AN ABSTRACT OF THE THESIS OF

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Dr. Gregory Rorrer

The cultivation of a cell suspension culture of the brown alga *L. saccharina* and a tissue culture of the green alga *Acrosiphonia coalita* were investigated in both 280 mL and 900 mL bubble-column photobioreactors at 13-14 °C using CO_2 in air as the sole carbon source for growth.

Growth rate and biomass productivity data for *L. saccharina* female gametophyte cell cultures were obtained over a broad range of process conditions, including initial cell density (27 to 200 mg DCW/L), initial nitrate concentration (0 to 508 mg $NaNO_3$/L), aeration rate (0.17 to 2.00 vvm), and incident light intensity (90 to 8600 lux). Maximum cell densities exceeding 1100 mg DCW/L could be attained within a 20 day cultivation period. Both specific growth rate and final biomass density data were correlated to incident light intensity using an exponential type model. Incident light intensity below 3500 lux limited the specific growth rate and final biomass density. Initial cell density had a significant effect on the final biomass density and no significant effect on the specific growth rate. The aeration rate affected the biomass growth rate and final biomass density, but the culture was not CO_2-transport limited. Initial nitrate concentrations above 64 mg $NaNO_3$/L in the GP2 medium had no significant effect on the specific growth rate and final biomass density, a result supported by biomass stoichiometry calculations.

Photolithotrophic cultivation of *Acrosiphonia coaltia* tissue cultures in the 280 mL bubble-column photobioreactor resulted in a maximum cell density of 630 mg DCW/L within a 20 day cultivation period at 12 °C and 4000 lux incident light intensity. However,

the cells clumped and settled in the photobioreactor. Based on the final cell density measurements, there was no advantage for bioreactor cultivation compared with static flask cultivation.

Cultivation of *Laminaria saccharina* Gametophyte Cell Cultures and *Acrosiphonia coalita* Tissue Cultures in a Bubble-Column Photobioreactor

by

Chunxing Zhi

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Major Professor, representing Chemical Engineering

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Chair of Department of Chemical Engineering

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NOMENCLATURE

C_{CO2}	Dissolved CO_2 concentration in the medium, mmole CO_2/L
C^*_{CO2}	Dissolved CO_2 concentration in equilibrium with the partial pressure of CO_2 in the aerating gas stream, mmole CO_2/L
D_{665}	Absorbance at light wavelength 665 nm
D_{652}	Absorbance at light wavelength 652 nm
D_{CO2}	Diffusion coefficient of CO_2 in liquid medium, cm^2/sec
D_{O2}	Diffusion coefficient of O_2 in liquid medium, cm^2/sec
I_k	Light intenisty at which the extended initial slope of the light curve intersects with μ_{max}, lux
I_k'	Light intenisty at which the extended initial slope of the light curve intersects with X_{fm}, lux
I_o	Incident light intensity, lux
k_La	Oxygen mass transfer coefficient for dissolved O_2, hr^{-1}
$(k_La)_{CO2}$	CO_2 volumetric mass transfer coefficient for dissolved CO_2, hr^{-1}
m_f	Dry weight of filter, filtered and washed biomass, g
m_{GP2}	Dry weight of 20 mL GP2 salt content on the filter, g
m_i	Dry weight of filter, g
P_g	Gas power input, W
Q_{CO2}	Volumetric CO_2 consumption rate, mole CO_2/L-hr
r_x	Growth rate of biomass, mg DCW/L-day
v_o	Aeration rate, ml/min
V_o	Volume of inoculum mixture, mL
V_m	Volume of GP2 medium, mL
V	Culture sample volume, mL
V_s	Superficial gas velocity, m/sec
V_t	Total culture volume in bioreactor, mL
X	Culture cell density, mg DCW/L

X_o	Initial dry cell density of the culture, mg DCW/L
$X_{c,o}$	The dry cell density of inoculum mixture, mg DCW/L
X_f	Final biomass density, mg DCW/L
X_{fm}	Maximum stationary phase biomass density at light saturation, mg DCW/L
$Y_{x/CO2}$	Growth yield coefficient based on CO_2, mg DCW/mole CO_2
$Y_{x/NO3}$	Growth yield coefficient based on nitrate, mg DCW/mole NO_3
β	Ratio of available nitrate in the medium to the nitrate consumed by the culture
μ	Specific growth rate, day^{-1}
μ_{max}	Specific growth rate at saturation, day^{-1}

Cultivation of *Laminaria saccharina* Gametophyte Cell Cultures and *Acrosiphonia coalita* Tissue Cultures in a Bubble-Column Photobioreactor

INTRODUCTION

Marine algae are one of the large plant families which contains over 1800 genera and 21,000 species and live in the oceans which cover approximately 71% of our earth's surface (Alexopoulos et al., 1967). Since the inter-species competition for space, nutrients, and light are fierce in the marine environment, marine plants have evolved unique chemical defense mechanisms to enhance survival or ward off predators. These chemical defense mechanisms are often the source of structurally unique nature products (Gerwick, 1987). One of the very important groups of natural metabolic products to find wide distribution in the marine algae are eicosanoids, which are 20-carbon fatty acids with at least one site of oxidation in addition to the carboxyl group. Recently, a metabolic pathway known as the arachidonic acid cascade has been discovered in some red and brown macroalgae (Gerwick et al., 1990). In the arachidonic acid cascade, arachidonic acid is enzymatically oxidized to form eicosanoids. These important bioactive compounds contain novel structural features which represent unique analogs of physiological important mammalian eicosanoids (Gerwick and Bernart, 1993). In fact, many commercial drugs are manufactured from eicosanoids including prostaglandins, thromboxanes, prostacylins, and leukotrienes (Nelson et al, 1982).

Traditionally, there are two ways of approaching the manufacture of natural products from plants. The first is to attempt the chemical synthesis of the desired biomedicinal. However, it is too difficult or too expensive to chemically synthesize biomedicinals despite the substantial advances in modern synthetic chemistry. The second approach is to farm the macroalga that produce the natural products in ocean and then extract the desired products from the harvested algae. Although this approach is industrially feasible for some natural products such as polysaccharides, most secondary

metabolites are usually in trace concentrations within the intact macroalgal plant. For example, eicosanoids typically constitute less than 5% of the lipid extract (Gerwick et al., 1990). Therefore, extraction of these secondary metabolites is very difficult and economically prohibitive.

A new approach for producing marine plant secondary metabolites is to cultivate the macroalga as a liquid cell suspension within a bioreactor under controlled conditions. This approach has three significant advantages. First, marine plants cells can be cultivated anywhere without restriction of weather or geographical conditions. Second, the biomass and product yields can potentially be controlled and optimized by manipulating the bioreactor cultivation parameters, such as medium composition, light intensity and aeration rate. Third, some secondary metabolites can be produced in higher quantities in suspension culture than that in the whole plant.

Despite the aforementioned advantages, there are no published reports on the cultivation of macroalgal cell suspension cultures in bioreactors to produce the biomedicinals. The reason is the biomedicinal discovery in seaweeds and cell culture methodology for seaweeds, the two areas underlying the bioprocess development of macroalgal cultivation for biomedicinals, have advanced significantly only in the past ten years. In general, cell cultures derived from marine seaweeds are established using techniques adapted from terrestrial plant cell and tissue culture. Cell cultures from a few species of red macroalgae have been developed by callus induction and protoplast isolation (Tait et al., 1990; Chen, 1989; Liu and Kloareg, 1991). However, these cultures are difficult to initiate, and the techniques are underdeveloped (Bulter and Evans, 1990). A new cell culture system for marine macroalgae that circumvents unproven callus induction and protoplast isolation techniques involves the isolation and culture of microscopic gametophyte cells. The isolation of gametophyte cells is relatively simpler than the callus induction and protoplast isolation. Some brown macroalgae of order Laminariales possess a reduced gametophyte life phase which can approximate the properties of a liquid cell suspension. Although unstudied, it is possible to use gametophytes of certain brown macroalgae to serve as cell suspension cultures for bioreactor cultivation. The female gametophytes from the brown alga *Laminaria*

saccharina are of particular interest because the parent plant is known to produce 15-lipoxygenase metabolites (Proteau and Gerwick, 1993). These cultures are photolithtrophic and possess a filamentous morphology.

It also may be possible to circumvent the development of cell suspension culture and consider the development of a tissue culture for bioreactor cultivation studies. *Acrosiphonia coalita* tissue culture is an ideal candidate for two reasons. First, it produces 15-lipoxygenase metabolites (Bernart et al., 1993). Second, it possesses a fine filamentous structure. The filaments can be easily isolated and potentially cultured as a semi-differentiated filamentous tissue suspended in liquid medium. In order to study the bioreactor cultivation of macroalgal cell cultures, *Laminaria saccharina* female gametophyte cell culture and *Acrosiphonia coalita* tissue culture will be cultivated in bubble-column photobioreactors. The specific objectives of this study are to:

1) Develop suitable bubble-column photobioreactors for *Laminaria saccharina* female gametophyte cell cultures and *Acrosiphonia coalita* tissue cultures.
2) Study the effect of initial cell density, aeration rate, initial nitrate concentration, and incident light intensity on the biomass growth kinetics of *Laminaria saccharina* female gametophyte cell cultures in 280 mL and 900 mL bubble-column photobioreactors.
3) Demonstrate the cultivation of *Acrosiphonia coalita* tissue culture suspension in a 280 mL bubble-column photobioreactor and compare the biomass productivity with static flask cultivation.

LITERATURE REVIEW

The following literature review focuses on gametophyte cell culture of *Laminaria saccharina* and photolithtrophic cultivation of marine microalgae in illuminated bioreactors.

Laminaria saccharina Gametophyte Culture

The lifecycle of *Laminarina saccharina* is shown in Figure 1. The algae alternates between a large sporophyte and microscopic gametophyte. The sporophyte is highly differentiated, but the gametophyte grows in a filamentous manner and remains relatively undifferentiated. Within sporangia on the central region of the sporophyte blade, meiosis occurs releasing zoospores. These spores then develop into either male or female gametophytes. The male gametophytes generate sperm from the antheridiom and the female gametophyte generates one egg on the oogonium. Once the sperm fertilizes the eggs, the sporophyte stage starts once again.

Basic techniques for isolation of gametophytes from Laminarina are described by Luning and Neushul (1978) and Luning (1980). Steele and Thursby (1988) showed that *L. saccharina* gametophytes could be cultured successfully in GP2 artificial seawater medium at a temperature of 12-15 °C. The GP2 medium contains no iron. This lack of iron inhibits gametophyte gametogenesis and egg formation, and thus provides faster vegetative growth (Montomura and Sakai, 1984).

Luning and Neushul (1978) studied the light and temperature demands for growth and reproduction of Laminarina gametophytes. They reported that the gametophytes of nine Laminarina species became fertile in the unicellular stage (female gametophytes) or in a few-celled stage (male gametophytes), when appropriate temperatures and a sufficiently high quantum irradiance in the blue part of the spectrum were supplied. Vegetative growth, leading to the formation of filamentous gametophytes was light-saturated at a

Life Cycle of Laminaria

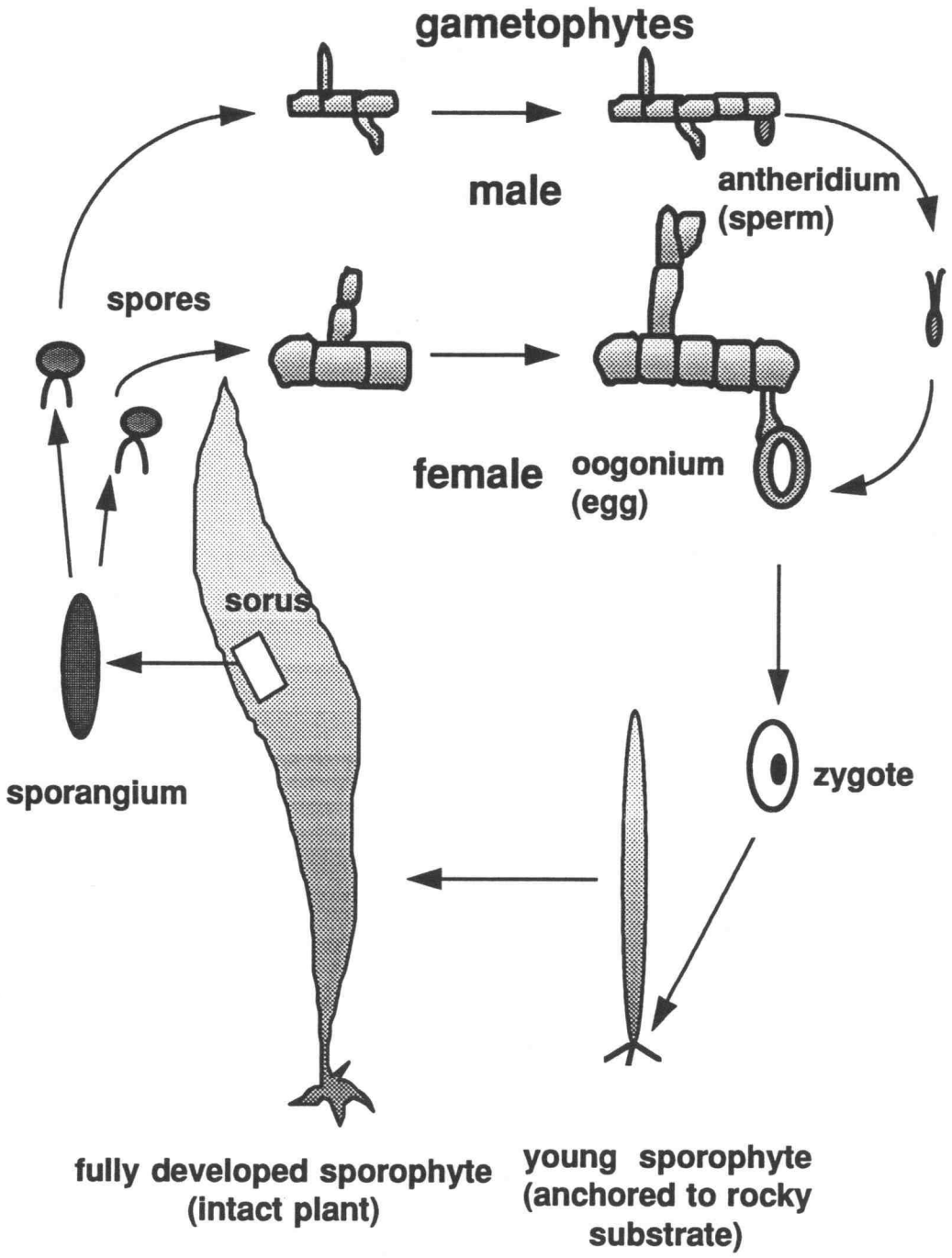

Figure 1. Lifecycle of the brown macroalga *Laminaria saccharina*.

relatively low illuminance of 1000 lux, whereas 2000-3000 lux (fluorescent cool white light) was needed to induce the majority of the gametophytes to become fertile. An illuminance of 8300 lux did not inhibit the development of the gametophyte from southern Californian species. Laminarina gametophytes of species from central and southern California differed in regard to their temperature optimum for growth (12-17 °C) and their upper temperature limit for reproduction (17 - 20 °C), depending on the location of their habitat.

Luning (1980) cultivated gametophytes of three Laminarina species, including *Laminarina saccharina*, in the 12:12 LD light fields at different temperature (from 2 °C to 21 °C). The results showed that the rate of vegetative growth did not depend on spectral distribution, and was light-saturated at 1000-1500 lux. However, the growth rate increased with increasing temperature up to 15 °C, and the decreased from 15 °C to 21 °C. At 22 °C, the gametophytes died. The cultivation of *Laminarina saccharina* exhibited the highest tolerance toward temperature, light intensity and UV spectral radiation.

Davison et al. (1991) investigated the temperature acclimation of respiration and photosynthesis in the sporophytes of brown alga *Laminarina saccharina*. Sporophytes grown at 15 °C contained significantly more chlorophyll a (chl a) than did similar sporophytes grown at 5 °C. The increase in chl a within sporophytes cultivated at 15 °C were associated with increased photosynthetic efficiencies and reduced respiration rates.

Photobioreactor Cultivation of Microalgae

There are no previous reports on bioreactor cultivation of sporophytic or gametophytic macroalgal cell suspensions. Therefore, bioreactor cultivation of microalgal cultures is reviewed since microalgae are photolithtrophic and may possess similar cell morphologies to macroalgae. The review specifically focuses on the effects of initial cell density, aeration rate, nitrate concentration, and light intensity on the microalgal culture growth in photobioreactors.

Microalgae dominate the open waters of the oceans and large lakes (Geider, 1992). There is significant interest in developing high-density microalgal cultures to produce high-value products, such as pharmaceuticals and genetically-engineered products (Javanmardian et al., 1991). The increased interest in the commercial exploitation of microalgae has led to a requirement for more efficient, economical and controllable algal biomass production systems, including closed-system photobioreactors.

Chrismadha et al. (1994) investigated the effect of cell density and light intensity on the growth and eicosapentaenoic acid (EPA) production of the diatom *Phaeodactylum tricornutum* in a helical tubular photobioreactor. They reported that algal growth rate decreased with increasing cell density up to a stage where the culture became unstable at constant incident light intensity. They proposed that light attenuation through the dense culture set the upper limit of operational maximum cell density. Higher incident light intensities increased this upper limit and also increased the growth rate. Further, their study showed that biomass productivity and EPA productivity could be maximized by optimizing cell density with respect to illumination intensity.

The effect of aeration rate on the growth rate of the microalga *Isochrysis galbana* was studied by Grima et al.(1992). Sanchez et al.(1993) performed similar studies for the microalga *Skeletonema costatum.* Grima et al. found that at aeration rates below 2.50 vvm, growth was limited by the CO_2 transferred to the cells, but at higher flow rates the transfer of CO_2 to culture medium was not limiting. However the excessive turbulence at high aeration rates may have produced some cell damage, especially to *I. galbana* which is a fragile flagellate. Sanchez et al. reported that the most favorable conditions for biomass production of *Skeletonema costatum* were achieved at an aeration rate of 1.5 vvm. They used a model analogous to the substrate-inhibition model to predict the variation of specific growth rate with the aeration rate.

Grima et al. (1992) investigated the effect of nitrate concentration on the EPA productivity and growth rate of microalga *Isochrysis galbana.* They found the specific growth rate is limited at initial nitrate concentrations below 0.5 mM. Above this concentration the specific growth rate was constant at 0.032 h^{-1}. As the nitrate availability increased, the final concentration of cells in the stationary phase increased. In a recent

review, Turpin (1991) stated that as growth becomes limited by any resource other than light, there is a decrease in the apparent quantum yield of cell-specific photosynthesis. Limitation of algal growth by N availability results in a decrease in N/C ratios, Chl, N, and protein per cell.

Almost all microalgae are photosynthetic. Light energy is an important factor in photosynthetic microalgal cultures, especially in high-density microalgal cell suspensions. Several studies showed that light intensity affected growth of microalgal cultures. The cultivation of marine microalga *Isochrysis galbana* Parke under different illumination conditions was studied by Grima et al. (1992). Their study showed that culture growth rate increased linearly with increased light intensity up to 124 W/m^2, followed by saturation effect up to 376 W/m^2, while higher intensities inhibited photosynthesis. Dermoun et al. (1991) studied the effects of several growth parameters including light intensity on the growth of unicellular red alga *Porphyridium cruentum* under non-nutrient-limited conditions. Dermoun et al. shown that the growth rate depended on light intensity at temperature ranging from 5°C to 35 °C. For each temperature, the growth rate increased with increasing of light intensity up to a maximum value 400 μmol photon/m^2s, then decreased. Like Dermoun's study, several studies involving the effect of light intensity on microalgal culture growth have developed models showing the dependence of microalgal culture growth rate on light intensity. Lee et al. (1986) cultivated the blue-green algae *Spirulina platensis* in a rectangular algal fermentor with uniform lighting on the front and back sides of the vessel. Two cell concentrations of 40 mg DCW/L and 50 mg DCW/L were investigated. Their results showed that when the light intensity increased, the average specific growth rate increased up to a maximum value of 0.12 h^{-1} at a saturation light intensity of about 400 μmol photon/m^2s, then decreased, indicating light inhibition. Several kinetic models including the Monod kinetic model, the Bannister model and the Aiba model were used to express the relationship between growth rate and light intensity. All of the models were consistent with the experiments results for light-limited growth. Sancho et al. (1991) studied kinetics of growth in the unicellular alga *Chlorella pyrenoidosa* culture under light-limited conditions. They reported that at low light intensities, a linear relationship was observed between the specific growth rate and the

light intensity. However, they also reported that at high values of light intensity, the specific rate become constant, implying the light saturated growth. Thus, light intensity affects microalgal culture growth and is often the limiting factor for microalgal cultivation.

