



The hydraulically integrated serial turbidostat algal reactor (HISTAR) for microalgal production

Kelly A. Rusch*, J. Michael Christensen

*Department of Civil and Environmental Engineering, Louisiana State University, CEBA Building,
Baton Rouge, LA 70803, USA*

Received 15 March 2002; accepted 9 December 2002

Abstract

A hydraulically integrated serial turbidostat algal reactor (HISTAR) for the mass production of microalgae was designed, constructed and preliminarily evaluated. The 9266-l experimental system consists of two enclosed turbidostats hydraulically linked to a series of six open continuous-flow, stirred-tank reactors (CFSTRs). The system was monitored and controlled using GENESIS process control software. A production study was performed using *Isochrysis* sp. (C-iso) to assess system stability and production potential under commercial-like conditions. The study was performed at the following target system parameters: system dilution rate of 0.49 per day, pH 7.6, nitrogen = 10 mg l⁻¹, phosphorus = 2 mg l⁻¹, and artificial illumination (photosynthetic photon flux density) from 1000 W metal halide lamps = 800 μmol s⁻¹ m⁻². At steady state conditions, daily harvested algal paste was 1454 g (wet), mean areal system productivity = 47.8 ± 3.04 g m⁻² per day (17.1 ± 1.09 g C m⁻² per day) and mean CFSTR6 density = 105.5 ± 6.71 mg l⁻¹.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Microalgal production; HISTAR; Continuous culture; Process control

* Corresponding author. Tel.: +1-225-5788528; fax: +1-225-5788662.

E-mail address: krusch@lsu.edu (K.A. Rusch).

1. Introduction

The use of microalgae for various applications has increased in recent decades. During the past several years, there has been significant research into the use of microalgal constituents for the pharmaceutical and pharmacological industries (Apt and Behrens, 1999; Kreitlow et al., 1999; Yamaguchi, 1997; Borowitzka, 1992, 1988; Lincoln et al., 1990; Ben-Amotz and Avron, 1989). A number of compounds, including vitamins, fatty acids, and antibiotic agents can be extracted from certain microalgal species (Centeno and Ballantine, 1999; Barwell, 1994). Additionally, the use of microalgae as a waste treatment and CO₂ sequestration method is increasing (Tang et al., 2002; Liehr et al., 1994; Holan and Volesky, 1994; Talbot et al., 1991).

The most abundant use for cultured microalgae is in the aquaculture industry. Although the development of artificial feeds and/or algal substitutes has advanced for many cultured species (Cahu and Infante, 2001; Lazo et al., 2000; Holt, 1993; Jones et al., 1993; LeRuyet et al., 1993; Walford et al., 1991; Coutteau et al., 1990), there are still many species that rely on live foods, especially during larval stages (Takeuchi, 2001; Shields, 2001; Hagiwara et al., 2001; LeRuyet et al., 1993; Benemann, 1992). As the United States' marine aquaculture industry begins to expand, the need for better live feed technologies is becoming quite apparent.

The technical issues that must be addressed irrespective of application include daily productivity and system stability. Both of these issues inherently incorporate several subissues including cost (fixed and variable costs), production runtime, required personnel oversight and contaminant control.

Current methods of microalgal culture rely on batch (static containers that are inoculated, grown to a specific density and harvested), semi-continuous or continuous cultures. As the need for microalgae increases, semi-continuous and continuous cultures are becoming the prevalent culture techniques. Most continuous cultures of microalgae are implemented within enclosed bioreactors to minimize airborne contaminant introduction and increase system control. There have been a number of recent advancements in continuous microalgal culture methods, including the flat plate glass reactor (Zou and Richmond, 1999), closed tubular photobioreactors made of glass or acrylic tubing (Molina-Grima et al., 1999; Miron et al., 1999), closed reactors with integrated internal lighting (Ogbonna et al., 1999), plastic bag cultures (Seasalter Shellfish, LTD, Personal communication) and the partitioned aquaculture system (PAS) originally developed as the waste treatment component for pond fish culture (Drapcho and Brune, 2000). All of these systems are enclosed bioreactors except the PAS, which operates as an open raceway culture. Each of these methods presents a unique approach for increasing productivity levels, but they do not necessarily address the potential for culture collapse due to inadvertent contaminants common to all static and continuous cultures (Moreno-Garrido and Canavate, 2001; DePauw et al., 1984; Goldman 1980; Heussler et al., 1978).

Continuous cultures tend to be operated over a much longer time than the typical batch culture, thus, the potential for contamination is greater. The inadvertent introduction of undesired microalgae or zooplankton into a culture might eventually lead to competition within the reactor and the eventual collapse of the desired

culture. Continuous cultures are susceptible to impacts from inadvertent contaminants because the contaminant specific growth rate and/or nutrient and light requirements are most probably similar to the desired microalgal species under cultivation. Although safeguard methods are available to reduce contamination, these approaches typically result in hindrances via light limitations, nutrient limitations, or some other limitation that can greatly reduce productivity. In addition, the treatment of the source water to a level of purity needed for enclosed reactors can become cost-prohibitive on a commercial level. Attempts to mitigate contaminants by limiting a requirement or overcoming growth rates via manipulation of the culture dilution rate will most often have the same impact on the cultured algae. Therefore, current methods used to reduce contamination problems center on keeping the contaminants out of the cultures through up front sterilization procedures, which can be costly.

