture systems.



*FIGURE 6. Average cell density (left) and culture length (right) of algal species cultured in the air-lift photobioreactor with culture crashes due to system malfunction removed.*