MATERIALS AND METHODS

Culture Maintenance

Laminaria saccharina Gametophyte Cell Culture

Laminaria saccharina female gametophyte cell cultures were obtained from Dr. Richard Steele, Environmental Protection Agency, Newport, Oregon and maintained in 250 ml Erlenmeyer flasks containing 100 ml GP2 artificial seawater medium. The composition of GP2 artificial seawater medium is shown in Table 1. The culture flasks were kept in an incubator at 13 ^{0}C under light intensity of 2000 lux and photoperiod of 16 hours light/8 hours light dark.

The cultures were subcultured approximately every six weeks at 25% v/v by the procedure described below. Five representative cultures were selected for subculture. Selection was based on a deep brown color and no sign of contamination. Each culture was poured into a sterilized blender cup and blended at "liquefy" setting for 10 to 15 seconds on an Osterizer blender. Then 25 ml of freshly blended culture was pipetted to a 250 ml sterile flask containing 75 ml of autoclaved GP2 medium. All techniques were carried out using sterile technique in the laminar flow hood. The newly inoculated flasks were placed in a low-temperature incubator and cultivated at 13 ^{0}C under 2000 lux, 16L:8D photoperiod.

Acrosiphonia coalita Tissue Culture

Acrosiphonia coalita tissue cultures developed from single filaments were obtained from Dr. Miriam Polne-Fuller, University of California at Santa Barbara, Marine Science Institute. The cultures were maintained in 250 ml Erlenmeyer flasks containing 100 ml PES medium. The composition of PES medium is shown in Table 2. Instant ocean salt was used as

Table 1. GP2 Artificial Seawater medium composition.

Compound	Chemical Formula	mg/L
Artificial Seawater		
Sodium chloride	NaCl	21,030
Sodium sulfate	Na_2SO_4	3,520
Potassium chloride	KCl	610
Potassium bromide	KBr	88
Sodium tetraborate decahydrate	$Na_2B_4O_7 \cdot 10H_20$	34
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	9,500
Calcium chloride dehydrate	$CaCl_2 \cdot 2H_2O$	1,320
Strontium chloride hexahydrate	$SrCl_2 \cdot 6H_2O$	20
Nutrients		
Sodium nitrate	$NaNO_3$	63.5
Sodium phosphate	$NaH_2PO_4 \cdot H_2O$	6.4
Sodium citrate dihydrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	0.52
Trace Metals:		
Sodium molybdate (VI) dihydrate	$Na_2MnO_4 \cdot 2H_2O$	0.012
Potassium iodide	KI	0.042
Zinc sulfate hepahydrate	$ZnSO_4 \cdot 7H_2O$	0.0112
Sodium orthovanadate	Na_3VO_4	0.0048
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0.0034
Vitamins:		
Thiamine-HCl		0.25
B_{12}		0.000125
Biotin		0.000125

the seawater base. The culture flasks were kept in an incubator at 14 °C under a light intensity of 4000 lux and photoperiod of 16 hours light/8 hours dark.

The *Acrosiphonia coalita* tissue cultures were subcultured every three weeks at approximately 25% v/v. All techniques were carried out using sterile technique in the laminar flow hood. Prior to subculture, the biomass filaments were diced up and washed with fresh PES medium to remove soluble debris as described below. The four best looking culture flasks were selected from each cell line. About 50 ml of clear medium was pipetted out from the 100 ml culture in the flask, and the tissue and remaining medium were poured into a 50 ml autoclaved centrifuge tube. The culture was centrifuged at 1000 rpm for 5 min. The medium supernate was pipetted out, and the centrifuged culture biomass was transferred to a sterile petri dish using a spatula. The biomass filaments were cut up into 1-2 mm lengths using a sterile razor blade in 2-5 ml PES medium. The diced filaments and medium were transferred using a wide-bore (3 mm) pipette to a 50 ml autoclaved centrifuge tube containing 40 ml fresh PES medium. In the first washing step, the culture was mixed thoroughly by hand for about 1 minute, and then allowed to stand for 5 minutes. The diced culture was then centrifuged at 1000 rpm for 5 minutes. In the second washing step, the supernate was removed with a pipette, and the pellet was resuspended in 50 ml fresh PES medium. The suspended culture was ready to subculture.

After the second washing, the diced tissue was subcultured. Specifically, 12.5 ml of the suspended culture in the 50 ml centrifuge tube was transferred to 100 ml of fresh PES liquid medium in a 250 ml flask. The entire procedure was repeated for the remaining 3 flasks. The inoculated flasks were placed in a low-temperature incubator and cultivated at 14 °C under 4000 lux, 16L:8D photoperiod.

The filament dicing method was modified to accommodate large culture volumes. The culture was loaded into an autoclaved 500 mL glass Mason jar equipped with two raze double-edged blades connected to a sealed shaft assembly. The shaft assembly was mounted to the lid of the jar and fitted to the drive on the Osterizer blender. The culture was blended at "liquefy" speed setting for 15 seconds.

Table 2. PES Enriched Seawater medium composition.

Compound	Chemical Formula	mg/L
Sodium nitrate	$NaNO_3$	70.0
Boric acid	H_3BO_3	5.72
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	0.02018
Iron (III) chloride hexahydrate	$FeCl_3 \cdot 6H_2O$	2.662
EDTA disodium dihydrate	$Na_2EDTA \cdot 2H_2O$	8.332
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0.7205
Glycerophosphate disodium hydrate	$Na_2(glycerophosphate) \cdot H_2O$	10.0
Zinc chloride	$ZnCl_2$	0.05212
Biotin		0.001
B_{12}		0.002
Thiamine-HCl		0.1
Instant Ocean Salt		33,000

Photobioreactor Cultivation

Bubble-Column Photobioreactor Design

Two bubble-column photobioreactor systems were fabricated. The first bioreactor shown in Figure 2 has an effective cultivation volume of 280 ml. The second bioreactor shown in Figure 3 has an effective cultivation volume of 900 ml.

Each bubble-column photobioreactor system, including the bioreactor assembly, illumination stage, and aeration system were installed within a low-temperature incubator. The glass reactor vessel for the 280 ml bioreactor consists of a 5.0 inch straight section and a 6.0 inch conical section with 1.8 inch I.D. for the straight section and 0.5 inch I.D. for the base. The body is sealed to the headplate using two Viton O rings, one above and one below the

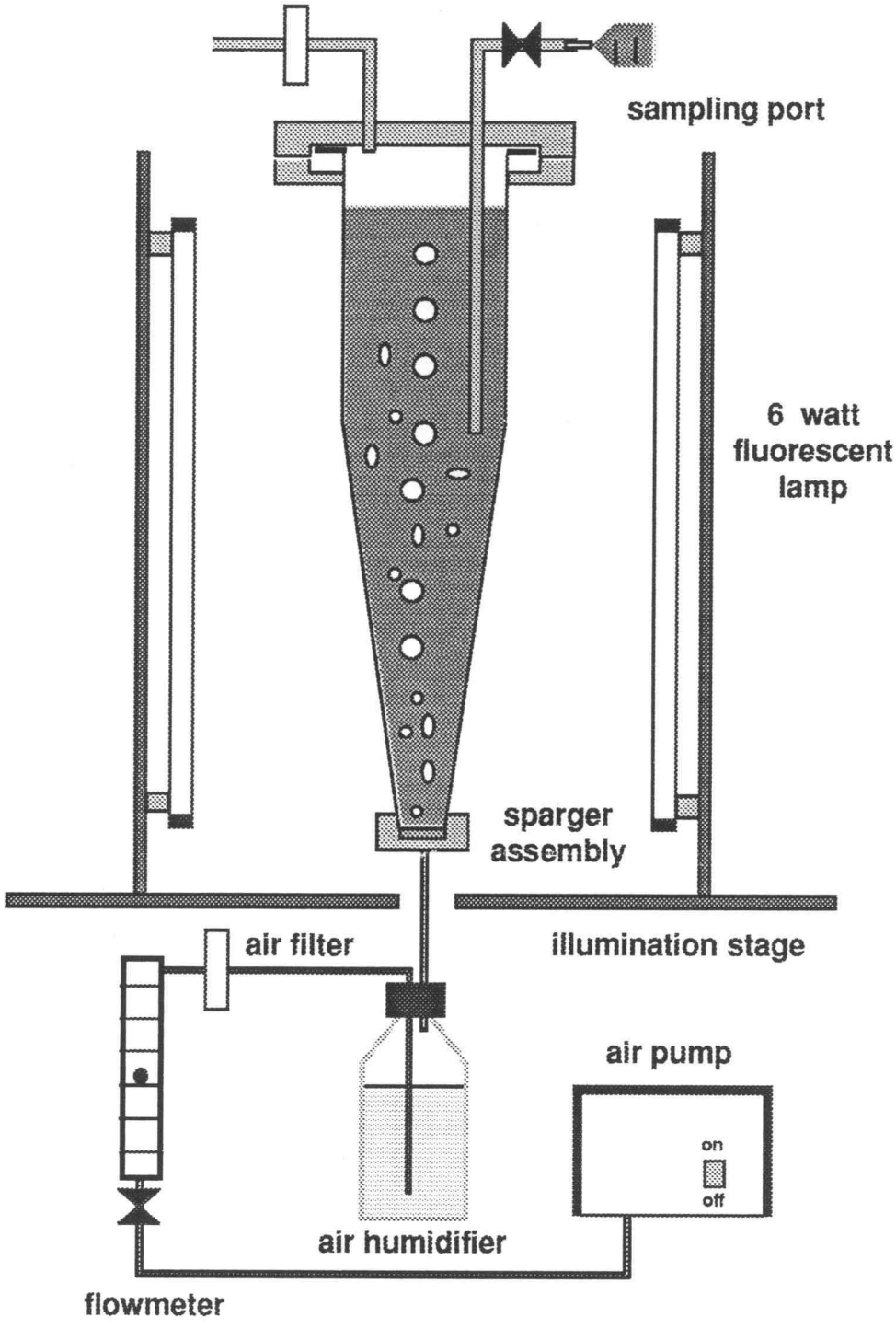

Figure 2. 280 mL bubble-column photobioreactor.

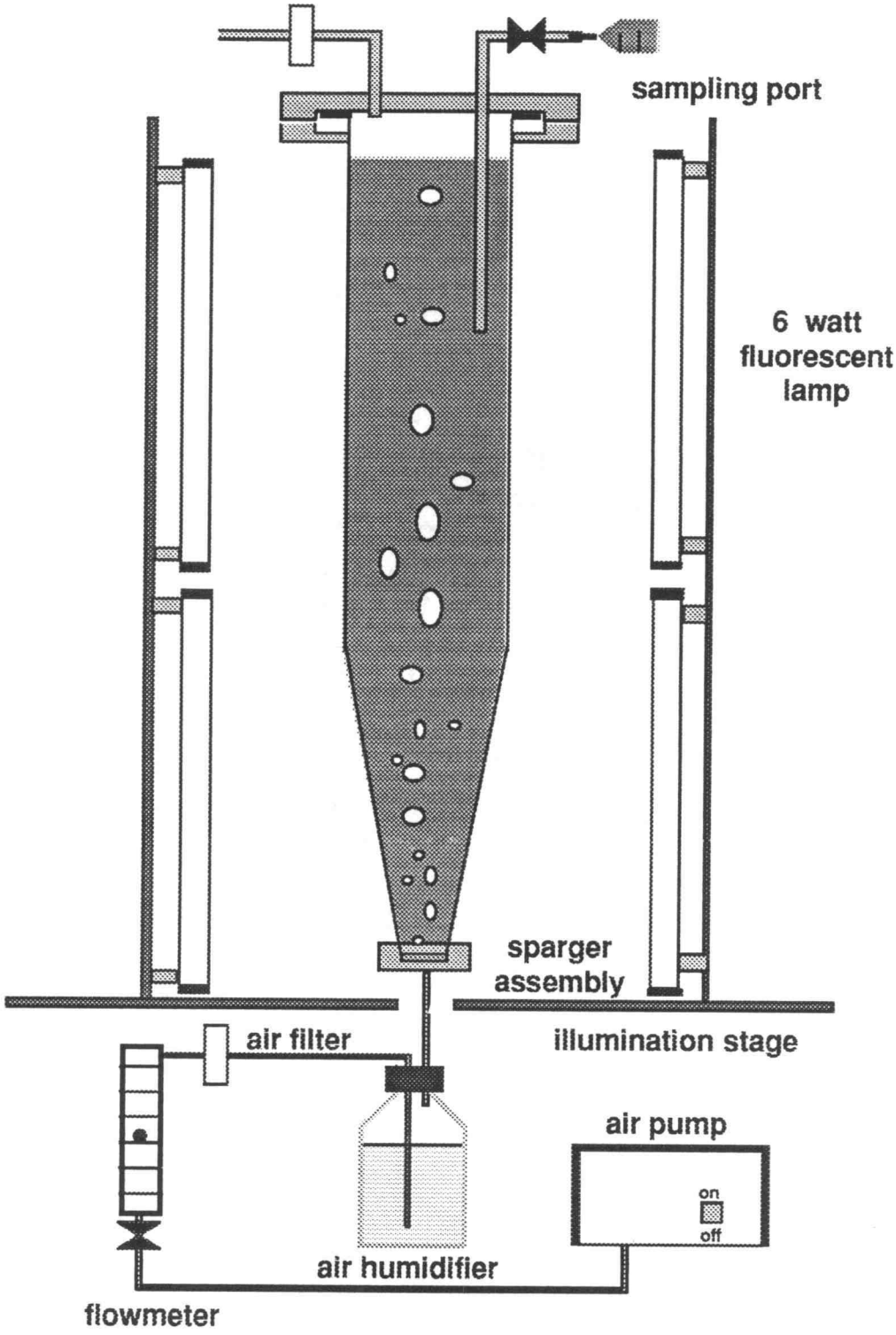

Figure 3. 900 mL bubble-column photobioreactor.

flange of the glass body. The head plate has two ports, each containing a stainless steel Swage-lok bored-through connector. The first port is for the air outlet, which is fitted with a 1/4 inch hose connection. The second port contains the culture sampling assembly. The sampling assembly consists of a 1/4 inch stainless steel tube. The tube penetrates to the culture solution at one end. The other end is connected to a 1/8 inch hose fitting connected to a 1/4 inch I.D. by 3/8 inch O.D. autoclavable silicone tubing. The air outlet port is connected to a 1/4 inch I.D. autoclavable silicone tubing and a 0.2 μ m Gelman sterilizing filter.

Ambient air is pumped through a 0.2 μ m sterile air filter, and then sparged to the culture. The sparger consists of a removable course size frit (40-60 μ m pore size with 1.4 cm diameter) installed in the bottom of vessel. The removable sparger frit facilitated the cleaning of the sparger assembly. The air flow is metered by a calibrated flowmeter.

The light stage consists of two 6 watt fluorescent tubes, mounted vertically on plexiglass plates. The plexiglass plates were fitted with referencing holes to set the position of the lamps relative to position and orientation of the bioreactor vessel.

The 900 mL photobioreactor system has the same headplate assembly, sparger assembly, and air flow system as the 280 mL photobioreactor. However, the straight section of the 900 mL glass reactor vessel is 19 inches vs. 5 inches for the 280 mL vessel. Also, the light stage consists of four 6 watt fluorescent tubes at 2 per side mounted vertically on plexiglass plates, as shown in Figure 3.

Operating Procedures

The operating procedures used for cultivation of *Laminaria saccharina* gametophyte cells in the 280 ml bubble-column bioreactor involved three major steps: bioreactor sterilization and inoculation, bioreactor culture sampling during a 20 day cultivation period, and bioreactor shut down. Prior to cultivation experiments, the bioreactor was disassembled and cleaned using phosphate-free Liquinox soap. The cleaned glass vessel was silanized using Sigmacote to help prevent biomass from sticking to the glass. The Sigmacote-treated vessel was dried for 24 hours to cure the silanizing

agent. After drying, the vessel was washed 10 times with deionized water. The vessel and headplate were re-assembled, and then autoclaved at 121 °C under 15 psig for 20 minutes.

The bioreactor was inoculated in the laminar flow hood using sterile technique. An inoculum mixture was typically obtained from 3 to 4 representative 250 ml flask cultures. The inoculum mixture was pooled to one 500 ml flask and blended for 5 to 10 seconds on "liquefy" setting in the Osterizer blender to disperse the culture. The dry cell density ($X_{c,0}$, mg DCW/L) of the inoculum mixture was then measured. The volume of inoculum mixture (V_0, ml) needed to inoculate the bioreactor to the desired initial density (X_o, mg DCW/L) was calculated by

$$X_o = X_{c,o} \frac{V_o}{(V_o + V_m)} = X_{c,o} \frac{V_o}{V_t} \qquad (1)$$

where V_m is the GP2 medium volume and V_t is the total culture volume. The inoculum mixture of volume V_o was then poured to the bioreactor, which contained GP2 medium cooled to 13 °C. After inoculation, the bioreactor was sealed in the laminar flow hood and then placed into the incubator on the illumination stage. The GP2 medium was adjusted to pH 8 prior to autoclaving.

The experimental design for the cultivation of *Laminaria saccharina* gametophyte cell cultures focused on studying the effect of initial nitrate concentration in GP2 medium, aeration rate, initial inoculum density and incident light intensity on the biomass growth kinetics in the bubble-column photobioreactor. The base run conditions are shown in Table 3.

After inoculation and start-up, two 5 ml culture samples were immediately removed from the bioreactor culture. During the 20 day cultivation period, culture samples were taken from the bioreactor for chlorophyll a concentration measurements at two day intervals. Prior to sampling, the silicone tube leading from the sampling port was unclamped. A 10 ml culture sample was withdrawn from the bioreactor through the sampling tube using the 20 ml syringe to provide suction.

Table 3. Base conditions for cultivation of *Laminaria saccharina* female gametophyte cell culture in the 280 mL bubble-column photobioreactor.

Process Condition	**Value and units**
Temperature	13-14 °C
Photoperiod	16L:8D
Incident light Intensity (I_o)	2600 lux
Medium	GP2
Medium pH	8.0*
Total Culture Volume (V_t)	280 ml
Aeration Rate (v_o)	97 ml/min
Cultivation Period	20 days

* Prior to autoclaving

The experiment was shut down after 20 days of cultivation. The bioreactor was taken out the incubator and the headplate was removed. The culture was poured into the blender cup, and blended in the Osterizer blender at "liquefy" setting for 10 to 15 seconds. After blending, the chl a concentration and dry cell density of the blended culture were measured.

For the 900 mL photobioreactor operating procedure, the dry cell density was measured at four day intervals during the 20 day cultivation time period. All other procedures were consistent with the 280 ml photobioreactor operating procedures.

Analysis Techniques

Biomass growth kinetics of the photosynthetic *Laminarina saccharina* female gametophyte cell cultures were followed by measuring of the chlorophyll a concentration

in the culture. The chlorophyll a concentration was measured by spectrophotometry at a wavelength of 665 nm using a Hitachi 100-10 spectrophotometer. Each 10 ml culture sample was divided into two 5 ml aliquots. Each 5 ml aliquot was vacuum filtered through a 20 μ m nylon mesh filter. The filter and cells were then placed in a 50 ml centrifuge tube and 5 ml HPLC grade methanol was added to the cells. The sample was vortexed at speed setting 4 for 30 seconds. Each sample was then stored in a dark refrigerator at 4 °C overnight. Before spectrophotometric measurement, the samples were vortexed again for 30 seconds.