Economical, large-scale microalgal culture designs must mesh ‘contaminant tolerance’ techniques as well as contaminant mitigation methods with productivity requirements. Researchers at Louisiana State University developed the Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR) to provide a robust system that superimposes suspended contaminant control on algal productivity. The purpose of this paper is to present this new philosophy for designing commercial, microalgal production systems. The theoretical basis is summarized and a physical description of HISTAR provided, along with illustrative production data.

2. Materials and methods

2.1. Theoretical system description

In operation, algal productivity and contaminant mitigation are accomplished by integrating an enclosed bioreactor (turbidostat) with a series of open-top continuous-flow, stirred-tank reactors (CFSTRs). The turbidostats are completely enclosed and controlled to provide an uni-algal and contaminant-free inoculum to the first CFSTR. By using the enclosed bioreactor as an inoculum source and not for productivity, the volume can be kept small relative to the rest of the culture system, thus, reducing the amount of ‘pristine or sterile’ source water required.

The use of a series of CFSTRs versus a single CFSTR allows for the separation of the dilution rates controlling the individual reactors versus the entire series of reactors. The dilution rate of an individual reactor, or local dilution rate,

$$D_n = Q_T / V_n, \quad (1)$$

where D_n is the local dilution rate for CFSTR n (per day), Q_T is the total flow rate ($Q_{tb} + Q_f$, l per day), Q_{tb} is the flow from turbidostats (l per day), Q_f is the source water flow into CFSTR1 (l per day) and V_n is the volume of CFSTR n (l), can be manipulated such that $D_n > U_c$ (net specific growth rate of contaminant, per day). While contaminant growth may occur within an individual reactor, the cells or

organisms are washed from reactor $n-1$ to reactor n before an exponential increase can occur, thereby, preventing culture takeover and eventual collapse.

The actual growth of the desired microalgal culture is controlled by the system dilution rate,

$$D_s = Q_T / \sum_{n=1}^N V_n, \quad (2)$$

where D_s is the system dilution rate (per day) and N is the total number of CFSTRs. The system dilution rate can be set operationally through the manipulation of D_n within an appropriate range or physically through the addition or removal of CFSTRs. While the number of reactors sets the D_s for a given D_n , careful consideration should be given to determining the number of CFSTRs for a particular application. The desired end result needs to be considered; high productivity and/or optimal biochemical composition. These two do not necessarily correspond to the same hydraulic regime within the system. Productivity increases as D_s increases, up to the 'washout' threshold, at which point, production quickly decreases to a new steady state level reflecting the output from the turbidostats. In contrast, the D_s will decrease as the number of CFSTRs is increased, thus, stagnating the culture in the later reactors and reducing productivity. The biochemical composition of an algal cell can be manipulated by varying environmental and operational factors, including cell residence time. Highly unsaturated fatty acids (HUFAs), which are of main importance in larviculture, tend to increase in overall percentage with longer culture retention times (lower D_s). Subsequently, additional CFSTRs may be warranted.

Eqs. (1) and (2) indicate that any slug input (desired algal species or contaminant) into the first CFSTR will grow as it moves through the system, but is ultimately flushed out the system for the condition $D_n > U$. However, a constant input into CFSTR1 will result in a steady state output from the final reactor. Thus, by providing a continual input of the desired algal species from the turbidostats into CFSTR1, while only allowing for the potential of a one-time pulse or a minutely small continuous input of a contaminant, steady state production of the desired algal species can be achieved with little to no impact from inadvertent suspended contaminants. The turbidostats still are prone to the same contamination issues as any typical continuous culture and must be protected. However, by linking these reactors to the series of CFSTRs, an amplification process is added that allows for the mass production of the algae without the added expense of maintaining sterile conditions throughout the entire system.

Rusch and Malone (1998) and Theegala (1997) performed in-depth mathematical analyses of the theoretical potential of HISTAR to maintain stable microalgal productivity levels while also providing a contaminant tolerant environment. Models were developed to investigate the transitional and steady state growth of microalgae and the transitional behavior of contaminants within the CFSTRs impacted by a one-time pulse (slug) input into any of the CFSTRs and the steady-state behavior of the system under a continuous loading of a contaminant to the first CFSTR

(Theegala et al., 1999). These models were then used to design the physical system discussed in the following sections.

2.2. Physical system description

HISTAR consists of six major components: (1) culture reactors; (2) air injection system; (3) CO₂ injection system; (4) nutrient injection system; (5) lighting (optional); and (6) automation and monitoring system (Figs. 1 and 2). Peripheral components include an algal collection device (centrifuge) and saltwater reconditioning system consisting of a sand filter, reservoir, UV light and cartridge filters (down to 0.2 µm) for water treatment and reuse. The saltwater reconditioning system facilitated reduced artificial salt usage, reducing production costs. A detailed description of the system is provided in Theegala (1997).

The experimental unit described in this paper consists of a 9266-l (2448 gal) HISTAR system interfaced with a continuous-duty centrifuge, a saltwater treatment/recirculation system and a process control unit. Each rectangular-shaped reactor (two turbidostats and six CFSTRs) is 1158 l (306 gal; actual tank capacity) with a rounded bottom to achieve proper hydraulic mixing. The irradiated surfaced area is 1.67 m², and the average culture depth is 0.71 m. All reactors are constructed of food-grade fiberglass and resins, with a white food-grade gel coat on the inside surface to reflect light back into the culture. The turbidostats are covered with clear acrylic (0.64 cm thick) to prevent the entrance of airborne contaminants. During normal operation, positive airflow is maintained using filtered compressed or blown air to prevent outside air from entering the tanks. Culture levels are maintained using a float valve, while a float switch signals the automation system to shut down



Fig. 1. The experimental HISTAR system contains two enclosed turbidostats (foreground) and six open-topped continuous-flow, stirred-tank reactors (CFSTRs, background).

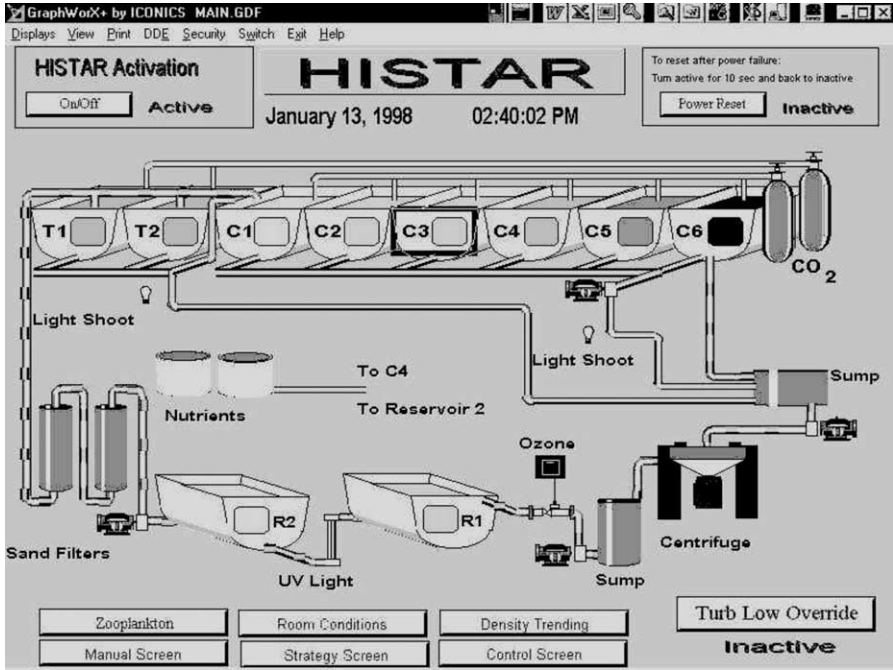


Fig. 2. The process control system consists of a computer, I/O plexors and GENESIS software. The control screen of the GENESIS control software allows the user to quickly assess operating conditions within the system.

operation if the turbidostat water level becomes too low. Microalgae harvested from the turbidostats are transferred through a monitoring unit (Fig. 3) to CFSTR1 via a diaphragm pump. A continuous flow of make-up water from the saltwater treatment

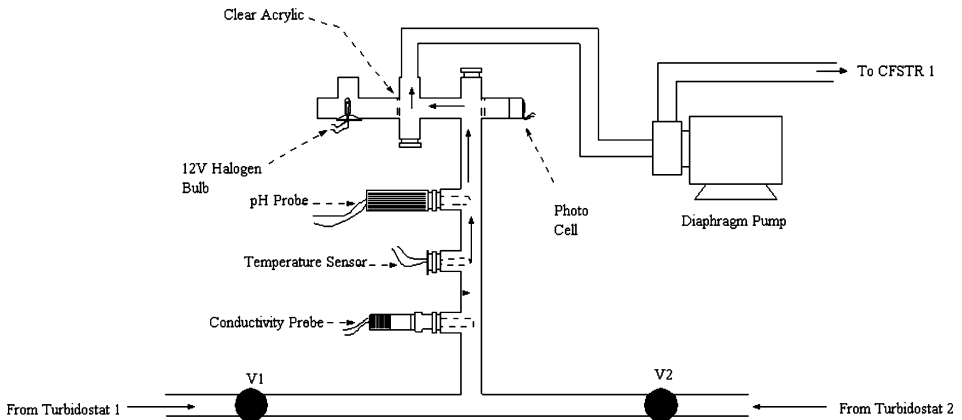


Fig. 3. The monitoring units collect samples from the culture reactors and transmit analog signals for pH, conductivity, temperature and culture density to the process control system.

system is added to CFSTR1, creating the driving force (Q_f) for serial movement of the algae from CFSTR1 to CFSTR6. The CFSTRs are open to the atmosphere and not as tightly controlled as the turbidostats, thereby reducing overall capital and operational costs. Culture conditions within each CFSTR are monitored via a monitoring unit of similar design as that of the turbidostat. Flow from an individual CFSTR into the monitoring unit is controlled by a solenoid valve.

System aeration and circulation are provided through a manifold connected to a 1.0-hp blower equipped with a 0.3 μm HEPA filter to minimize the potential for airborne contaminants. Flow meters, installed above each tank, facilitate measurement and control of airflow rates through air diffuser assemblies located along the bottom of each tank. Carbon dioxide injection into the air diffuser assembly is dependent on the pH measurements collected by the monitoring units and transmitted to the process control and monitoring unit.

Two 114-l nutrient metering tanks provide continuous addition of Galliard's F/2 media to the turbidostats and CFSTR1. Artificial illumination is provided by a 1000-W metal halide lamp suspended 45 cm above each CFSTR reactor, resulting in an average surface photosynthetic photon flux density (PPFD) of approximately 800 $\mu\text{mol s}^{-1} \text{m}^{-2}$. The turbidostats are illuminated by 250-W metal halide lamps over each reactor.