The conversion factor of 16.29 mg chl a/L/AU unit was used for estimating chlorophyll concentration with 100% methanol extracts of filtered algal biomass (Porra et al. 1989). The specific equations for chl a and chl b concentration are

$$\text{Chl a} = 16.29D_{665} - 8.54D_{652} \quad (2)$$

$$\text{Chl b} = -13.58D_{665} + 30.66D_{652} \quad (3)$$

where D_{665} and D_{652} are the absorbances at light wavelength 665 and 652 nm in a 1-cm pathlength spectrophotometer cell. In *L. saccharina*, chl b is not present and D_{652} was omitted in equation (2).

The pH of culture sample was measured at four day intervals using a combination pH electrode and pH meter.

At the beginning and the end of the cultivation, the dry cell density of the culture was measured in triplicate. Prior to the measurement, each Millipore filter of 47 mm diameter and 0.45 μ m pore size (catalog # HAWP 04700) was dried in a covered glass petri dish at 70 °C for 24 hours, and then weighed. Each 20 ml culture sample was vacuum filtered, and then the biomass was washed. The filter and biomass were dried at 70 °C for 24 hours, and then weighed. The weight of the dry cell mass was determined by the weight difference, and the dry cell density was determined from the dry cell mass and the volume of the culture sample.

Three different techniques were used for washing the filtered biomass: distilled water washing (DD Water), GP2 medium washing (GP2-1), and no washing (GP2-2).

The GP-1 and GP2-2 washing techniques were better than the DD water washing technique because DD water washing can lyse the cells. The equations for calculation of dry cell density (X) by each washing method were:

$$X_{(DD\ H2O)} = \frac{m_f - m_i}{V} \tag{4}$$

$$X_{(GP2\text{-}1,2)} = \frac{m_f - m_i - m_{GP2}}{V} \tag{5}$$

$$X_{(GP2\text{-}1,2)} = X_{(DD\ H2O)} \cdot 1.13 + 112 \tag{6}$$

where m_i is the dry weight of filter, m_f is the dry weight of filter and filtered and washed biomass, and m_{GP2} is the dry weight of 20 mL GP2 salt content on the filter, and V is the culture sample volume. Equations (4) and (5) were used to calculate the dry cell density for each washing technique and equation (6) was used to convert the DD water washing dry cell density to the GP2 washing based dry cell density. The development of equation (6) is described in Appendix B.

RESULTS

Bubble-Column Photobioreactor Cultivation of *Laminarina saccharina* Female Gametophyte Cell Cultures

Base Run Conditions and Repeatability of Growth Kinetics

Female gametophyte cell cultures of the marine brown alga *L. saccharina* were cultivated in a 280 bubble-column photobioreactor. The base cultivation conditions are shown in Table 3. Three cell cultivation runs were performed at the same conditions to assess the repeatability of the growth kinetics of *Laminaria saccharina* female gametophyte cells. Because the culture was dilute and the reactor volume was small (280 ml), dry cell density was measured only at the beginning and end of the cultivation period. Intermediate growth curve points were measured as the chl a concentration in the culture (mg chl a/L). The growth curves are compared in Figure 4. Figure 5 shows the semi-log plots for the curves. The specific growth rate (μ) was determined from the slope of the exponential growth phase data. The average specific growth rate was 0.11 ± 0.021(1s) day^{-1} for the three combined runs. Table 4 shows the final cell density (X_f), ratio of X_f/X_o, specific growth rate (μ) for the three runs.

From Table 4 and Figures 4 and 5, we can see the cultivation is repeatable. All three cultivations give a lag phase of about 4 days and a growth phase of about 10 days followed by a stationary phase of 6 days. The repeatibility of the growth curve can be affected by many environmental factors, even when the process conditions are constant, such as the age of the cells, the inoculum density and even the specific bioreactor used for cultivation.

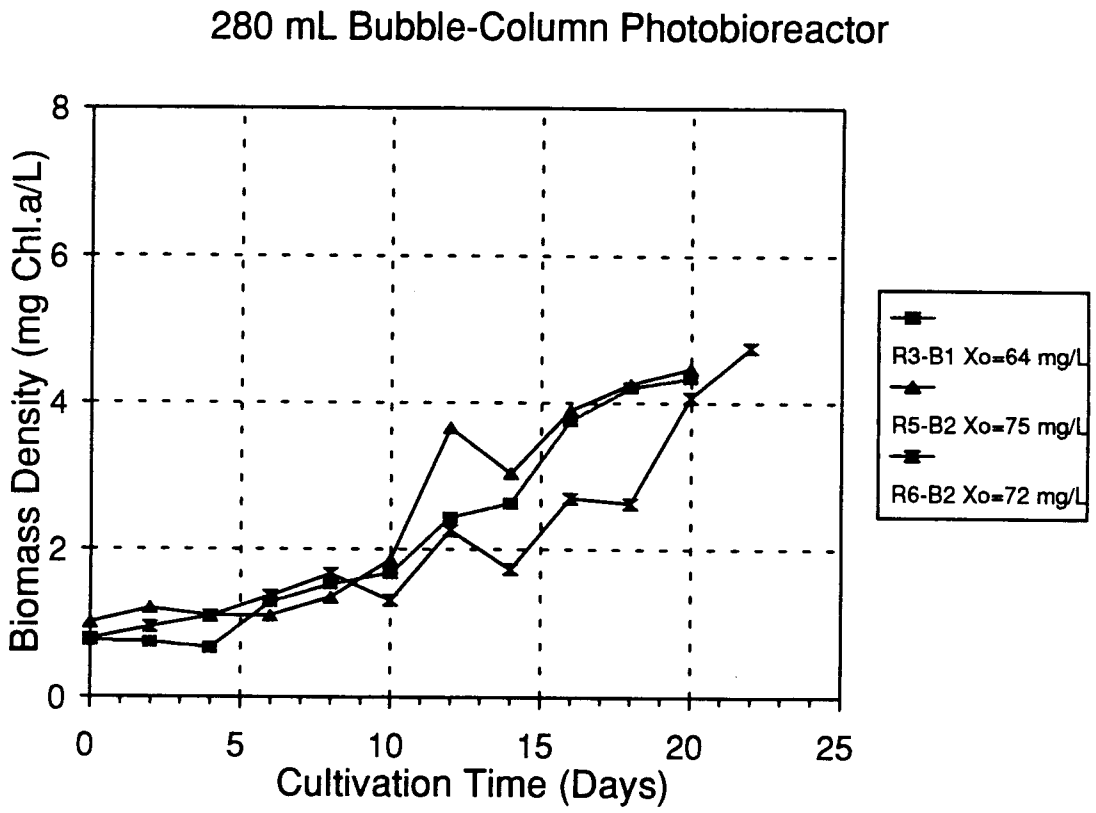

Figure 4. Repeatibility of biomass growth kinetics in 280 mL bubble-column bioreactor.

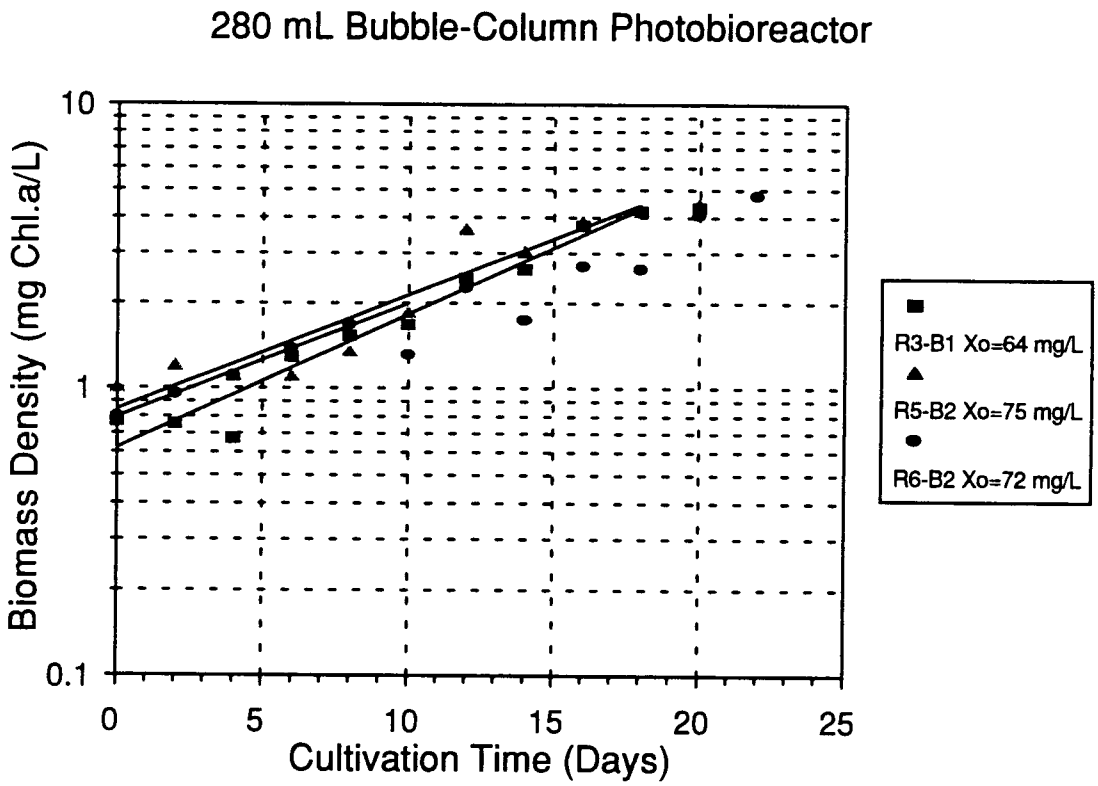

Figure 5. Repeatibility of biomass growth kinetics in 280 mL bubble-column bioreactor (semi-log plot).

Table 4. Repeatibility of growth parameters in the 280 mL bubble-column Photobioreactor.

Run # and Bioreactor #	Cell Line & Age of Inoculum	X_o (mg/L)	X_f (mg/L)	X_f/X_o	$\mu \pm 1s$ (1/day)
Run #3-B1	DS-2, 48 days	64	566	8.84	0.12±0.015
Run #5-B2	LS-3, 41 days	75	458	6.11	0.15±0.034
Run #6-B2	L-5, 31 days	72	470	6.53	0.07±0.013
Average			498	7.16	0.11±0.021

Effect of Process Variables on Growth Kinetics

Initial Cell Density

The effect of initial cell density (X_o) on the growth curve for the 280 ml bubble-column photobioreator at a constant incident light intensity of 2600 lux is shown in Figure 6, and semi-log plots for the growth curves are shown in Figure 7. The cultivation at a low initial cell density of 26 mg DCW/L in Figure 7 was allowed to proceed for a longer time than the standard 20 days to insure that the stationary phase of growth was achieved. The cultivation parameters are presented in Table 5. From Figure 6, increasing the initial cell density from 30 to 117 mg DCW/L significantly decreased the length of the lag phase.

The effects of X_o on the specific growth rate and final biomass density are shown in Figure 7 and Table 5. Increasing the initial cell density increased the final biomass density, but did not significantly affect the specific growth rate. However, An optimum in the final cell density was found within the initial cell density range of 100 to 130 mg DCW/L at constant incident light intensity of 2600 lux.

The increase in final biomass density with increasing initial cell density can be explained by the growth pattern of the *L. saccharina* female gametophyte cell culture in the bubble-column bioreactor. These cultures grow as loosely clumped filaments as

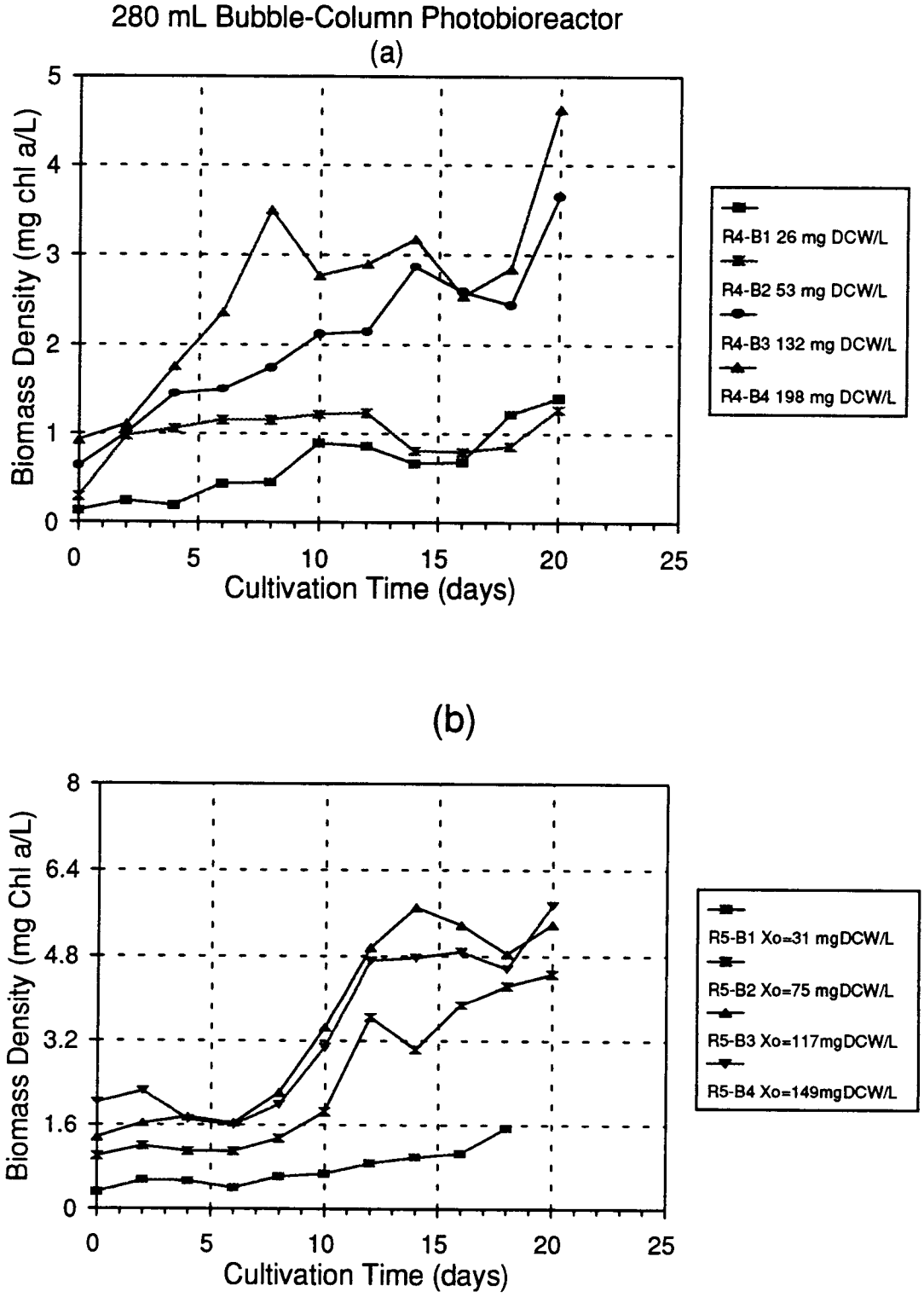

Figure 6. Effect of initial cell density on the growth curve, (a) Run 4, (b) Run 5.

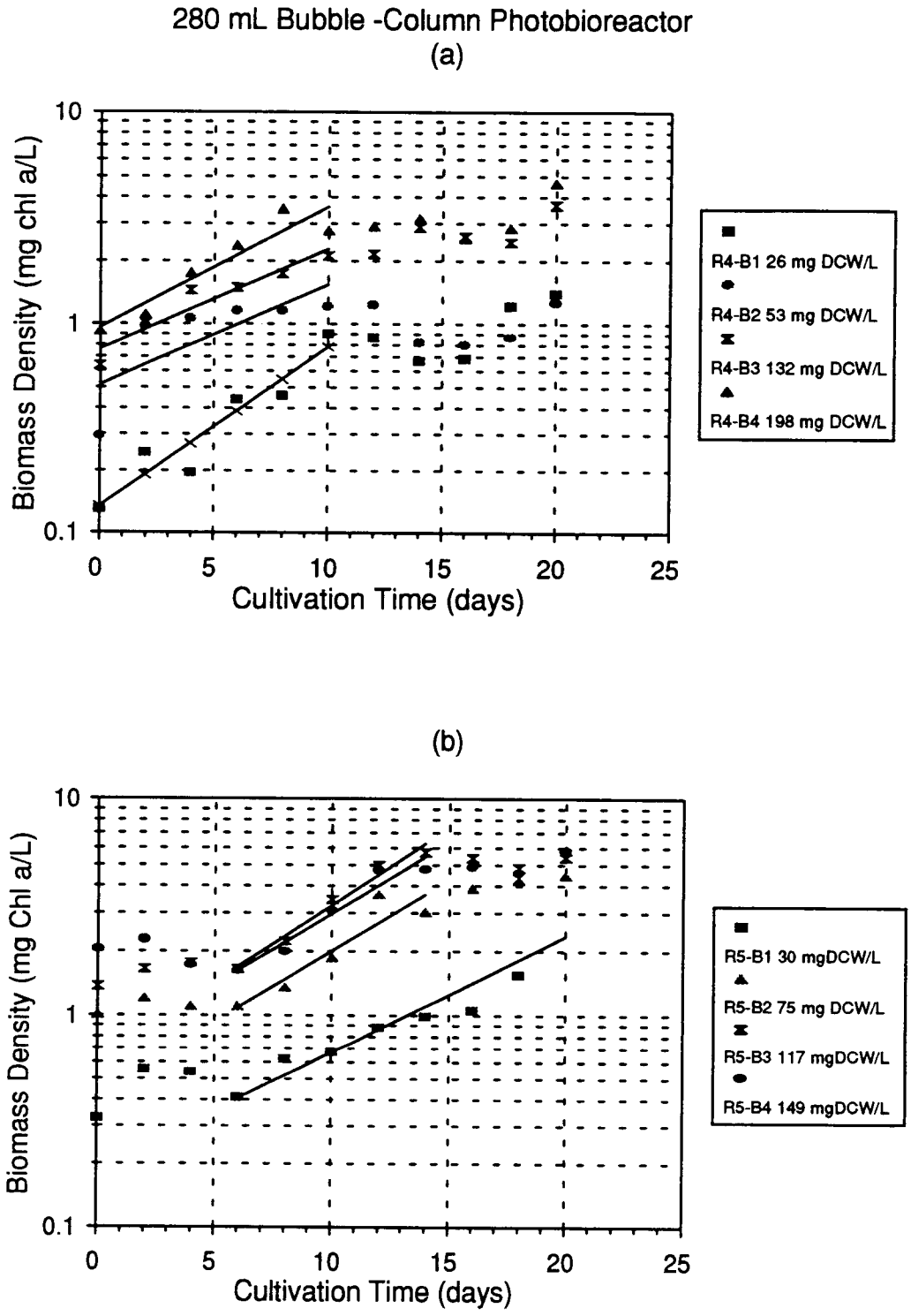

Figure 7. Effect of initial cell density on the growth curve, (a) Run 4, (b) Run 5 (Semi-log plots).

Table 5. Effect of initial cell density on the growth parameters.

Run # and Bioreactor #	Initial Inoculum Density X_o (mgDCW/L)	X_f (mg DCW/L)	X_f/X_o	$\mu \pm 1s$ (1/day)
R4-B1	27	595	22.04	0.18±0.029
R4-B2	67	572	8.54	0.11±0.048
R4-B3	133	731	5.50	0.11±0.018
R4-B4	200	742	3.71	0.13±0.024
R5-B1	31	763	24.61	0.13±0.014
R5-B2	76	458	6.03	0.15±0.034
R5-B3	119	1180	9.92	0.16±0.014
R5-B4	145	900	6.21	0.15±0.021
R7-B2	113	765	6.77	0.13±0.008
R8-B1	113	895	7.92	0.11±0.010

opposed to single cells. The inoculum culture for the bioreactor was blended to disperse the filamentous cell mass to uniform clumps. Thus at inoculation the culture was very uniform, but during the lag phase some of the finely blended filament clumps combined back together. When the initial cell density increased, the number of the cell clumps serving as nucleation sites for cell growth increased. This implies that the final biomass density should linearly increase with increasing initial cell density. Although an increase was observed, it was not linear.