2.3. System operation

HISTAR is configured to operate under conditions similar to those found in a commercial setting. Culture temperature, which is not internally controlled, is dependent on external air temperature and heat inputs from the aeration system. Due to the nature of the design, the cultures are not maintained in an axenic state throughout the system. The system is monitored and controlled with a desktop computer, GENESIS for Windows software (Iconics, Inc., Foxborough, MA), and Dutec I/O plexors (Jackson, MI) for input/output control. GENESIS (Fig. 2) is capable of full system control and data collection, storage, and trending. The process control unit collects data from the two monitoring units servicing the turbidostats and CFSTRs. The turbidostats are monitored with each harvest, while a sample is automatically sent to the monitoring unit from each CFSTR once per hour. The data obtained from both monitoring units includes cell density estimations, pH, temperature and conductivity.

Real-time biomass densities are estimated via a photocell consisting of a 12-V halogen light source and a photovoltaic cell on opposite ends of a culture collection chamber within the monitoring unit. Biomass density estimations are based on a linear relationship between the current (given in millivolts) generated by the photovoltaic cell in response to the light output of the halogen bulb versus a total suspended solids (TSS) measurement. The TSS procedure is modified for saltwater microalgae. The modification includes a pre- and post-rinsing of the filter pad with 30 ml 0.05 M ammonium formate to ensure all salts are dissolved and do not add to the mass of the algae. The biomass density estimates for the turbidostat cultures are used by GENESIS to automatically adjust the harvest from these reactors. Harvest

events occur every twenty minutes per reactor, with the volume harvested determined based on the biomass density estimations. The harvest cycles are staggered, facilitating inoculum input into CFSTR1 every 10 min. The basis for the harvest algorithm is to maintain stable, steady state cultures within the turbidostat, which does not necessarily translate into maximum biomass density. pH measurements for both the turbidostats and the CFSTRs are used to automatically adjust CO₂ additions within the cultures. Temperature and conductivity are monitored, but not used as an automatic control measure. Conductivity measurements were converted to salinity and used to make daily, manual adjustments within the saltwater reservoir.

2.4. System evaluation

Initial system evaluation focused on two areas. The first study was performed to validate the completely mixed design assumption and investigate headloss across the series of CFSTRs. This assumption was experimentally validated through several tracer studies using Rhodamine-WT dye. This study also provided the opportunity to investigate the potential 'washout' capabilities of the series of CFSTRs (assuming a no growth term). CFSTR1 was hydraulically isolated from CFSTRs 2–6 and used as the control tank. The flushing flow (Q_f) entering CFSTR2 was set at 5.8 l min⁻¹, with the resulting theoretical $D_n = 8.0$ per day (culture volume = 1044 l). Once the system had hydraulically reached steady state, 9.5 ml of Rhodamine-WT dye was introduced into CFSTRs 1 and 2. Samples were collected from each reactor every 3 h and measured for fluorescence until values dropped below the detection limits of the fluorometer. A calibration curve was developed to convert fluorescence to dye concentration (ml-dye l⁻¹). Dye concentrations were converted to percent of initial concentration ($C_o = 100$). The concentrations were also adjusted for the varying volumes of each reactor created through headloss. A model was developed to predict the transitional concentration within each reactor. The theoretical and experimental data were plotted, and a standard error of prediction was calculated to estimate fit of the model and validate the completely mixed assumption.

The second area of focus was the investigation of system behavior during full, automatic operation. While this study did not focus on system optimization, the ultimate goal is for at least a 10-fold increase in daily production (mass per day) between the turbidostat and CFSTRs. The study was performed using *Isochrysis* sp. (C-iso) as a representative algal species used in the aquaculture industry. Each turbidostat was inoculated with 12 l of C-iso and allowed to acclimate for 1–2 day(s) prior to activation of the process control system. The flushing flow (Q_f) from the saltwater reservoir into CFSTR1 was set at 1.97 l min⁻¹, resulting in an approximate $D_n = 2.91$ per day and $D_s = 0.49$ per day (culture volume = 975 l). Salinity was maintained at 16‰ (Instant Ocean®). Nutrients were dosed to the turbidostats with each harvest and to CFSTR1 continuously, resulting in an average nitrogen and phosphorus concentration of 10 and 2 mg l⁻¹, respectively. The target CFSTR pH of 7.6 was maintained through CO₂ additions every 10 min. The turbidostats were harvested automatically every 20 min on an alternating basis, with the harvest time

ranging from 20 to 50 s determined from recent past culture conditions. No attempt was made to optimize biomass density or productivity.

Data collected by the process control/monitoring unit were used to calculate actual turbidostat and total flows, local and system dilution rate and retention time, turbidostat biomass concentrations and mass transfer rates and system harvested biomass and areal system productivity.

Areal system productivity was calculated as a function of algal biomass and carbon fixation rate:

$$P_a = \frac{\text{TSS}(d)}{\tau_s}, \quad (3)$$

$$P_{ac} = \frac{\text{TSS}(d)}{2.79\tau_s}, \quad (4)$$

where, P_a is the daily areal productivity based on algal biomass (g dry biomass m^{-2} per day), TSS is the average daily biomass concentration (dry biomass) in CFSTR6, d is the mean culture depth (0.71 m), τ_s is the system retention time ($1/D_s$), P_{ac} is the daily areal productivity based on carbon fixation rate (g C m^{-2} per day) and 2.79 is the approximate conversion between dry biomass and carbon content of a typical algal cell (Stumm and Morgan, 1996).

Overall daily arithmetic means and standard errors were calculated for biomass density, harvested biomass, areal system productivity, pH, temperature and salinity. Overall arithmetic means and standard deviations were calculated for the turbidostat and flushing flowrates. Significant differences between CFSTRs 1–6 for pH, temperature and salinity were investigated using single-factor ANOVA. The results were considered significant for $P < 0.05$.

3. Results and discussion

3.1. Dye study

The experimental data collected from the dye study matched theoretical values to within a standard error of prediction of 0.014, indicating that the reactors do behave as completely mixed systems (Fig. 4). These results will permit all subsequent system modeling and design to assume completely mixed conditions. During the course of the study, a 15.6 cm headloss developed across the six CFSTRs, resulting in a liquid volume ratio for CFSTR6:CFSTR1 of 0.66. Subsequently, the theoretical D_n was adjusted to account for the varying liquid volumes. The adjusted D_n equaled 9.7 ± 1.58 per day or 21% greater than the desired local dilution rate. These data were used to make design modifications within the piping system connecting each of the CFSTRs. The initial diameter of the interconnecting pipes was 1.91 cm, resulting in a flow velocity of 24 cm s^{-1} . The pipe diameter was increased to 2.53 cm, resulting in a flow velocity of 19.1 cm s^{-1} and a headloss of $< 3.81 \text{ cm}$ across the series of CFSTRs. The initial design philosophy was to maintain velocities within the

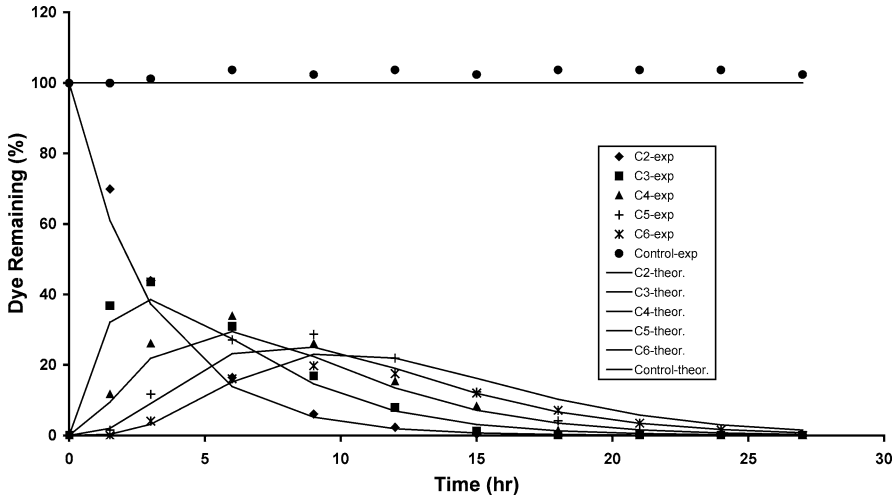


Fig. 4. The dye study resulted in a standard error of prediction of 0.014 between the theoretical and experimental analyses. These results validate the completely-mixed assumption of the culture reactors.

connecting pipes $> 10 \text{ cm s}^{-1}$ to prevent the upstream migration (or recontamination of CFSTR $n-1$) of inadvertent contaminants.

3.2. Production run evaluation

The *Isochrysis* sp. (C-iso) study was conducted for 3 weeks. During the 1st week, several power outages occurred, resulting in discontinuous data collection. Thus, several days have been excluded from the analyzed data sets.

The turbidostats were harvested every 20 min, with the volume harvested dependent on the culture density over several harvest events. Over the course of the study, the two turbidostats did not behave similarly. Turbidostat one (T1) was harvested at a mean of $23.2 \pm 13.55 \text{ s}$ per harvest cycle for a mean flow rate (Q_{tb}) of 300 l per day. This corresponds to a mean daily culture retention time and algal transfer to CFSTR1 of 2.91 day and 6.8 g per day, respectively. Turbidostat two (T2) was harvested at a mean of $48.7 \pm 8.44 \text{ s}$ for a mean flow rate (Q_{tb}) of 573 l per day and a mean daily culture retention time of 1.52 day. The corresponding algal transfer from T2 to CFSTR1 was 36.3 g per day. The algal culture in T1 never achieved the same biomass densities as T2. The reason for this is possibly attributed to light bleed over from CFSTR1 to turbidostat two. While the two cultures varied in productivity, the data indicate the ability of the process control algorithms to maintain steady state biomass levels within each reactor via the manipulation of the volume harvested per harvest event.

The combined T1 and T2 flow (873 l per day) into CFSTR1 represented 23.5% of the total flow ($Q_T = Q_{tb} + Q_f = 3710 \text{ l per day}$) through the system. The local (D_n) and system (D_s) dilution rates approximated at the start of the study using Q_f were recalculated to include the contribution of the turbidostats. The recalculation

resulted in a D_n and D_s of 3.81 per day ($\tau_n = 0.26$ day or 6.3 h) and 0.64 per day ($\tau_s = 1.57$ day), respectively. The relative impact of the turbidostat flow on local and system dilution rates is mainly determined by Q_f . As Q_f increases, the percent impact of the turbidostat decreases, resulting in dilution rates closer to the initial target values.

The series of CFSTRs function as an amplification unit, with increasing biomass observed as the culture moves through the system (Fig. 5). The system did behave as anticipated, with each successive reactor having a greater biomass concentration than the preceding one. Under ideal conditions, the difference in biomass concentration between CFSTR1 and CFSTR6 would be maximized to optimize productivity. On Day 1 (after system acclimation), the CFSTR6/CFSTR1 biomass concentration ratio was 15.8. By the end of the study, the ratio had decreased to 1.3. While it would be impossible to maintain the same ratio throughout the entire production period due to physiological limitations of the algae, decreasing the impact of controllable operational parameters such as nutrients, light (to a point), temperature and pH would result in minimizing decreasing biomass ratios through the system. The ultimate result would be a decrease in unit production.