The growth rate was not affected by the initial cell density in the bubble-column bioreactor. This observation has also been reported in the microalgal bioreactor cultivation studies of *Phaeodactylum tricornutum* (Chrismadha et al. 1994).

Initial Nitrate Concentration

Macroalgal plants are photolithotrophic and require light and an inorganic carbon source (e.g. dissolved CO_2 or HCO_3^-) for growth (Cole and Sheath, 1992). Next to carbon, nitrogen and phosphorous are the most important elements for culture growth. Nitrogen, supplied to the liquid medium in the form of nitrate ion, is ultimately assimilated into proteins, enzymes, and genetic material.

In this set of experiments, female gametophyte cell cultures of the marine brown alga *L. saccharina* were cultivated in a 280 ml bubble-column photobioreactor at different nitrate concentrations ranging from 0 to 548 mg $NaNO_3$/L in the GP2 medium. The effect of nitrate concentration on the growth curves is shown in Figure 8. The specific growth rate was computed from the linear portion of the semi-log plot of the growth kinetic data given in Figure 8. Table 6 shows the final cell density (X_f), X_f/X_o ratio, and the specific growth rate (μ) at each initial nitrate concentration. For the data given in Table 6, the nitrate concentration had no statistically significant effect on the growth curve and final dry cell density at nitrate concentrations of 64 mg $NaNO_3$/L and greater. Therefore, the nitrate supply in the GP2 medium was not the limiting nutrient in this system. Control experiments with no nitrate in the GP2 medium (0 mg $NaNO_3$/L) showed no significant culture growth (Table 6).

An ion meter was used to measure the nitrate concentration in the GP2 medium during the bubble-column bioreactor cultivation of the *L. saccharina* female gametophyte cell culture to determine if all the nitrate was consumed by the culture, indicating nitrogen limitation. Unfortunately, the ion meter was not sensitive at nitrate concentrations below 100 mg NaNO3/L (see Figure D-1 in Appendix D). Therefore, nitrate consumption was not measured directly.

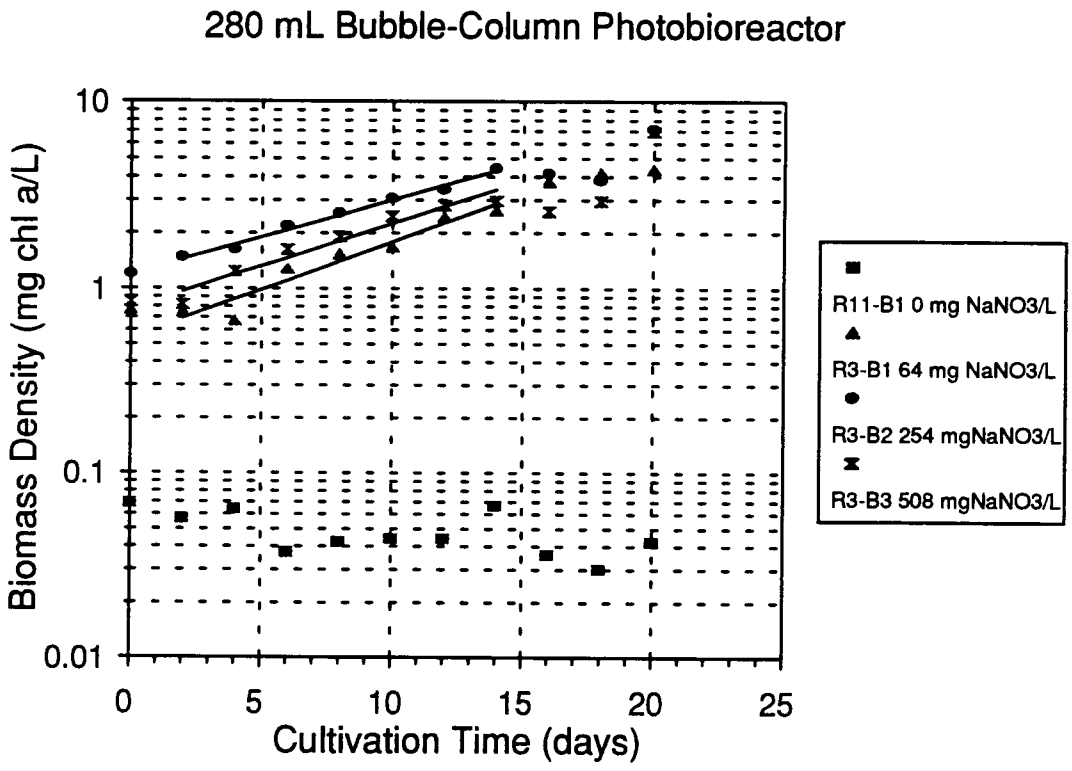

Figure 8. Effect of initial nitrate concentration on the growth curve (semi-log plot).

Table 6. Effect of initial nitrate concentration on the growth parameters.

Run # and Bioreactor #	Nitrate Conc. (mg $NaNO_3$/L)	X_o (mg $NaNO_3$/L)	X_f (mg $NaNO_3$/L)	X_f/X_o	$\mu \pm 1s$ (1/day)
R11-B1	0	75	150	2.00	0.02±0.030
R3-B1	64	64	566	8.84	0.10±0.025
R3-B2	254	75	653	8.71	0.10±0.005
R3-B4	508	90	619	6.88	0.12±0.012

Aeration Rate

Aeration rate is an important variable for photolithotrophic cultivation of macroalgal cells because it supplies the carbon dioxide necessary for photosynthesis. Aeration in a bubble column bioreactor maintains a uniform distribution of macroalgal cells, thereby providing all the cells with an even exposure to light. Increasing the aeration rate also increases the fluid motion over the cells and improves dissolved nutrient mass transfer to the cells. In addition, aeration can indirectly regulate the pH, since carbon dioxide carried by the air dissolves into the culture medium and is speciated into bicarbonate (HCO_3^-). However, high aeration rates create excessive turbulence and cause hydrodynamic shear damage to the cells or blow out culture onto the walls and headplate of the vessel.

Four cultivations were carried out at aeration rates ranging from 97 mL air/min (0.35 vvm) to 350 mL air/min (1.4 vvm). The effect of aeration rate on the cultivation parameters are presented in Table 7. The effect of aeration rate on the growth curves and the semi-log plots of these growth curves are shown in Figure 9 and Figure 10.

From Figure 9 and Table 7, increasing the aeration rate increased the culture growth rate and final dry cell density. However, the aeration rate at 2.0 vvm blew the culture onto the walls of the reactor, and thus decreased the biomass productivity. The

Table 7. Effect of aeration rate on the growth parameters.

Run # and Bioreactor #	Aeration Rate (vvm.)/(mL/min)		X_o (mg/L)	X_f (mg/L)	X_f/X_o	$\mu \pm 1s$ (1/day)
R6-B1	0.17	48	72	417	5.79	0.08±0.030
R6-B2	0.35	97	72	470	6.53	0.06±0.020
R6-B3	1.00	280	72	785	10.90	0.14±0.030
R6-B4	2.00	560	72	455	6.32	0.09±0.050
R7-B1	0.17	48	113	590	5.22	0.12±0.013
R7-B2	0.35	97	113	765	6.77	0.13±0.008
R12-B1	1.00	280	113	1010	8.94	0.15±0.028
R7-B4	1.40	400	113	870	7.70	0.11±0.013

results show that the best aeration rate was around 1.0 vvm with a specific growth rate of 0.15 ± 0.029 day^{-1}. An increase in biomass productivity with the increasing of aeration rate was also observed for the microalgae photobioreactor cultivation of *Skeletonema costatum* (Sanchez et al, 1993) and *Isochrysis galbana* (Grima et al, 1992).

Incident Light Intensity

Incident light intensity is a very important factor for the photolithtrophic cell growth since it provides the energy for the photosynthesis. The effect of incident light intensity (100 to 8200 lux) on the growth curve based on chl a concentration in the 280 bubble-column bioreactor is shown in Figure 11. The final biomass density (X_f), and final chlorophyll a content in the biomass at each illumination intensity are presented in Figure 12. Table 8 shows effect of incident light intensity on the growth parameters.

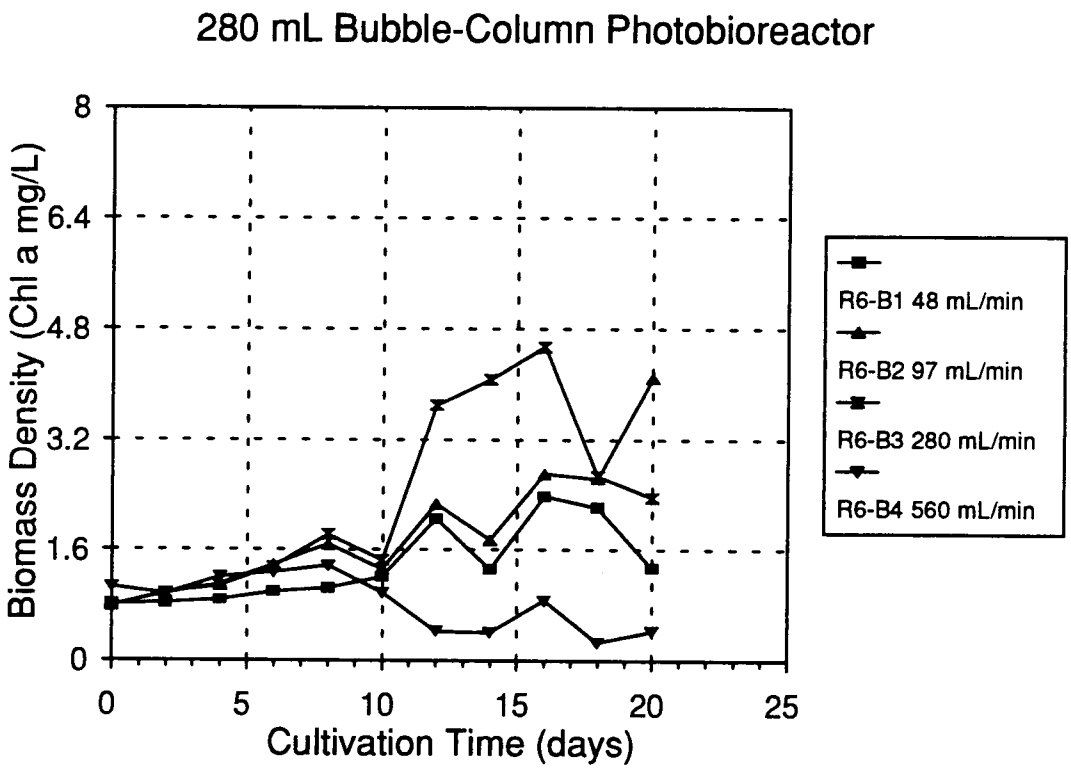

Figure 9. Effect of aeration rate on the growth curve (Run 6).

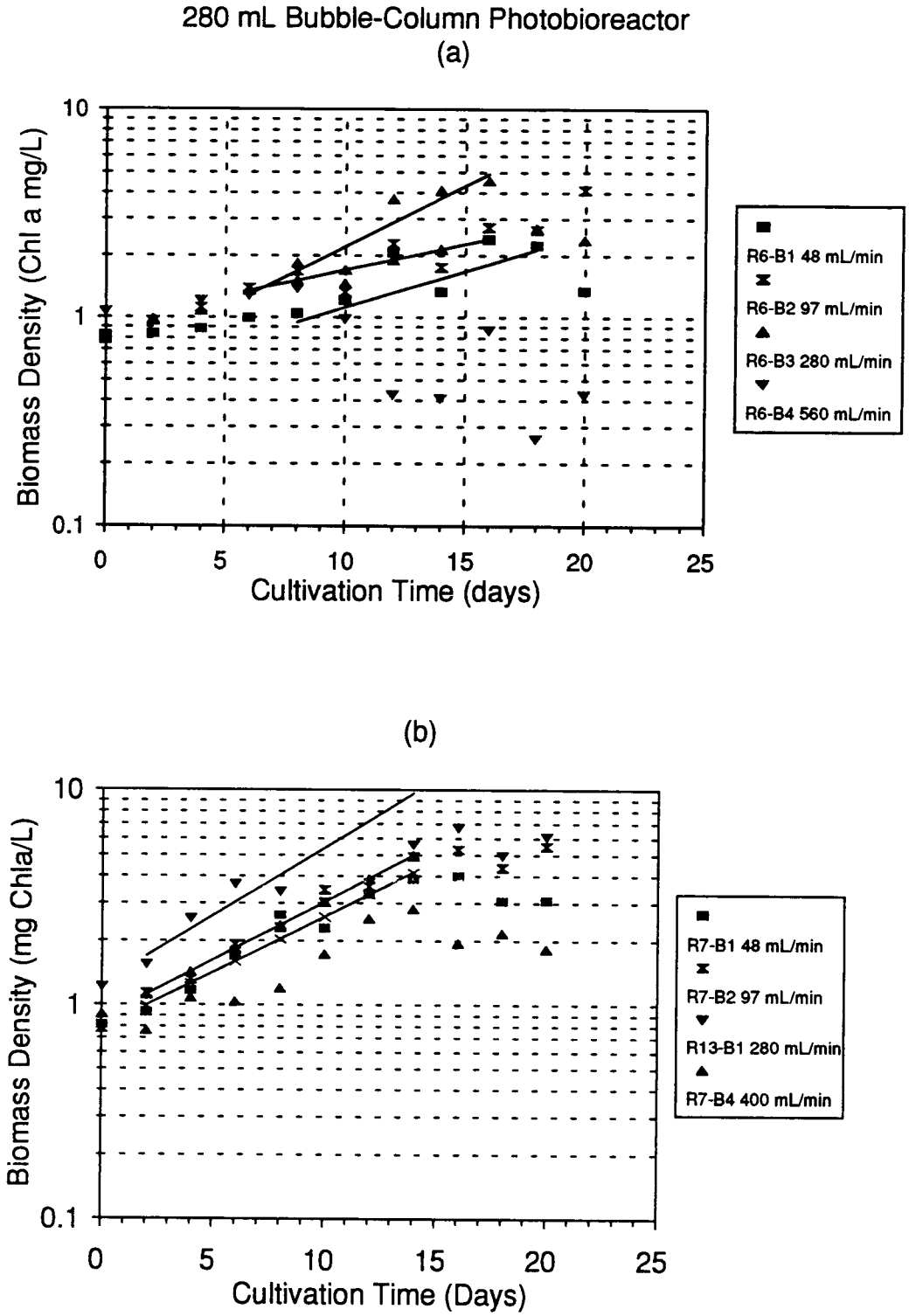

Figure 10. Effect of aeration rate on the growth curve (Run 6 and 7, semi-log plot).

Increasing the incident light intensity improved the final biomass density from 370 mg DCW/L at 90 lux to 1313 mg DCW/L at 8000 lux at an initial cell density of 113 mg DCW/L. However, the chlorophyll pigment was inversely proportional to the incident light intensity. The chl a content in the biomass decreased from 1.82 to 0.49 mg chl a/100 mg DCW over the same range of incident light intensity. It is known that algae lower the concentration of light-harvesting pigments in the cell biomass in response to increased illumination intensity when growth is not light saturated. Thus the chl a concentration in the culture (Figure 11) was not a accurate indicator of biomass growth and so was not used to determine culture growth kinetics. Therefore, dry cell density measurements were required to compare biomass growth kinetics at different light intensities. However, dry cell densities measurements require a large cultivation volume to account for the culture volume which must be removed by sampling.

A 900 mL photobioreactor was fabricated to provide culture volumes sufficient for measurement of the growth curve based on dry cell density so that the biomass growth kinetics at different light intensities could be obtained. With this photobioreactor, samples for dry cell biomass density measurements were obtained at four day intervals.

The effect of incident light intensity ranging from 1000 to 8600 lux on the growth curve is shown in Figure 13. Semi-log plots of these growth curves are shown in Figure 14. A plot of chlorophyll a content and biomass density versus cultivation time at an incident light intensity of 2600 lux is shown in Figure 15. The effect of incident light intensity on the growth parameters are presented in Table 9.

From Figure 13, 14 and Table 9, the specific growth rate and final biomass density increased with the increasing incident light intensity until saturation was observed, implying that incident light intensity was a limiting factor to the culture growth. This result has been obtained for several microalgae photobioreactor cultivation studies (Chrismadha et al, 1994, Grima et al, 1992 and 1994, Lee et al, 1990).

Table 8. Effect of incident light intensity on the final cell density in 280 mL photobioreactor.

Run # & Bioreactor #	Incident Light Intensity I_o (lux)	X_o (mg/L)	X_f (mg/L)	X_f/X_o
R8-B1	90	113	370	3.27
R8-B2	1400	113	737	6.52
R8-B3	2600	113	1123	9.94
R8-B4	8000	113	1313	11.6

Table 9. Effect of incident light intensity on the growth parameters in 900 mL photobioreactor.

Run # and Bioreactor #	Incident Light Intensity I_o (lux)	X_o (mg/L)	X_f (mg/L)	X_f/X_o	$\mu \pm 1s$ (1/day)
R13-B2	1000	139	863	6.20	0.12 ± 0.03
R9-B2	2600	139	910	6.55	0.11 ± 0.03
R13-B3	5000	139	600	4.32	0.14 ± 0.02
R10-B2	8600	140	1088	7.77	0.19 ± 0.02

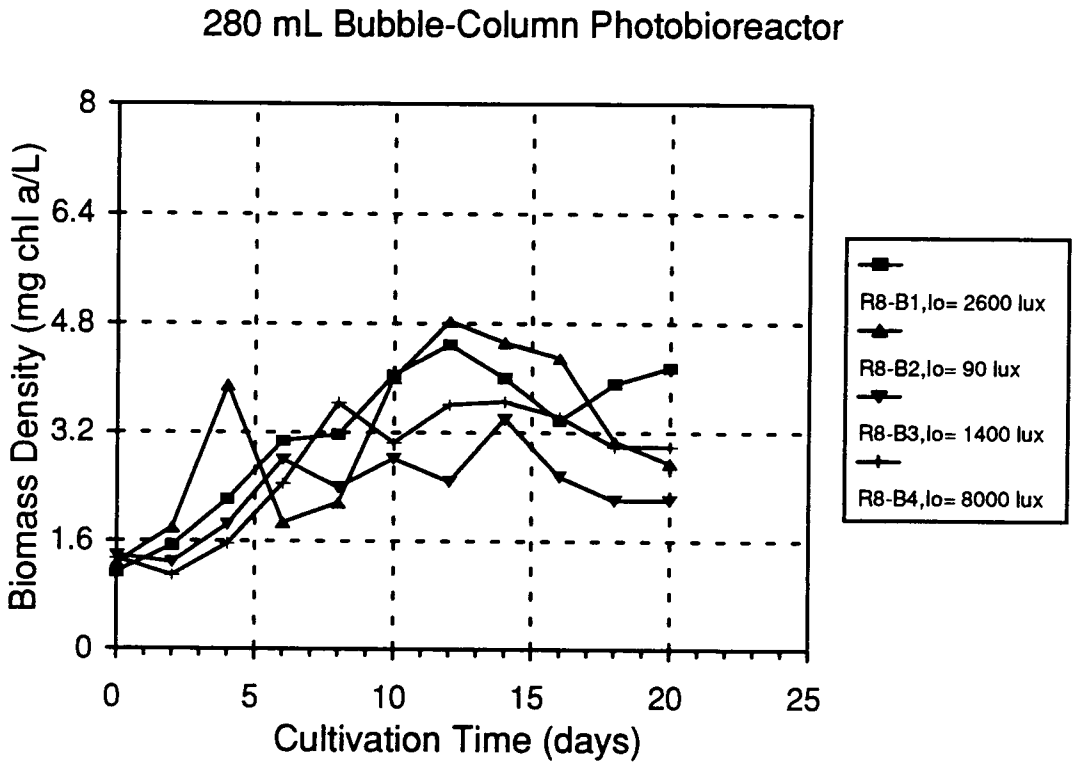

Figure 11. Effect of incident light intensity on the growth curves in 280 mL photobioractor.

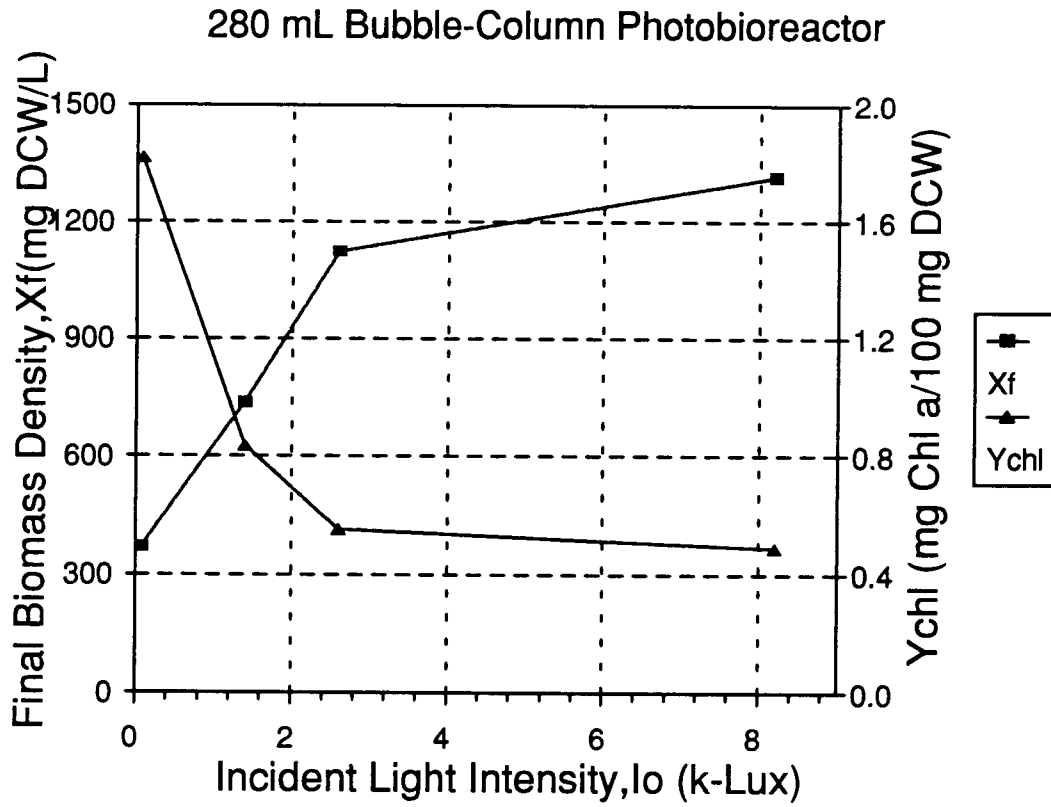

Figure 12. Final biomass density (X_f) and biomass chlorophyll a content (Y_{chl}) versus incident light intensity (I_o).

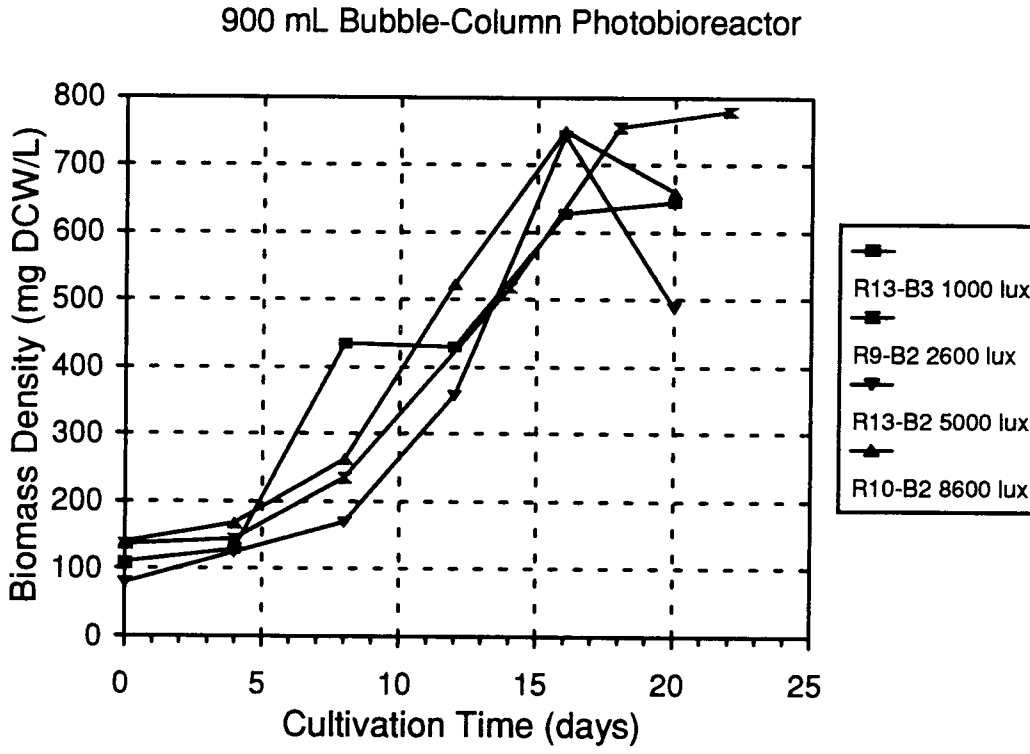

Figure 13. Effect of incident light intensity on the growth curves in 900 mL photobioreactor.

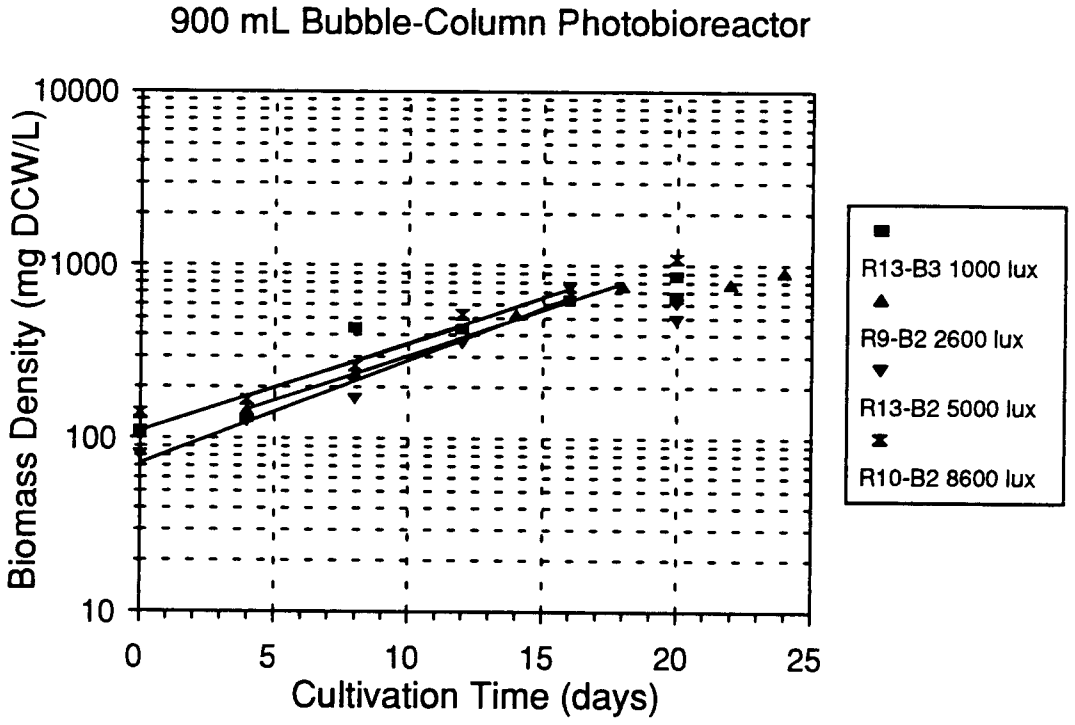

Figure 14. Effect of incident light intensity on the growth curves in the 900 mL photobioreactor (semi-log plot).

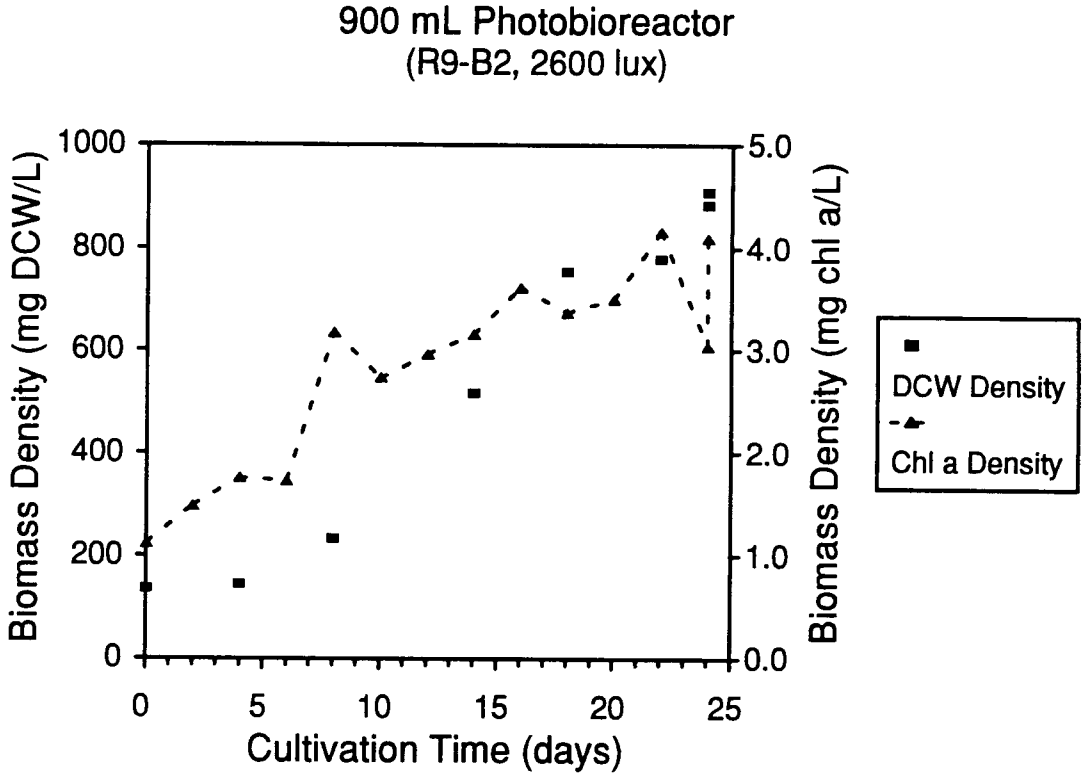

Figure 15. Dry cell density and chlorophyll a content versus cultivation time.

Bubble-Column Photobioreactor Cultivation of *Acrosiphonia coalita* Tissue Culture

Acrosiphonia colatia tissue cultures were cultivated in a 280 ml bubble-column photobioreactor. All photolithotrophic cultivations were carried in PES medium at 12 °C, aeration rate of 97 air/min (0.35 vvm), and incident light intensity of 4000 lux for 20 days. Because the culture was dilute and the reactor volume was small (280 ml), dry cell density was measured only at the beginning and the end of the cultivation period. There was no measurement of biomass density of intermediate points.

Two cultivation systems were compared in a 20 day cultivation time period: the 280 mL bubble-column photobioreactor and a static flask (100 mL culture in 250 mL flask). The cultivation conditions for the the bubble column photobioreactor were essentially same as cultivation conditions for the static flask. Three runs were performed at consistent initial cell density and inoculum source. The final cell densities for the three runs are shown in Figure 13. From Figure 13, it can be clearly seen that bubble-column bioreactor has no special advantage over the static flask for the biomass production. Furthermore, the biomass was not uniformly suspended in the bubble-column bioreactor. In hte bubble-column bioreactor, the *Acrosiphonia coalia* tissue culture filaments clumped to a large particles after 3 to 5 day cultivation, and stuck to the bottom or floated to the top of the vessel.

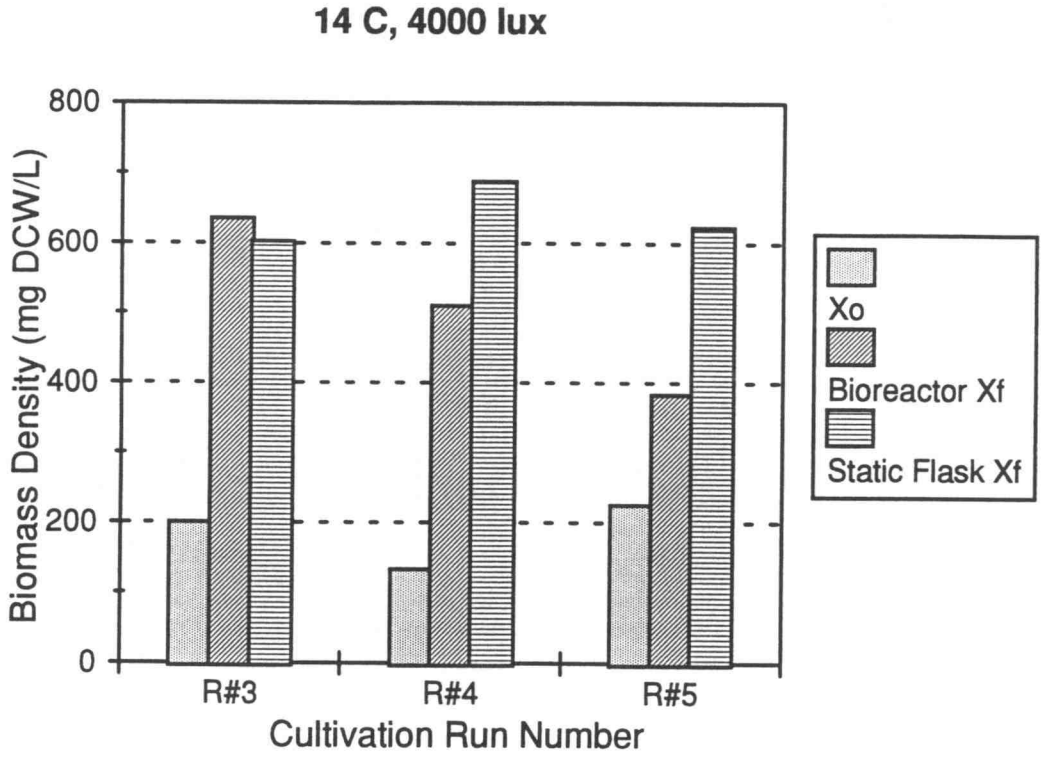

Figure 16. Comparison of two cultivation systems for *Acrosiphonia colita* tissue cultures.

DISCUSSION

L. saccharina female gametophyte cells are clumped and filamentous. The kinetics of growth, product yield and substrate utilization by filamentous organisms are complex (Bailey and Ollis, 1977).

In the Results section it was shown that increasing initial cell density increased the final biomass density. The effect of initial cell density on single-celled microalgal culture growth has been studied previously (Chrismadha et al. 1994). The effect of initial cell density on biomass growth obtained in this research results from the complex growth pattern of the *L. saccharina* female gametophyte cell culture in the bubble-column bioreactor. Therefore, it is complicated to mathematically model the effect of the initial cell density on the biomass growth of clumped filamentous cells. Thus, the discussion will focus on the effect of aeration rate, initial nitrate concentration, and incident light intensity on the biomass growth. Provided below are some simple models to explain the experimental data for these three process variables.

Aeration Rate. Aeration rate is an important variable for the photolithtrophic culture because it supplies the carbon dioxide necessary for photosynthesis. Aeration also helps to uniformly suspend the culture, and provide the cells with an even exposure to light. In the Results section, aeration rate affected the final biomass density and specific growth rate. The optimum aeration rate was around 1 vvm.

The dissolved CO_2 used for photosynthesis is supplied to the liquid phase CO_2 by the aerating gas stream in accordance with the two-film theory for mass transfer. The transfer rate of CO_2 (CO_2-TR) is balanced by the volumetric CO_2 consumption rate (Q_{CO2}) of the biomass:

$$CO_2\text{-TR} = Q_{CO2} = \frac{r_x}{Y_{x/CO2}} = \frac{\mu X}{Y_{X/CO2}} \qquad (7)$$

where r_x is the growth rate of biomass (mg DCW/L-day), μ is the specific growth rate (day^{-1}), X is the culture cell density (mg DCW/L), and $Y_{x/CO2}$ is growth yield coefficient based on CO_2 , defined as the mass of biomass produced per mole of CO_2 consumed (mg DCW/mole CO_2). The transfer rate of CO_2 from the bulk gas to the bulk liquid phase is

$$CO_2\text{-TR} = (k_La)_{CO2} (C^*_{CO2} - C_{CO2}) \quad (8)$$

where C^*_{CO2} is the dissolved CO_2 concentration in equilibrium with the partial pressure of CO_2 in the aerating gas stream (mmole CO_2/L), and $(k_La)_{CO2}$ is the CO_2 volumetric mass transfer coefficient (hr^{-1}). At a CO_2 partial pressure of 35 Pa at 1 atm total pressure, C^*_{CO2} is equal to 0.0145 mM for 35 ppt seawater at 13 °C (Raven, 1984). The maximum CO_2-TR at C_{CO2} equal to zero must always be greater than Q_{CO2} to avoid CO_2 transfer limited growth,

$$(CO_2\text{-TR})_{max} = (k_La)_{CO2} C^*_{CO2} \geq \frac{\mu}{Y_{X/CO2}} X_f \quad (9)$$

Grima et al. showed that $(k_La)_{CO2}$ can be estimated from oxygen mass transfer coefficient k_La by

$$(k_La)_{CO2} = (k_La) \left[\frac{D_{CO_2}}{D_{O_2}} \right]^{0.5} \quad (10)$$

For seawater-based medium at 20°C, the ratio of the diffusivities D_{CO2}/D_{O2} is 0.91 (Grima et al., 1993). This value D_{CO2}/D_{O2} was also presumed valid at 13 °C, because the temperature dependency of the diffusivities would tend to cancel each other out when expressed as a ratio. Therefore $(k_La)_{CO2}$ can be estimated if k_La is known for oxygen mass transfer.

In bubble columns, for $0<V_s<0.15$ m/s and $100<P_g/V_t<1100$ W/m^3, Botton et al. (1980) correlated the k_La for O_2 transfer with aeration rate as

$$\frac{k_L a}{0.08} = \left(\frac{P_g / V_t}{800}\right)^{0.75} \qquad \text{with} \quad \frac{P_g}{v} = 800\left(\frac{V_s}{0.1}\right)^{0.75} \tag{11}$$

where V_s is the superficial gas velocity (m/sec), P_g is the gas power input (W), and V_t is the total volume of liquid (mL). The gas velocity V_s is computed from the volumetric flowrate and the average diameter of the reactor.

We can use biomass stoichiometry to estimate $Y_{x/CO2}$. Atkinson & Smith (1983) proposed following photosynthesis equation for macroalgae:

$$550\ CO_2 + 580\ H_2O + 30\ HNO_3 + H_3PO_4 \rightarrow$$
$$(CH2O)_{550}(NH_3)_{30}(H_3PO_4) + 610\ O_2 \tag{12}$$

From equation (12), $Y_{x/CO2}$ is equal to 0.71 g DCW/g CO_2 (31.2 g DCW/mole CO_2). A plot of Q_{CO2} and $(CO_2\text{-}TR)_{max}$ versus aeration rate is shown in Figure 17. From this plot, we see that CO_2 transfer by the aerating gas is not a limiting factor for the biomass growth. However, the aeration rate still had an effect on the final biomass density and specific growth rate. Although aeration rate may not limit CO_2 transfer, it may reduce the resistance to mass transfer of other nutrients.