The daily harvest from CFSTR6 averaged 1454 (wet weight) or 291 g (dry weight). Daily mean harvest based on the average steady state biomass density in CFSTR6 ($105.5 \pm 6.71 \text{ mg l}^{-1}$, Fig. 5) and Q_T results in a calculated harvest of $392.1 \pm 24.90 \text{ g}$. The difference between actual and calculated harvests may be attributed to the accuracy of the photocell (approximately $\pm 10\%$) and the incomplete removal of all of the algae from the centrifuge bowl. The calculated harvest based on mean algal concentration in CFSTR6 represents more than a 15-fold increase in harvest over that obtained from the turbidostats, demonstrating the amplification potential of the CFSTRs.

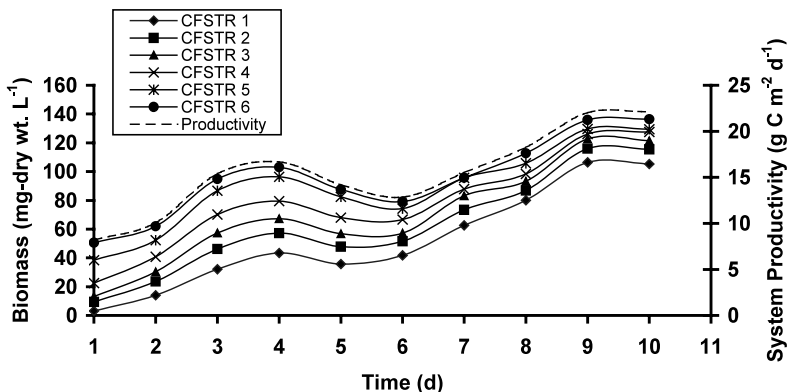


Fig. 5. The density in each of the six CFSTRs was estimated by the photocell located in the monitoring unit. The plotted values represent daily means.

Areal system productivity averaged 47.8 ± 3.04 g dry biomass m^{-2} per day (17.1 ± 1.09 g C m^{-2} per day, Fig. 5) for a system retention time of 1.57 days, culture depth of 0.71 m and surface irradiance of $800 \mu mol s^{-1} m^{-2}$. While different algal species will have varying physiological characteristics that directly impact potential productivity, global comparisons can be made between different production systems using areal productivity values. The daily areal productivity level observed in this study is in range with values reported in literature for various reactor designs operated under widely varying conditions and culturing a broad spectrum of algal species (Hai et al., 2000; Drapcho and Brune, 2000; Zou and Richmond, 1999; Muller-Fuega et al., 1998; Watanabe and Hall, 1996; Goldman, 1979).

Each CFSTR was monitored every hour. Salinity, temperature and pH are illustrated as daily means for the culture period (Fig. 6), while the overall means and standard errors are presented in Table 1. No significant differences existed between the CFSTRs for salinity ($P = 0.4726$), temperature ($P = 0.1577$) and pH ($P = 0.2512$). The target salinity was 16‰, with adjustments being made to the saltwater reservoir on a daily basis. For the majority of the study, the salinity remained stable. The deviations exhibited during the first few days of the study reflect initial salinity levels that were slightly higher than the target (CFSTRs 4–6) and a correction to bring the salinity back in line with the target (CFSTRs 1–3). The response is reflective of the 1.57-day lag between CFSTR1 and CFSTR6. Temperature was monitored, but not controlled. The increase in culture temperature followed by the decrease mimics the pattern observed for the room temperature. When the metal halide lamps were activated at the start of the study, the room temperature started to increase due to poor room ventilation. The culture temperature exhibited a lag in air temperature rise by approximately 2 days. Fig. 6 also illustrates a decline in culture temperature as the algae move through the CFSTRs. The water entering CFSTRs, in combination with the algal culture from the turbidostats, acts to heat the culture in the first several CFSTRs due to heat input from the pumps and the greenhouse effect of the covers of the turbidostats.

Carbon dioxide addition occurred every ten minutes based on the mean pH level recorded for CFSTRs 1–6 during the previous 1-h period. As indicated by Fig. 6, the effect of biomass density on pH was minimal across the CFSTRs. The pH in CFSTR1 and CFSTR6 averaged 7.61 ± 0.110 and 7.63 ± 0.131 , respectively. pH in CFSTR6 was 0.02 units higher and slightly more variable than that observed in CFSTR1. However, the differences were not significant ($P < 0.05$). The target pH at the start of the study was 7.6, and the overall pH across the series of CFSTRs was only 0.66% greater than this target. These data indicate the capabilities of the process control system in maintaining consistent environmental conditions within the CFSTRs.

The results presented clearly indicate the potential of HISTAR for stable, continuous production of microalgae. These results and this paper provide a foundation for further investigations focused on optimizing system design and operating conditions, which will lead to increased photosynthetic efficiency and reduced production costs.

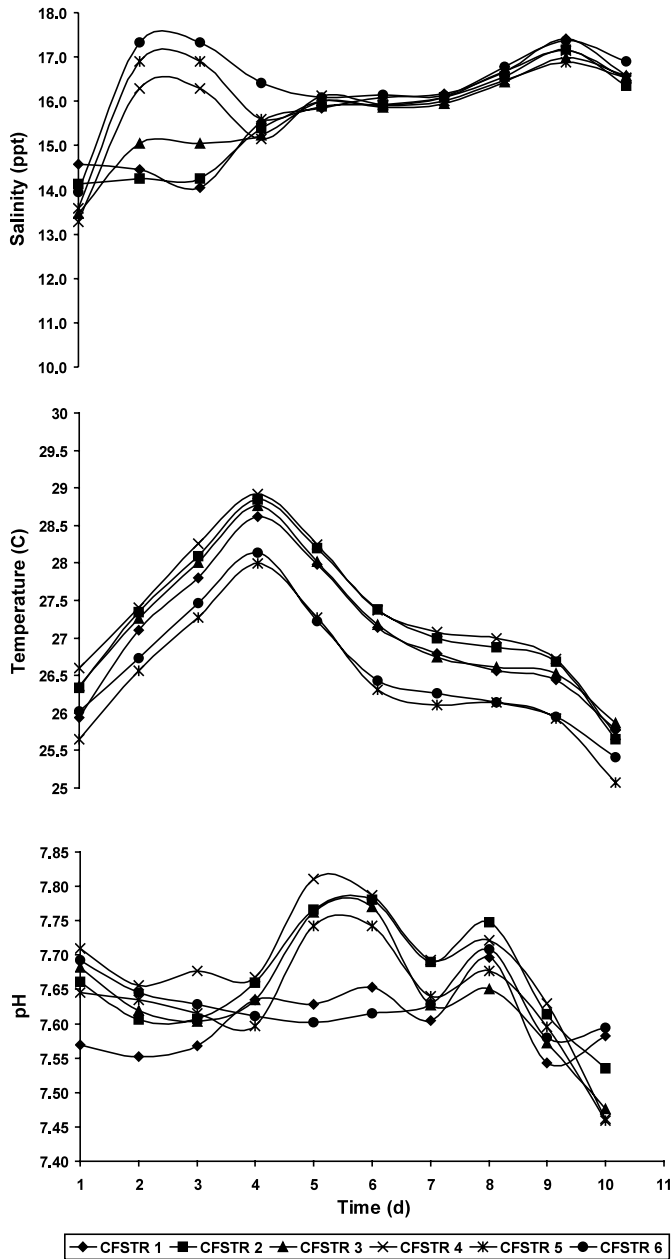


Fig. 6. Salinity, temperature and pH were monitored by the process control system. Salinity and temperature were not controlled, while pH was maintained by continual carbon dioxide addition. The plotted values represent daily means.

Table 1

Salinity, pH and temperature within each CFSTR were monitored by the process control system every hour

CFSTR #	Salinity (ppt)	Temperature (°C)	pH
1	15.7±0.34	27.0±0.29	7.60±0.016
2	15.6±0.31	27.2±0.30	7.67±0.25
3	15.7±0.22	27.1±0.28	7.65±0.027
4	15.9±0.17	27.3±0.30	7.68±0.30
5	16.1±0.16	26.4±0.27	7.63±0.026
6	16.4±0.17	26.6±0.26	7.63±0.013

The data presented are representative of the overall mean and standard error ($n = 10$).

Acknowledgements

This work was funded by the National Coastal Research and Resource Development Institute (Contract #AQ97.053-7404-02).

References

- Apt, K.E., Behrens, P.W., 1999. Commercial developments in microalgal biotechnology. *J. Phycol.* 35, 215–226.
- Barwell, C.J., 1994. Pharmacologically-active amines in some marine algae and algal food products. *J. Home Consum. Hortic.* 1, 77–82.
- Ben-Amotz, A., Avron, M., 1989. The biotechnology of mass culturing *Dunaliella* for products of commercial interest. In: Cresswell, R.C., Rees, R.C., Shah, T.A.V. (Eds.), *Algal and Cyanobacterial Biotechnology*. Longman, Harlow, pp. 90–114.
- Benemann, J.R., 1992. Microalgae aquaculture feeds. *J. Appl. Phycol.* 4, 233–245.
- Borowitzka, M.A., 1988. Vitamins and fine chemicals from micro-algae. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), *Micro Algal Biotechnology*. Cambridge University Press, pp. 169–174.
- Borowitzka, M.A., 1992. Algal biotechnology products and processes-matching science and economics. *J. Appl. Phycol.* 4, 267–279.
- Cahu, C., Infante, J.Z., 2001. Substitution of live food by formulated diets in marine fish larvae. *Aquaculture* 200, 161–180.
- Centeno, P.O., Ballantine, D.L., 1999. Effects of culture conditions on production of antibioticly active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). I. Light. *J. Appl. Phycol.* 11 (2), 217–224.
- Coutteau, P., Lavens, P., Sorgeloos, P., 1990. Baker's yeast as a potential substitute for live algae in aquaculture diets: *Artemia* as a case study. *J. World Aqua. Soc.* 21, 14–17.
- DePauw, N., Morales, J., Persoone, G., 1984. Mass culture of microalgae in aquaculture systems: progress and constraints. *Hydrobiologia* 116–117, 121–124.
- Drapcho, C.M., Brune, D.E., 2000. The partitioned aquaculture system: impact of design and environmental parameters on algal productivity and photosynthetic oxygen production. *J. Aqua. Eng.* 21, 151–168.
- Goldman, J.C., 1979. Outdoor algal mass cultures-II. Photosynthetic yield limitations. *Water Res.* 13, 119–136.
- Goldman, J.C., 1980. Physiological aspects in algal mass cultures. In: Shelef, G., Soeder, C.J. (Eds.), *Algae Biomass, Production and Use*. Elsevier, Amsterdam, pp. 343–359.