Initial Nitrate Concentration. Nitrogen, supplied in the form of inorganic nitrate, is essential for growth of algal cultures. From Results section, nitrate concentrations from 64 to 540 mg/L did not limit the biomass growth.

The biomass yield coefficient based on nitrate is $Y_{x/NO3}$, and from equation (12), $Y_{x/NO3}$ is estimated as 9.2 g DCW/g NO_3. The nitrate concentration reduction in the cultivation medium is equal to the nitrate consumed by the cell culture

$$C_{NO3,o}\text{-}C_{NO3,f} = \frac{X_f - X_o}{Y_{x/NO3}} \tag{13}$$

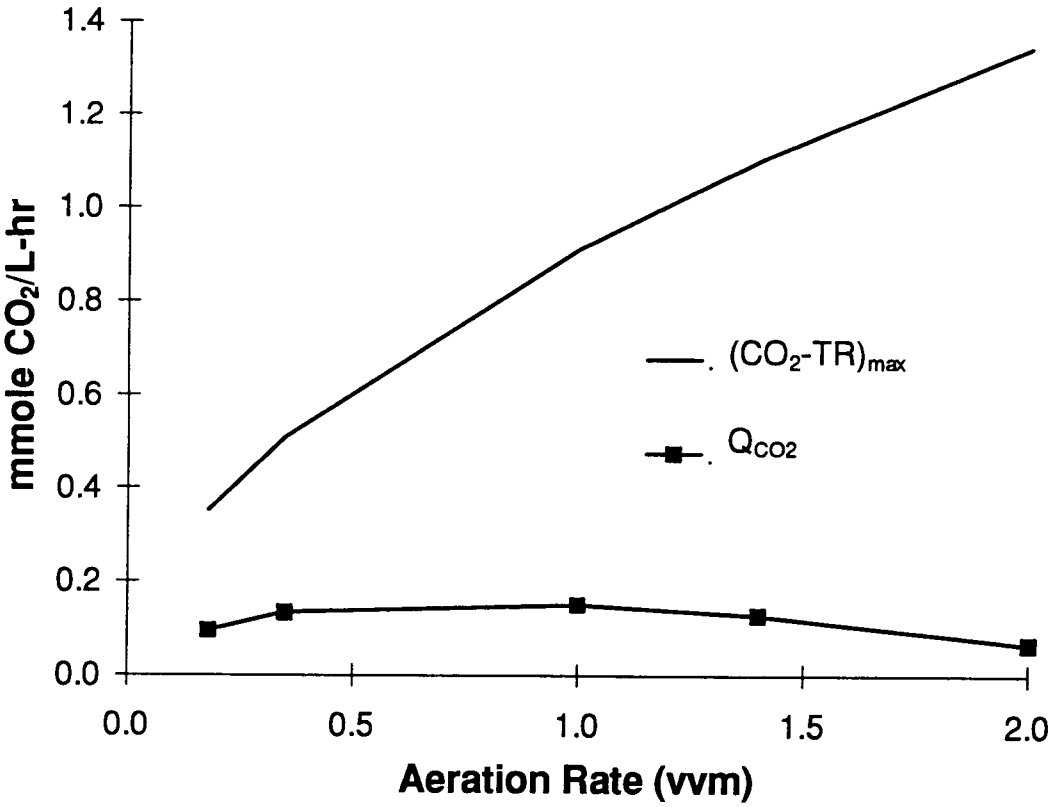

Figure 17. Comparison of maximum CO_2 transfer rate $(CO_2\text{-}TR)_{max}$ and CO_2 consumption rate(Q_{CO2}).

where $C_{NO3,o}$ is the initial nitrate concentration (mg $NaNO_3$/L) in the medium and $C_{NO3,f}$ is the final nitrate concentration (mg $NaNO_3$/L) in the medium. Based on equation (13), define β as the ratio of available nitrate in the medium to the nitrate consumed by the culture:

$$\beta = \frac{C_{NO3o} * Y_{NO3}}{X_f - X_o} \tag{14}$$

In equation (14), β must be greater than or equal to 1 to avoid stoichiometrically limiting growth. The plot of β versus $C_{NO3,o}$ is shown in Figure 18. From this plot, we can see that cultivation is not stoichiometrically limited by the nitrate after the initial nitrate concentration is above 64 mg $NaNO_3$/L. Below this initial nitrate concentration, the cultivation is stoichiometrically limited by the nitrate.

Incident light intensity. Light is the energy source for photosynthesis of the *L. saccharina* female gametophyte cell culture. From the Results section, incident light intensity is a limiting factor for the growth of *L. saccharina* female cell cultures.

Several models have been proposed to correlate μ to light intensity. The exponential model proposed by Oorshot et al (1955), given by

$$\mu = \mu_{max} (1-e^{-Io/Ik}) \tag{15}$$

correlates μ to I_o, where μ_{max} (day^{-1}) is the specific growth rate at saturation, and I_k (lux) is the light intensity at which the extended initial slope of the light curve intersects with μ_{max}. Equation (15) was fitted to μ versus I_o data for the 900 mL bioreactor given in Figure 18. The parameters obtained by non-linear regression are presented in Table 10. The prediction of μ versus I_o for the 900 mL photobioreactor is shown in Figure 18.

The stationary phase biomass density (final dry cell density X_f) variation with I_o is analogous to μ versus I_o. At low light intensity, X_f increases as I_o increases until saturation is achieved. Below saturation, the light intensity received by the culture limits

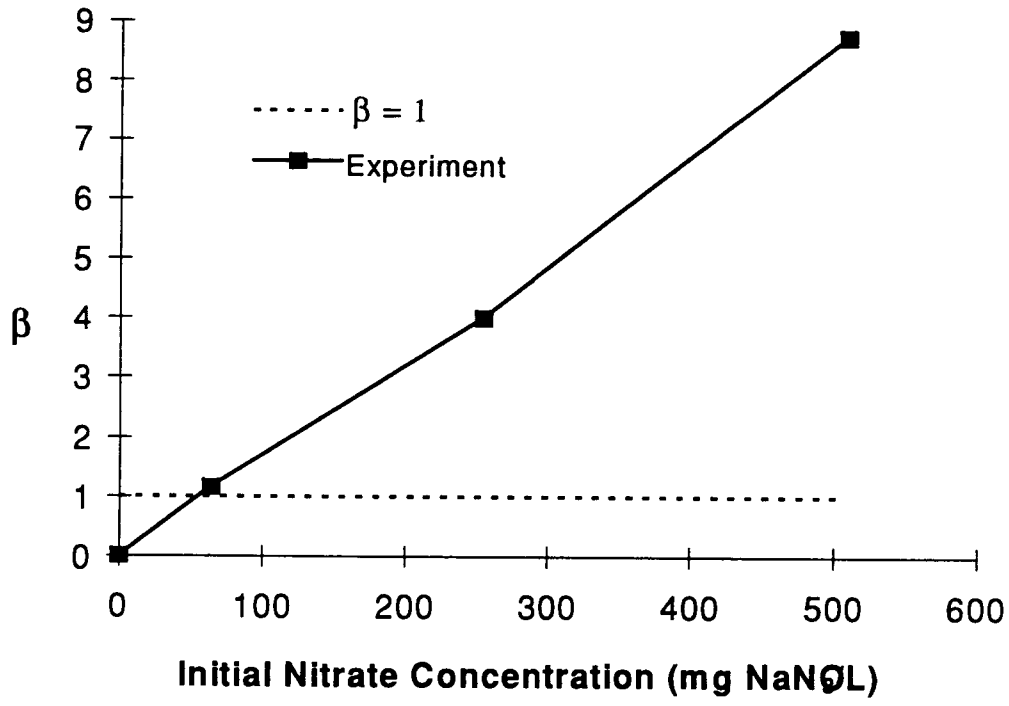

Figure 18. Plot of β versus initial nitrate concentration (C_{NO3o}).

biomass growth. At the saturation light intensity, the stationary phase is achieved when other medium nutrients are depleted. The relationship between X_f and I_o is also expressed as an exponential-type equation, given by

$$X_f = X_{fm} (1-e^{-Io/Ik'}) \qquad (16)$$

where X_{fm} is the maximum stationary phase biomass density at light saturation (mg DCW/L), and I_k' is the light intensity at which the extended initial slope of the light curve intersects with X_{fm} (lux). The parameter values obtained by non-linear regression for the 280 mL and 900 mL photobioreactors are presented in Table 10. Plots for prediction of X_f versus I_o for the 280 mL and 900 mL photobioreactors are shown in Figures 19 and 20 respectively.

From Table 10, X_{fm} for the 900 mL photobioreactor was significantly lower than Xfm for the 280 mL photobioreactor. This reduction in biomass productivity can be explained by the poor mixing in the 900 mL bioreactor. From Figures 18 to 20, both μ and X_f apparently increased with increasing I_o up to 3500-5000 lux, with a saturation effect at higher light intensities. Incident light intensities below 3500 lux limited the growth of *L. saccharina* female gametophyte cell culture.

Table 10. Parameters of equation (15) and equation (16).

	280 mL Bioreactor	900 mL Bioreactor
μ_{max} (day^{-1})	____	0.13
I_k (lux)	____	393
X_{fm} (mg DCW/L)	1318	866
I_k'(lux)	1474	384

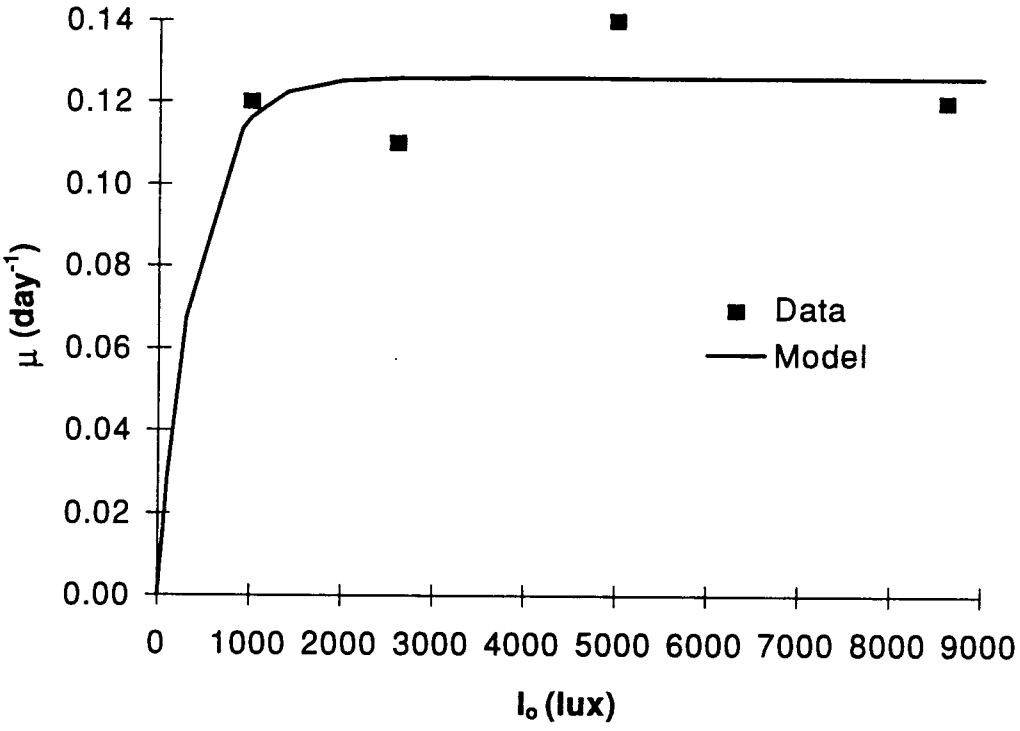

Figure 19. Specific growth rate (μ) versus incident light intensity (I_o) in the 900 mL bioreactor.

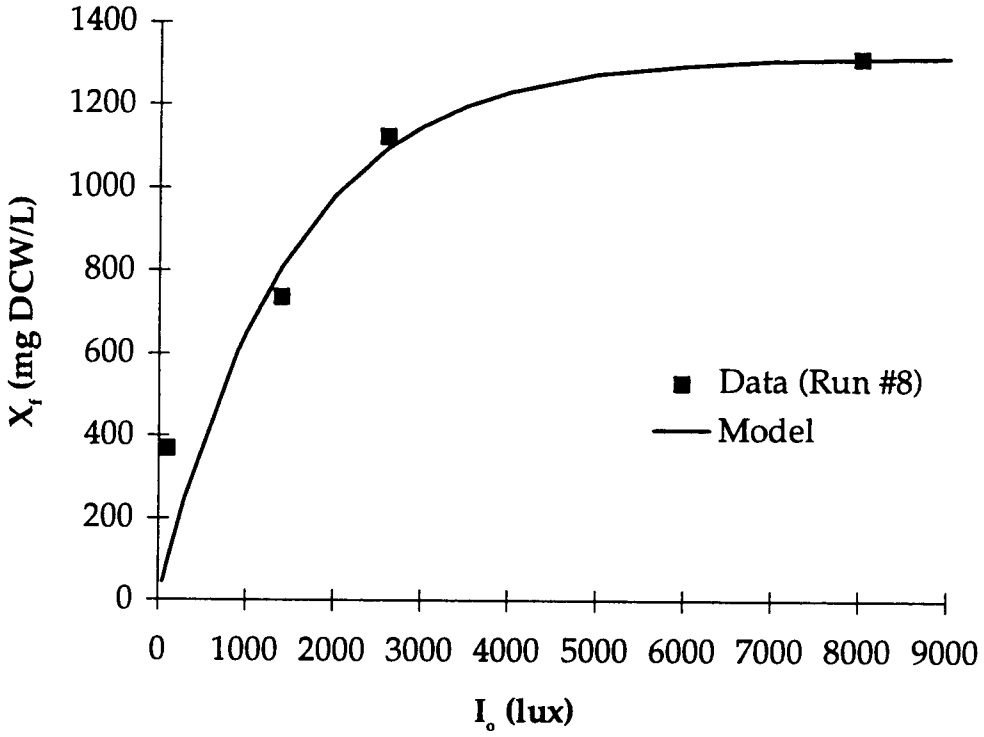

Figure 20. Maximum cell density (X_f) versus incident light intensity (I_o) in the 280 mL bioreactor.

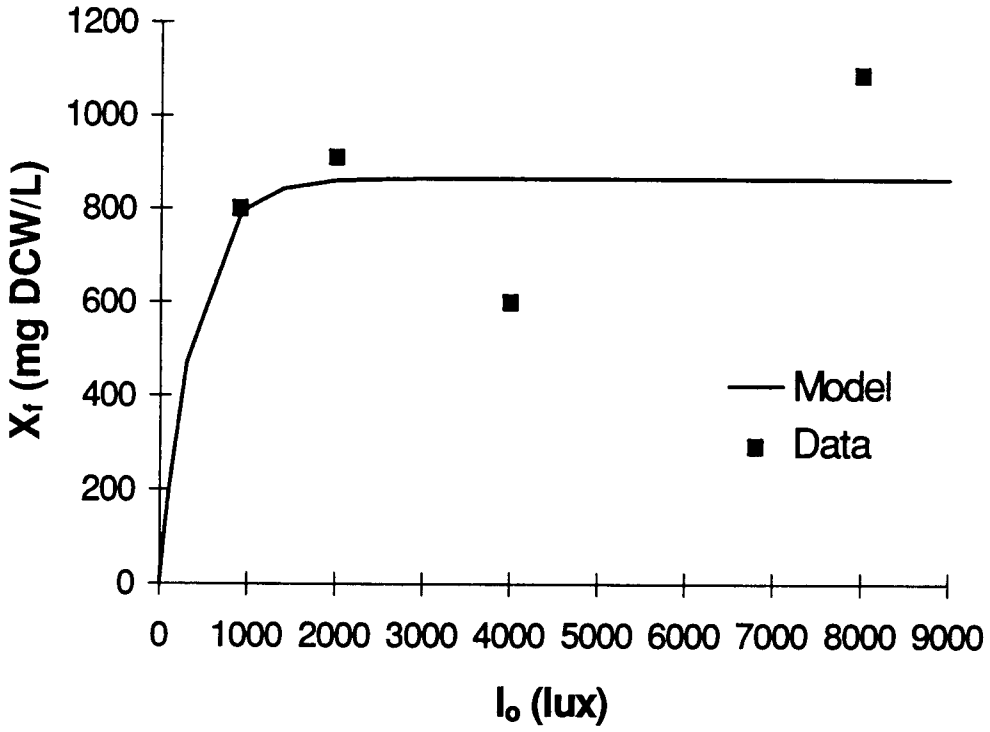

Figure 21. Maximum cell density (X_f) versus incident light intensity (I_o) in the 900 mL bioreactor.

In summary, the cultivation of macroalgal cell suspension cultures of *L. saccharina* female gametophyte cells was feasible in a bubble-column photobioreactor at 13-14 °C using CO_2 in air as the sole carbon source for growth.

Growth rate and biomass productivity data were obtained over a broad range of process conditions, including initial cell density (27 to 200 mg DCW/L), initial nitrate concentration (0 to 508 mg $NaNO_3$/L), aeration rate (0.17 to 2.00 vvm), and incident light intensity (90 to 8600 lux). Maximum cell densities exceeding 1100 mg DCW/L could be attained within 20 day cultivation period. Both specific growth rate and final biomass density data were correlated to incident light intensity using an exponential type model. Incident light intensity below 3500 lux limited the specific growth rate and final biomass density. Initial cell density had a significant effect on the final biomass density and no significant effect on the specific growth rate. The aeration rate affected the biomass growth rate and final biomass density, but the culture was not CO_2-transport limited. Initial nitrate concentrations above 64 mg $NaNO_3$/L in the GP2 medium had no significant effect on the specific growth rate and final biomass density, a result supported by biomass stoichiometry calculations.

Photolithotrophic cultivation of *Acrosiphonia coaltia* tissue culture was also possible in a bubble-column photobioreactor at 12 °C and 4000 lux incident light intensity. However, since the cells were clumped in the photobioreactor, there was no obvious advantage for the photobioreactor cultivation compared with static flask cultivation.

Future research needs to focus on several points. First, cultivation experiments at optimal process conditions for each process variable should be performed to see if biomass productivity is maximized. Recommended process conditions are initial cell density of 120 mg DCW/L, initial nitrate concentration of 64 mg $NaNO_3$/L, aeration rate of 1.00 vvm, and incident light intenisty of 5000 lux. Second, the eicosanoid productivity should be measured during the cultivation period. The effect of process variables on the eiconsanoid productivity should also be investigated. Finally, a new photobioreactor should be developed to alleviate clumping of the *Acrosiphonia coalita* tissue during cultivation. Further experiments should also consider the feeding of arachidonic acid to *L. saccharina* and *Acrosiphonia colita* cultures to stimulate the production of eicosanoids.

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APPENDICES

Appendix A

Tabulated Data

Table A-1. Laminarina saccharina Gametophyte Cell Cultivation Run 1

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**			**Process Parameters**		
Bioreactor Run#:	1	Inoculum Volume:	30.0	mL	Sparger Type:	Fixed	
Bioreactor #:	1, 2, 3	Inoculum Source:	DS-1-1		Flowmeter Setting:	50	Flowmeter 052530, 05
Date Started:	7/27/93	Age of Inoculum:	36.0	days	Aeration Rate:	97	mL/min
Time Started:	3:00 pm	NaHCO3 Conc.GP2 Medium:	0, 340, 680	mg/L	Setpoint Temperature:	13	C
		NaNO3 Conc.GP2 Medium:	63.5	mg/L	Illuminator Position:	4.5	inch from centerline
		GP2 Medium Volume:	250.0	mL	Illumination Intensity:	2600	Lux
		Total Culture Volume:	280.0	mL	Photoperiod:	16 hr ON 8 hr OFF	

	AU (665 nm)					mg chl a/L				
Day	BIOR #1	BIOR #2	BIOR #3	JGM's #1	JGM's #2	BIOR #1	BIOR #2	BIOR #3	JGM's #1	JGM's #2
0	0.039	0.047	0.026	0.031	0.041	0.635	0.766	0.424	0.505	0.668
2	0.043	0.084	0.044	0.061	0.057	0.700	1.368	0.717	0.994	0.929
4	0.040	0.090	0.033	0.099	0.098	0.652	1.466	0.538	1.613	1.596
6	0.054	0.079	0.051	0.146	0.147	0.880	1.287	0.831	2.378	2.395
8	0.067	0.082	0.042	0.201	0.177	1.091	1.336	0.684	3.274	2.883
10	0.128	0.098	0.064	0.299	0.251	2.085	1.596	1.043	4.871	4.089
12	0.146	0.172	0.074	0.340	0.260	2.378	2.802	1.205	5.539	4.235
14	0.248	0.289	0.081	0.352	0.269	4.040	4.708	1.319	5.734	4.382
16	0.168	0.181	0.067		0.292	2.737	2.948	1.091		4.757
18	0.272	0.223	0.076	0.437	0.355	4.431	3.633	1.238	7.119	5.783

Table A-2. Laminarina saccharina Gametophyte Cell Cultivation Run 2

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**			**Process Parameters**		
Bioreactor Run#:	2	Inoculum Volume:	30.0	mL	Sparger Type:	Fixed	
Bioreactor #:	1, 2, 3	Inoc. Source (cell line#):	DS-2-5,-2-6,-2-7		Flowmeter Setting:	50	Flowmeter 052530,0580
Date Started:	8/19/93	Age of Inoculum:	24.0	days	Aeration Rate:	97	mL/min
Time Started:	6:00 pm	NaHCO3 Conc.GP2 Medium:	340.0	mg/L	Setpoint Temperature:	13	C
		NaNO3 Conc.GP2 Medium:	0, 63.5, 127	mg/L	Illuminator Position:	4.5	inch from centerline
		GP2 Medium Volume:	250.0	mL	Illumination Intensity:	2600	Lux
		Total Culture Volume:	280.0	mL	Photoperiod:	16 hr ON 8 hr Off	

	AU (665 nm)			mg chl a/L			pH Value		
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #1	BIOR #2	BIOR #3	BIOR #1	BIOR #2	BIOR #3
0	0.012	0.019	0.012	0.195	0.310	0.195	8.350	8.460	8.500
2	0.017	0.023	0.020	0.277	0.375	0.326	8.230	8.260	8.320
4	0.017	0.020	0.024	0.277	0.326	0.391	8.300	8.260	8.340
6	0.031	0.032	0.042	0.505	0.521	0.684	8.220	8.240	8.300
8	0.029	0.047	0.054	0.472	0.766	0.880	8.180	8.250	8.310
10	0.040	0.056	0.057	0.652	0.912	0.929	8.290	8.370	8.420
12	0.050	0.088	0.064	0.815	1.434	1.043	8.230	8.270	8.280
14	0.062	0.061	0.085	1.010	0.994	1.385	8.250	8.200	8.240
16	0.029	0.041	0.067	0.472	0.668	1.091	8.240	8.350	8.380
18	0.034		0.064	0.554		1.043	8.150		8.280
20	0.049	0.161	0.202	0.798	2.623	3.291	8.240	8.270	8.340

Table A-3. Laminarina saccharina Gametophyte Cell Cultivation Run 3

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	
Bioreactor Run#:	3	Vessel Size:	280	280	280	mL
Bioreactor #:	1, 2, 3	Inoculum Volume:	30	30	30	mL
Date Started:	9/15/93	Inoculum Source:	DS-2-16,17	DS-2-12,15	DS-2-10,11	
Time Started:	1:00 pm	Age of Inoculum:	48	48	48	days
		GP2 Medium Volume:	250	250	250	mL
		Total Volume:	280	280	280	mL
		NaHCO3 Conc. GP2 Medium	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	254.0	508.0	mg/L

Day	AU (665 nm) BIOR #1	BIOR #2	BIOR #3	R11B1	mg chl a/L BIOR #1	BIOR #2	BIOR #3	pH Value BIOR #1
0	0.047	0.073	0.052	0.068	0.766	1.189	0.847	7.12
2	0.046	0.090	0.050	0.056	0.749	1.466	0.815	7.17
4	0.041	0.099	0.076	0.063	0.668	1.613	1.238	7.10
6	0.079	0.133	0.100	0.037	1.287	2.167	1.629	7.05
8	0.094	0.155	0.117	0.042	1.531	2.525	1.906	7.10
10	0.103	0.186	0.150	0.044	1.678	3.030	2.444	7.08
12	0.149	0.206	0.171	0.044	2.427	3.356	2.786	7.42
14	0.161	0.268	0.180	0.066	2.623	4.366	2.932	7.36
16	0.230	0.251	0.159	0.036	3.747	4.089	2.590	7.38
18	0.258	0.234	0.180	0.030	4.203	3.812	2.932	7.39
20	0.266	0.436	0.421	0.042	4.333	7.102	6.858	

Table A-3. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 3

Process Parameters	BIOR #1	BIOR #2	BIOR #3	
Sparger Type:	fixed	fixed	removable	
Flowmeter Setting:	50	50	15	
Flowmeter S/N:	52530	58047	59360	
Aeration Rate:	97	97	94	mL/min
Setpoint Temperature:	13	13	13	C
Illuminator Position:	5	5	5	inch from centerline
Illumination Intensity:	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	hr ON:hr OFF

BIOR #2	BIOR #3	ln (mg chl a/L) BIOR #1	BIOR #2	BIOR #3	Predicted mg chl a/L BIOR #1	BIOR #2	BIOR #3
6.82	7.06	-0.267	0.173	-0.166			
7.65	7.42	-0.289	0.383	-0.205	0.690	1.439	0.962
7.39	7.28	-0.404	0.478	0.214	0.873	1.725	1.187
7.29	7.30	0.252	0.773	0.488	1.106	2.067	1.464
7.40	7.27	0.426	0.926	0.645	1.400	2.478	1.805
7.39	7.28	0.518	1.109	0.893	1.772	2.971	2.226
7.40	7.30	0.887	1.211	1.024	2.244	3.561	2.746
7.59	7.58	0.964	1.474	1.076	2.840	4.268	3.386
7.48	7.44	1.321	1.408	0.952			
7.62	7.85	1.436	1.338	1.076			
		1.466	1.960	1.925			

Table A-3. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 3

BIOR #1

mg chl a/L vs time, 2 to 14 days		
Regression Output:		
Constant		-0.607
Std Err of Y Est		0.158
R Squared		0.925
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.118	1/day
Std Err of Coef.	0.015	

BIOR #2

mg chl a/L vs time, 2 to 14 days		
Regression Output:		
Constant		0.183
Std Err of Y Est		0.049
R Squared		0.987
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.091	1/day
Std Err of Coef.	0.005	

BIOR #3

mg chl a/L vs time, 2 to 14 days		
Regression Output:		
Constant		-0.248
Std Err of Y Est		0.121
R Squared		0.944
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.105	1/day
Std Err of Coef.	0.011	

Table A-4. Laminarina saccharina Gametophyte Cell Cultivation Run 4

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Bioreactor Run#:	4	Vessel Size:	280	280	280	280	mL
Bioreactor #:	1, 2, 3, 4	Inoculum Volume:	10	20	50	75	mL
Date Started:	10/11/93	Inoculum Source:	L-3-5,9,10,10,12,23				
Time Started:	3:30pm	Age of Inoculum:	70	70	70	70	days
		GP2 Medium Volume:	270	260	230	205	mL
		Total Volume:	280	280	280	280	mL
		NaHCO3 Conc. GP2 Medium	0.0	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	63.5	63.5	63.5	mg/L

	AU (665 nm)				mg chl a/L				pH Value
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1
0	0.008	0.018	0.039	0.057	0.130	0.293	0.635	0.929	7.30
2	0.015	0.060	0.062	0.068	0.244	0.977	1.010	1.108	7.17
4	0.012	0.065	0.089	0.108	0.195	1.059	1.450	1.759	7.05
6	0.027	0.071	0.092	0.145	0.440	1.157	1.499	2.362	7.03
8	0.028	0.071	0.107	0.215	0.456	1.157	1.743	3.502	7.02
10	0.055	0.075	0.130	0.170	0.896	1.222	2.118	2.769	7.04
12	0.053	0.076	0.132	0.178	0.863	1.238	2.150	2.900	7.01
14	0.041	0.050	0.176	0.195	0.668	0.815	2.867	3.177	7.10
16	0.042	0.049	0.159	0.156	0.684	0.798	2.590	2.541	7.19
18	0.075	0.053	0.150	0.175	1.222	0.863	2.444	2.851	
20	0.086	0.078	0.224	0.284	1.401	1.271	3.649	4.626	7.55

Table A-4. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 4

Process Parameters	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Sparger Type:	fixed	fixed	removable	removable	
Flowmeter Setting:	50	50	15	15	
Flowmeter S/N:	52530	58047	59360	26947	
Aeration Rate:	97	97	94	101	mL/min
Setpoint Temperature:	13	13	13	13	C
Illuminator Position:	5	5	5	5	inch from centerline
Illumination Intensity:	2600	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	16:8	hr ON:hr OFF

BIOR #2	BIOR #3	BIOR #4	Day	ln (mg Chl a/L) BIOR #1	BIOR #2	BIOR #3	BIOR #4	mg Chl a/L predicted BIOR #1	BIOR #2	BIOR #3	BIOR #4
7.48	7.97	7.94	0	-2.038	-1.227	-0.454	-0.074	0.134	0.512	0.759	0.963
7.42	7.72	7.82	2	-1.409	-0.023	0.010	0.102	0.190	0.639	0.945	1.254
7.33	7.71	7.65	4	-1.632	0.057	0.371	0.565	0.270	0.797	1.177	1.631
7.38	7.76	7.89	6	-0.821	0.145	0.405	0.860	0.384	0.994	1.467	2.122
			8	-0.785	0.145	0.556	1.253	0.546	1.239	1.827	2.761
			10	-0.110	0.200	0.750	1.019	0.777	1.545	2.276	3.592
			12	-0.147	0.214	0.766	1.065				
			14	-0.404	-0.205	1.053	1.156				
			16	-0.380	-0.225	0.952	0.933				
	7.66	7.68	18	0.200	-0.147	0.893	1.048				
7.73	7.92	7.89	20	0.337	0.240	1.294	1.532				

Table A-4. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 4

BIOR #1

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-2.013
Std Err of Y Est		0.245
R Squared		0.900
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.176	1/day
Std Err of Coef.	0.029	

BIOR #2

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.669
Std Err of Y Est		0.405
R Squared		0.565
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.110	1/day
Std Err of Coef.	0.048	

BIOR #3

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.276
Std Err of Y Est		0.148
R Squared		0.907
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.110	1/day
Std Err of Coef.	0.018	

BIOR #4

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.037
Std Err of Y Est		0.199
R Squared		0.885
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.132	1/day
Std Err of Coef.	0.024	

Table A-5. Laminarina saccharina Gametophyte Cell Cultivation Run 5

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Bioreactor Run#:	5	Vessel Size:	280	280	280	280	mL
Bioreactor #:	1, 2, 3, 4	Inoculum Volume:	19	47	73	93	mL
Date Started:	12/11/93	Inoculum Source:	LS-3-2,3,5,6	LS-3-2,3,5,	LS-3-2,3,5,	LS-3-2,3,5,6	
Time Started:	9:30 pm	Age of Inoculum:	41	41	41	41	days
		GP2 Medium Volume:	261	233	207	187	mL
		Total Volume:	280	280	280	280	mL
		NaHCO3 Conc. GP2 Medium	0.0	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	63.5	63.5	63.5	mg/L

	AU (665 nm)				mg chl a/L			
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	0.020	0.062	0.084	0.125	0.326	1.010	1.368	2.036
2	0.034	0.074	0.101	0.138	0.554	1.205	1.645	2.248
4	0.033	0.068	0.108	0.105	0.538	1.108	1.759	1.710
6	0.025	0.068	0.101	0.099	0.407	1.108	1.645	1.613
8	0.038	0.083	0.136	0.122	0.619	1.352	2.215	1.987
10	0.041	0.114	0.212	0.189	0.668	1.857	3.453	3.079
12	0.054	0.224	0.304	0.290	0.880	3.649	4.952	4.724
14	0.061	0.186	0.350	0.293	0.994	3.030	5.702	4.773
16	0.065	0.239	0.330	0.300	1.059	3.893	5.376	4.887
18	0.095	0.261	0.297	0.280	1.548	4.252	4.838	4.561
20		0.274	0.331	0.353		4.463	5.392	5.750
22	0.247				4.024			
24	0.182				2.965			
26	0.147				2.395			
28	0.231				3.763			

Table A-5. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 5

Process Parameters	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Sparger Type:	fixed	fixed	removable	removable	
Flowmeter Setting:	50	50	15	15	
Flowmeter S/N:	52530	58047	59360	26974	
Aeration Rate:	97	97	94	101	mL/min
Setpoint Temperature:	13	13	13	13	C
Illuminator Position:	5	5	5	5	inch from centerline
Illumination Intensity:	2600	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	16:8	hr ON:hr OFF

Day	ln (mg Chl a/L) BIOR #1	ln (mg Chl a/L) BIOR #2	BIOR #3	BIOR #4	mg Chl a/L predicted BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	-1.121	0.010	0.314	0.711				
2	-0.591	0.187	0.498	0.810				
4	-0.621	0.102	0.565	0.537				
6	-0.898	0.102	0.498	0.478	0.403	1.088	1.676	1.606
8	-0.480	0.302	0.795	0.687	0.517	1.469	2.330	2.176
10	-0.404	0.619	1.239	1.125	0.664	1.984	3.237	2.948
12	-0.128	1.294	1.600	1.553	0.852	2.680	4.498	3.993
14	-0.006	1.109	1.741	1.563	1.094	3.619	6.250	5.410
16	0.057	1.359	1.682	1.587	1.404			
18	0.437	1.447	1.577	1.518	1.803			
20	1.392	1.496	1.685	1.749	2.314			
22	1.087				2.971			
24	0.873							
28	1.325							

Table A-5. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 5

BIOR #1

mg chl a/L vs. time, 6 to 22 days		
Regression Output:		
Constant		-1.658
Std Err of Y Est		0.199
R Squared		0.929
No. of Observations		8.000
Degrees of Freedom		6.000
X Coefficient(s)	0.125	1/day
Std Err of Coef.	0.014	

BIOR #2

mg chl a/L vs. time, 6 to 14 days		
Regression Output:		
Constant		-0.817
Std Err of Y Est		0.215
R Squared		0.867
No. of Observations		5.000
Degrees of Freedom		3.000
X Coefficient(s)	0.150	1/day
Std Err of Coef.	0.034	

BIOR #3

mg chl a/L vs. time, 6 to 14 days		
Regression Output:		
Constant		-0.470
Std Err of Y Est		0.091
R Squared		0.978
No. of Observations		5.000
Degrees of Freedom		3.000
X Coefficient(s)	0.164	1/day
Std Err of Coef.	0.014	

BIOR #4

mg chl a/L vs. time, 6 to 14 days		
Regression Output:		
Constant		-0.437
Std Err of Y Est		0.134
R Squared		0.945
No. of Observations		5.000
Degrees of Freedom		3.000
X Coefficient(s)	0.152	1/day
Std Err of Coef.	0.021	

Table A-6. Laminarina saccharina Gametophyte Cell Cultivation Run 6

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification	
Bioreactor Run#:	6
Bioreactor #:	1,2,3,4
Date Started:	15/12/93
Time Started:	11:30 am

Culture Loading	
Inoculum Volume:	33 ml
Inoc. Source (cell line#):	L-5-3,5,6,8
Age of Inoculum:	31 days
NaHCO3 Conc.GP2 Medium:	0 mg/L
GP2 Medium Volume:	247 ml
NaNO3 Conc.GP2 Medium:	63.5 mg/L

	AU (665 nm)				mg chl a/L			
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	0.050	0.049	0.048	0.065	0.815	0.798	0.782	1.059
2	0.051	0.059	0.060	0.059	0.831	0.961	0.977	0.961
4	0.054	0.068	0.066	0.074	0.880	1.108	1.075	1.205
6	0.061	0.085	0.083	0.078	0.994	1.385	1.352	1.271
8	0.064	0.103	0.112	0.084	1.043	1.678	1.824	1.368
10	0.074	0.081	0.089	0.060	1.205	1.319	1.450	0.977
12	0.126	0.139	0.227	0.026	2.053	2.264	3.698	0.424
14	0.081	0.107	0.250	0.025	1.319	1.743	4.073	0.407
16	0.146	0.166	0.279	0.053	2.378	2.704	4.545	0.863
18	0.136	0.162	0.164	0.016	2.215	2.639	2.672	0.261
20	0.082	0.251	0.145	0.026	1.336	4.089	2.362	0.424
20	0.269	0.293	0.247	0.301	4.382	4.773	4.024	4.903

Table A-6. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 6

Process Parameters	BIOR#1	BIOR#2	BIOR#3	BIOR#4	
Sparger Type:	Fixed	fixed	removable	removable	
Flowmeter Setting:	25	50	40	60	
Flowmeter S/N:	52530	58047	59360	26947	
Aeration Rate:	49	97	280	560	mL/min
Setpoint Temperature:	13	13	13	13	C
Illuminator Position:	5	5	5	5	inch from centerline
Illumination Intensity:	2600	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	16:8	hr ON:hr OFF

Day	ln (mg chl a/L)				mg chl a/L Predicted			
	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	-0.205	-0.225	-0.246	0.057				
2	-0.185	-0.040	-0.023	-0.040		0.996	0.862	1.008
4	-0.128	0.102	0.072	0.187	0.841	1.132	1.097	1.127
6	-0.006	0.325	0.302	0.240	0.978	1.287	1.395	1.259
8	0.042	0.518	0.601	0.314	1.137	1.462	1.774	1.408
10	0.187	0.277	0.371	-0.023	1.323	1.662	2.257	1.573
12	0.719	0.817	1.308	-0.859	1.538	1.888	2.871	
14	0.277	0.556	1.404	-0.898	1.789	2.146	3.651	
16	0.866	0.995	1.514	-0.147	2.080	2.439	4.644	
18	0.795	0.970	0.983	-1.345	2.420	2.771	5.907	
20	0.290	1.408	0.860	-0.859				

Table A-6. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 6

BIOR #1

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.476
Std Err of Y Est		0.206
R Squared		0.751
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.076	
Std Err of Coef.	0.019	

BIOR #2

mg chl a/L vs. time, 2 to 16 days		
Regression Output:		
Constant		-0.132
Std Err of Y Est		0.166
R Squared		0.805
No. of Observations		8.000
Degrees of Freedom		6.000
X Coefficient(s)	0.064	
Std Err of Coef.	0.013	

BIOR #3

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.389
Std Err of Y Est		0.220
R Squared		0.893
No. of Observations		8.000
Degrees of Freedom		6.000
X Coefficient(s)	0.12	
Std Err of Coef.	0.02	

BIOR#4

mg chl a/L vs. time, 2 to 8 days		
Regression Output:		
Constant		-0.103
Std Err of Y Est		0.062
R Squared		0.889
No. of Observations		4.000
Degrees of Freedom		2.000
X Coefficient(s)	0.06	
Std Err of Coef.	0.01	

Table A-7. Laminarina saccharina Gametophyte Cell Cultivation Run 7

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification	
Bioreactor Run#:	7
Bioreactor #:	1,2,3,4
Date Started:	13/01/94
Time Started:	11:30 am

Culture Loading	
Inoculum Volume:	30 ml
Inoc. Source (cell line#):	DS-4-6,1,9
Age of Inoculum:	60 days
NaHCO3 Conc.GP2 Medium:	0 mg/L
GP2 Medium Volume:	250 ml
NaNO3 Conc.GP2 Medium:	63.5 mg/L