- Hagiwara, A., Gallardo, W.G., Assavaaree, M., Kotani, T., de Araujo, A.B., 2001. Live food production in Japan: recent progress and future aspects. *Aquaculture* 200, 111–127.
- Hai, T., Ahlers, H., Gorenflo, V., Steinbuchel, A., 2000. Axenic cultivation of anoxygenic phototrophic bacteria, cyanobacteria, and microalgae in a new closed tubular glass photobioreactor. *Appl. Microbiol. Biotechnol.* 53, 383–389.
- Holan, Z.R., Volesky, B., 1994. Biosorption of lead and nickel by biomass of marine algae. *Biotechnol. Bioeng.* 43, 1001–1009.
- Holt, G.J., 1993. Feeding larval red drum on microparticulate diets in a closed recirculating water system. *J. World Aqua. Soc.* 24, 225–230.
- Heussler, P., Castillo, S., Merino, F.M., 1978. Parasite problems in the outdoor cultivation of *Scenedesmus*. *Arch. Hydrobiol. Beih.* 11, 223–227.
- Jones, D.A., Kamarudin, M.S., LeVay, L., 1993. The potential for replacement of live feeds in larval culture. *J. World Aqua. Soc.* 24, 199–210.
- Kreitlow, S., Mundt, S., Lindequist, U., 1999. Cyanobacteria—a potential source of new biologically active substances. *J. Biotech.* 70, 61–63.
- Lazo, J.P., Dinis, M.T., Holt, G.J., Faulk, C., Arnold, C.R., 2000. Co-feeding microparticulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). *Aquaculture* 200, 339–351.
- LeRuyet, J.P., Alexandre, J.C., Thebaud, L., Mugner, C., 1993. Marine fish larvae feeding: formulated diets or live prey? *J. World Aqua. Soc.* 24, 211–224.
- Liehr, S.K., Chen, H.-J., Lin, S.-H., 1994. Metals removal by algal biofilms. *Water Sci. Tech.* 30 (11), 59–68.
- Lincoln, R.A., Strupinski, K., Walker, J.M., 1990. Pharmacologically active compounds from algae. *Br. Phycol. J.* 25, 92.
- Miron, A.S., Gomez, A.C., Camacho, F.G., Grima, E.M., Chisti, Y., 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *J. Biotech.* 70, 249–270.
- Molina-Grima, E., Fernandez, F.G.A., Camacho, F.G., Chisti, Y., 1999. Photobioreactors: light regime, mass transfer, and scale up. *J. Biotech.* 70, 231–247.
- Moreno-Garrido, I., Canavate, J.P., 2001. Assessing chemical compounds for controlling predator ciliates in outdoor mass cultures of the green algae *Dunaliella salina*. *J. Aqua. Eng.* 24, 107–114.
- Muller-Fuega, A., Le Guedes, R., Herve, A., Durand, P., 1998. Comparison of artificial light photobioreactors and other production systems using *Porphyridium cruentum*. *J. Appl. Phycol.* 10, 83–90.
- Ogbonna, J.C., Soejima, T., Tanaka, H., 1999. An integrated solar and artificial light system for internal illumination of photobioreactors. *J. Biotech.* 70, 289–297.
- Rusch, K.A., Malone, R.F., 1998. Microalgal production using a hydraulically integrated serial turbidostat algal reactor (HISTAR): a conceptual model. *J. Aqua. Eng.* 18, 251–264.
- Shields, R.J., 2001. Larviculture of marine finfish in Europe. *Aquaculture* 200, 55–88.
- Stumm, W., Morgan, J.J., 1996. *Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters*, 3rd ed.. Wiley-Interscience, New York, p. 1022.
- Takeuchi, T., 2001. A review of feed development for early life stages of marine finfish in Japan. *Aquaculture* 200, 203–222.
- Talbot, P., Thebault, J.M., Dauta, A., 1991. A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. *Water Res.* 25, 465–473.
- Tang, Y.-Z., Gin, K.Y.H., Aziz, M.A., 2002. Equilibrium model for cadmium adsorption by green algae in a batch reactor. *J. Environ. Eng.* 128 (4), 304–312.
- Theegala, C.S., 1997. A computer automated hydraulically integrated serial turbidostat algal reactor (HISTAR): mathematical modeling and experimental analysis, Louisiana State University, Ph.D. dissertation.
- Theegala, C.S., Malone, R.F., Rusch, K.A., 1999. Contaminant washout in a hydraulically integrated serial turbidostat algal reactor (HISTAR). *J. Aqua. Eng.* 19, 223–241.

- Walford, J., Lim, T.M., Lam, T.J., 1991. Replacing live foods with microencapsulated diets in the rearing of Seabass (*Lates calcarifer*) larvae: do the larvae ingest and digest protein membrane microcapsules? *J. Aqua.* 69, 105–113.
- Watanabe, Y., Hall, D.O., 1996. Photosynthetic production of the filamentous cyanobacterium *Spirulina platensis* in a cone-shaped helical tubular photobioreactor. *Appl. Microbiol. Biotechnol.* 44, 693–698.
- Yamaguchi, K., 1997. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *J. Appl. Phycol.* 8, 487–502.
- Zou, N., Richmond, A., 1999. Effect of light path length in outdoor flat plate reactors on output rate of cell mass and of EPA in *Nannochloropsis* sp. *J. Biotech.* 70, 351–356.