	AU (665 nm)				mg chl a/L			
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	0.050	0.048	0.052	0.056	0.815	0.782	0.847	0.912
2	0.057	0.070	0.069	0.047	0.929	1.140	1.124	0.766
4	0.072	0.081	0.078	0.067	1.173	1.319	1.271	1.091
6	0.104	0.118	0.083	0.064	1.694	1.922	1.352	1.043
8	0.162	0.142	0.089	0.074	2.639	2.313	1.450	1.205
10	0.141	0.212	0.082	0.107	2.297	3.453	1.336	1.743
12	0.202	0.220	0.045	0.156	3.291	3.584	0.733	2.541
14	0.239	0.303	0.036	0.173	3.893	4.936	0.586	2.818
16	0.246	0.325	0.039	0.120	4.007	5.294	0.635	1.955
18	0.188	0.268	0.018	0.134	3.063	4.366	0.293	2.183
20	0.189	0.337	0.016	0.113	3.079	5.490	0.261	1.841
20	0.374	0.331	0.365	0.451	6.092	5.392	5.946	7.347

Table A-7. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 7

Process Parameters	BIOR#1	BIOR#2	BIOR#3	BIOR#4	
Sparger Type:	Fixed	Fixed	Removable	Removable	
Flowmeter Setting:	25	50	40	50	
Flowmeter S/N:	52530	58047	59360	26947	
Aeration Rate:	49	97	280	560	mL/min
Setpoint Temperature:	13	13	13	13	C
Illuminator Position:	5	5	5	5	inch from centerline
Illumination Intensity:	2600	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	16:8	hr ON:hr OFF

	ln (mg chl a/L))				mg chl a/L Predicted			
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
0	-0.205	-0.246	-0.166	-0.092				
2	-0.074	0.131	0.117	-0.267	0.995	1.118	1.048	0.750
4	0.159	0.277	0.240	0.087	1.262	1.434	1.189	0.933
6	0.527	0.653	0.302	0.042	1.602	1.840	1.348	1.161
8	0.970	0.839	0.371	0.187	2.032	2.361	1.529	1.444
10	0.832	1.239	0.290	0.556	2.579	3.029	1.734	1.797
12	1.191	1.276	-0.311	0.933	3.272	3.887	1.967	2.235
14	1.359	1.597	-0.534	1.036	4.152	4.987		2.781
16	1.388	1.667	-0.454	0.670				
18	1.119	1.474	-1.227	0.781				
20	1.125	1.703	-1.345	0.610				

Table A-7. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 7

BIOR#1

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.243
Std Err of Y Est		0.141
R Squared		0.941
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.119	1/day
Std Err of Coef.	0.013	

BIOR#2

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.138
Std Err of Y Est		0.082
R Squared		0.981
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.125	1/day
Std Err of Coef.	0.008	

BIOR#3

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.079
Std Err of Y Est		0.081
R Squared		0.889
No. of Observations		5.000
Degrees of Freedom		3.000
X Coefficient(s)	0.063	
Std Err of Coef.	0.013	

BIOR#4

mg chl a/L vs. time, 4 to 16 days		
Regression Output:		
Constant		-0.506
Std Err of Y Est		0.132
R Squared		0.939
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.109	1/day
Std Err of Coef.	0.012	

Table A-8. Laminarina saccharina Gametophyte Cell Cultivation Run 8

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Bioreactor Run#:	8	Vessel Size:	280	280	280	280	mL
Bioreactor #:	1, 2, 3, 4	Inoculum Volume:	44	44	44	44	mL
Date Started:	2/15/94	Inoculum Source:	DS-5-2,4,7	DS-5-2,4,7	DS-5-2,4,7	DS-5-2,4,7	
Time Started:	11:30 am	Age of Inoculum:	38	38	38	38	days
		GP2 Medium Volume:	236	236	236	236	mL
		Total Volume:	280	280	280	280	mL
		NaHCO3 Conc. GP2 Medium	0.0	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	63.5	63.5	63.5	mg/L

	AU (665 nm)				mg chl a/L				pH value
Day	BIOR #1	BIOR #2	BIOR #3	BIOR#4	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1
0	0.070	0.078	0.085	0.082	1.140	1.271	1.385	1.336	7.500
2	0.094	0.110	0.079	0.067	1.531	1.792	1.287	1.091	
4	0.136	0.240	0.113	0.096	2.215	3.910	1.841	1.564	
6	0.189	0.115	0.172	0.150	3.079	1.873	2.802	2.444	7.680
8	0.195	0.133	0.147	0.224	3.177	2.167	2.395	3.649	
10	0.249	0.246	0.173	0.187	4.056	4.007	2.818	3.046	
12	0.276	0.297	0.152	0.222	4.496	4.838	2.476	3.616	7.900
14	0.246	0.278	0.209	0.225	4.007	4.529	3.405	3.665	
16	0.208	0.264	0.157	0.211	3.388	4.301	2.558	3.437	
18	0.241	0.189	0.135	0.184	3.926	3.079	2.199	2.997	7.850
20	0.255	0.169	0.135	0.184	4.154	2.753	2.199	2.997	
20	0.382	0.413	0.378	0.394	6.223	6.728	6.158	6.418	7.940

Table A-8. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 8

Process Parameters	BIOR #1	BIOR #2	BIOR #3	BIOR #4	
Sparger Type:	fixed	fixed	removable	removable	
Flowmeter Setting:	50	50	15	15	
Flowmeter S/N:	52530	58047	59360	26947	
Aeration Rate:	97	97	94	101	mL/min
Setpoint Temperature:	13	13	13	13	C
Illuminator Position:	5	6 (with net)	6	3	inch from centerline
Illumination Intensity:	2600	90	1400	8000	Lux
Photoperiod:	16:8	16:8	16:8	16:8	hr ON:hr OFF

			ln (mg chl a/L)				Predicted mg chl a/L			
BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4	BIOR #1	BIOR #2	BIOR #3	BIOR #4
7.650	7.680	7.750	0.131	0.240	0.325	0.290	1.278	1.487	1.311	1.153
			0.426	0.583	0.252	0.087	1.608	1.780	1.549	1.423
			0.795	1.363	0.610	0.447	2.023	2.130	1.830	1.756
7.690	7.690	7.740	1.125	0.628	1.030	0.893	2.544	2.550	2.163	2.166
			1.156	0.773	0.873	1.294	3.200	3.051	2.555	2.673
			1.400	1.388	1.036	1.114	4.026	3.652	3.019	3.299
7.910	7.960	8.000	1.503	1.577	0.907	1.285	5.064	4.371		4.071
			1.388	1.510	1.225	1.299				
			1.220	1.459	0.939	1.235				
7.950	7.900	7.950	1.368	1.125	0.788	1.098				
			1.424	1.013	0.788	1.098				
7.900	7.800	7.700	1.828	1.906	1.818	1.859				

Table A-8. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 8

BIOR #1

mg chl a/L vs. time, 0 to 12 days		
Regression Output:		
Constant		0.245
Std Err of Y Est		0.122
R Squared		0.952
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.115	1/day
Std Err of Coef.	0.012	

BIOR #2

mg chl a/L vs. time, 0 to 12 days		
Regression Output:		
Constant		0.397
Std Err of Y Est		0.353
R Squared		0.591
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.090	1/day
Std Err of Coef.	0.033	

BIOR #3

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		0.271
Std Err of Y Est		0.168
R Squared		0.811
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.083	1/day
Std Err of Coef.	0.020	

BIOR #4

mg chl a/L vs. time, 0 to 12 days		
Regression Output:		
Constant		0.142
Std Err of Y Est		0.218
R Squared		0.839
No. of Observations		7.000
Degrees of Freedom		5.000
X Coefficient(s)	0.105	1/day
Std Err of Coef.	0.021	

Table A-9. Laminarina saccharina Gametophyte Cell Cultivation Run 9

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	
Bioreactor Run#:	9	Vessel Size:	280	900	280	mL
Bioreactor #:	1, 2, 3	Inoculum Volume:	45	130	45	mL
Date Started:	4/11/94	Inoculum Source:	DS-6-1,2,4,	DS-6-1,2,4,	DS-6-1,2,4,7	
Time Started:	1:30 pm	Age of Inoculum:	46	46	46	days
		GP2 Medium Volume (mL):	235	770	235	mL
		Total Volume (mL)	280	900	280	mL
		NaHCO3 Conc. GP2 Medium:	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	254.0	63.5	64.0	mg/L
		FeCl3 Conc. GP2 Medium:			0.088	mg/L

	AU (665 nm)			mg Chl a/L					X (mg DCW/L)		
Day	BIOR #1	BIOR #2	BIOR #3	BIOR #1	BIOR #2	BIOR #3	Day	Notes	BIOR #1	BIOR #2	BIOR #3
							0	Xo - inoculum	139.0	136.0	139.0
0	0.062	0.069	0.058	1.010	1.124	0.945	0	Xo	139.0	137.0	139.0
2	0.077	0.091	0.068	1.254	1.482	1.108	2				
4	0.081	0.108	0.094	1.319	1.759	1.531	4			145.0	
6	0.119	0.106	0.121	1.939	1.727	1.971	6				
8	0.193	0.195	0.155	3.144	3.177	2.525	8			235.0	
10	0.136	0.168	0.196	2.215	2.737	3.193	10				
12	0.064	0.182	0.179	1.043	2.965	2.916	12				
14	0.071	0.194	0.127	1.157	3.160	2.069	14			518.0	
16	0.073	0.222	0.123	1.189	3.616	2.004	16				
18	0.060	0.207	0.132	0.977	3.372	2.150	18			755.0	
20	0.102	0.215	0.129	1.662	3.502	2.101	20				
22	0.310	0.256	0.296	5.050	4.170	4.822	22	Xf, B-1, B-3	435.0	780.0	880.0
24		0.187			3.046		24			885.0	
24		0.252			4.105		24	Xf, B-2		910.0	

Table A-9. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 9

Process Parameters	BIOR #1	BIOR #2	BIOR #3	
Sparger Type:	removable	removable	removable	
Flowmeter Serial #:	58047	59360	26947	
Flowmeter Setting:	50	38	15	
Aeration Rate:	97	275	101	mL/min
Temperature:	13	13	13	C
Illuminator Position:	5	5	5	inch from centerline
Incident Light Flux:	2600	2600	2600	Lux
Photoperiod:	16:8	16:8	16:8	hr ON:hr OFF

Wt% Chl a BIOR # 2	pH value BIOR #1	BIOR #2	BIOR #3	ln (mg Chl a/L) Day	BIOR #1	BIOR #2	BIOR #3	Predicted mg Chl a/L BIOR #1	BIOR #2	BIOR #3	Days	ln (X) BIOR #2	X, predicted BIOR #2
0.820	7.35	7.21	7.86	0	0.010	0.117	-0.057	1.015	1.163	0.914	0	4.920	
				2	0.227	0.394	0.102	1.242	1.409	1.176	4	4.977	146.7
1.213	7.61	7.31	7.55	4	0.277	0.565	0.426	1.520	1.707	1.512	8	5.460	237.0
				6	0.662	0.546	0.679	1.860	2.068	1.945	14	6.250	487.0
1.352	7.55	7.75	7.90	8	1.145	1.156	0.926	2.276	2.506	2.502	18	6.627	787.1
				10	0.795	1.007	1.161	2.785	3.036	3.219	22	6.659	
				12	0.042	1.087	1.070				24	6.813	
0.610	7.75	7.66	7.80	14	0.145	1.151	0.727						
				16	0.173	1.285	0.695						
0.447				18	-0.023	1.216	0.766						
				20	0.508	1.253	0.743						
0.535													
0.344													
0.451													

Table A-9. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 9

BIOR #1

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		0.015
Std Err of Y Est		0.211
R Squared		0.800
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.101	1/day
Std Err of Coef.	0.025	

BIOR #2

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		0.151
Std Err of Y Est		0.161
R Squared		0.861
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.0960	1/day
Std Err of Coef.	0.0193	

BIOR #3

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.090
Std Err of Y Est		0.036
R Squared		0.995
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.126	1/day
Std Err of Coef.	0.004	

BIOR #2

mg DCW/L vs. time, 4 to 18 days		
Regression Output:		
Constant		4.508
Std Err of Y Est		0.054
R Squared		0.997
No. of Observations		4.000
Degrees of Freedom		2.000
X Coefficient(s)	0.120	1/day
Std Err of Coef.	0.005	

Table A-10. Laminarina saccharina Gametophyte Cell Cultivation Run 10

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	
Bioreactor Run#:	10	Vessel Size:	280	900	280	mL
Bioreactor #:	1, 2, 3	Inoculum Volume:	38	120	38	mL
Date Started:	5/24/94	Inoculum Source:	LS-6-1,2,3,	LS-6-1,2,3	LS-6-1,2,3	
Time Started:	10:00 am	Age of Inoculum:	40	40	40	days
		GP2 Medium Volume (mL):	242	780	242	mL
		Total Volume (mL)	280	900	280	mL
		NaHCO3 Conc. GP2 Medium:	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	63.5	63.5	mg/L
		FeCl3 Conc. GP2 Medium:	0.0	0.0	0.0	mg/L

	AU (665 nm)			mg Chl a/L			Day	Notes	X (mg DCW/L)		
Days	BIOR #1	BIOR #2	BIOR #3	BIOR #1	BIOR #2	BIOR #3			BIOR #1	BIOR #2	BIOR #3
0	0.046	0.061	0.057	0.749	0.994	0.929	0	Xo	140.0	140.0	140.0
2	0.072	0.077	0.057	1.173	1.254	0.929	2				
4	0.100	0.074	0.113	1.629	1.205	1.841	4			168.0	
6	0.053	0.145	0.182	0.863	2.362	2.965	6				
8	0.094	0.164	0.132	1.531	2.672	2.150	8			263.0	
10	0.056	0.240	0.174	0.912	3.910	2.834	10				
12	0.045	0.243	0.132	0.733	3.958	2.150	12			523.0	
14	0.047	0.266	0.160	0.766	4.333	2.606	24				
16	0.036	0.234	0.155	0.586	3.812	2.525	16			750.0	
18	0.033	0.217	0.151	0.538	3.535	2.460	18				
20	0.040	0.222	0.157	0.652	3.616	2.558	20	Xf, B-1, B-3	128.0	660.0	550.0
20	0.152	0.355	0.264	2.476	5.783	4.301	20	Xf, B-2		1088.0	

Table A-10. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 10

Process Parameters	BIOR #1	BIOR #2	BIOR #3	
Sparger Type:	removable	removable	removable	
Flowmeter Serial #:	58047	59360	26947	
Flowmeter Setting:	50	38	15	
Aeration Rate:	97	275	101	mL/min
Temperature:	13	13	13	C
Illuminator Position:	6 (with net)	5	3	inch from centerline
Incident Light Flux:	990	3000	8500	Lux
Photoperiod:	16:8	16:8	16:8	hr ON:hr OFF

Wt% Chl a	pH Value			ln (mg Chl a/L)				Predicted mg Chl a/L			ln (X)	X, predicted
BIOR # 2	BIOR #1	BIOR #2	BIOR #3	Day	BIOR #1	BIOR #2	BIOR #3	BIOR #1	BIOR #2	BIOR #3	Days	BIOR #2	BIOR #2
0.710	7.67	7.84	7.00	0	-0.289	-0.006	-0.074	1.009	0.907	0.948	0	4.942	
				2	0.159	0.227	-0.074	1.043	1.200	1.211	4	5.124	173.6
0.718	7.54	7.80	7.77	4	0.488	0.187	0.610	1.077	1.588	1.548	8	5.572	280.1
				6	-0.147	0.860	1.087	1.113	2.100	1.978	12	6.260	452.0
1.016	7.50	8.00	7.79	8	0.426	0.983	0.766	1.151	2.778	2.527	16	6.620	729.2
				10	-0.092	1.363	1.042	1.189	3.674	3.228	20	6.992	1176.6
0.757	7.35	7.75	7.71	12	-0.311	1.376	0.766						
				14	-0.267	1.466	0.958						
0.508				16	-0.534	1.338	0.926						
				18	-0.621	1.263	0.900						
0.548				20	-0.428	1.285	0.939						
0.532													

Table A-10. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 10

BIOR #1

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		0.009
Std Err of Y Est		0.350
R Squared		0.037
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.016	1/day
Std Err of Coef.	0.042	

BIOR #2

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.097
Std Err of Y Est		0.162
R Squared		0.929
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.140	1/day
Std Err of Coef.	0.019	

BIOR #3

mg chl a/L vs. time, 0 to 10 days		
Regression Output:		
Constant		-0.053
Std Err of Y Est		0.278
R Squared		0.773
No. of Observations		6.000
Degrees of Freedom		4.000
X Coefficient(s)	0.123	1/day
Std Err of Coef.	0.033	

BIOR #2

mg DCW/L vs. time, 4 to 20 days		
Regression Output:		
Constant		4.678
Std Err of Y Est		0.105
R Squared		0.986
No. of Observations		5.000
Degrees of Freedom		3.000
X Coefficient(s)	0.120	1/day
Std Err of Coef.	0.008	

Table A-11. Laminarina saccharina Gametophyte Cell Cultivation Run 11

Bubble-Column Bioreactor Cell Growth and Process Measurements

Run Identification		**Culture Loading**	BIOR #1	BIOR #2	BIOR #3	
Bioreactor Run#:	13	Vessel Size:	280	900	900	mL
Bioreactor #:	1, 2, 3	Inoculum Volume:	38	120	38	mL
Date Started:	10/05/94	Inoculum Source:	LS-D-5-1,3,4,6			
Time Started:	3:30 pm	Age of Inoculum:	48	48	48	days
		GP2 Medium Volume (mL):	232	737	737	mL
		Total Volume (mL)	280	900	900	mL
		NaHCO3 Conc. GP2 Medium:	0.0	0.0	0.0	mg/L
		NaNO3 Conc.GP2 Medium:	63.5	63.5	63.5	mg/L
		FeCl3 Conc. GP2 Medium:	0.0	0.0	0.0	mg/L

	AU (665 nm)			mg Chl a/			X (mg DCW/L)			mg chl a/L	
Days	BIOR #1	BIOR #2	BIOR #3	BIOR #1	Day	Notes	BIOR #1	BIOR #2	BIOR #3	BIOR #2	BIOR #3
								140.0	140		
0	0.075	0.070	0.069	1.222	0	Xo	113.0	80.0	110.0	1.140	1.124
2	0.095	0.07	0.108	1.548	4			125.0	130.0	1.564	2.232
4	0.156	0.096	0.137	2.541	8			170.0	435.0	1.417	7.331
6	0.225			3.665	12			358.0	430.0	3.910	4.773
8	0.208	0.087	0.45	3.388	16			743.0	628.0	9.660	6.597
10	0.186			3.030	20	Xf, B-1, B-	1010.0	488.0	645.0	5.539	6.337
12	0.23	0.24	0.293	3.747	20	Xf, B-2		530.0	888.0	6.435	8.161
14	0.344			5.604							
16	0.411	0.593	0.405	6.695							
18	0.304			4.952							
20	0.375	0.34	0.389	6.109							
20	0.492	0.395	0.501	8.015							

Table A-11. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 11

Process Parameter	BIOR #1	BIOR #2	BIOR #3	
Sparger Type:	removabl	removabl	removable	
Flowmeter Serial #:	53918	26947	59360	
Flowmeter Setting:	35	35	35	
Aeration Rate:	250	275	275	mL/min
Temperature:	13	13	13	C
Illuminator Position:	5	3	5 (net)	inch from centerline
Incident Light Flux:	2600	5000	1000	Lux
Photoperiod:	16:8	16:8	16:8	hr ON:hr OFF

Wt% Chl BIOR # 2	pH Value BIOR #1	BIOR #2	BIOR #3	Day	ln (mg Chl a/L) BIOR #1	BIOR #2	BIOR #3	Predicted BIOR #1	Days	ln (X) BIOR #2	BIOR #3	X, predicted BIOR #2	BIO #3
1.425	7.90	7.95	8.00	0	0.200	0.131	0.117	1.268	0	4.38	4.70	71.2	109.3
				2	0.437			1.695	4	4.83	4.87	123.5	174.5
1.251	8.20	8.28	8.39	4	0.933	0.447	0.803	2.265	8	5.14	6.08	214.3	278.6
				6	1.299			3.028	12	5.88	6.06	371.8	445.0
0.834				8	1.220	0.349	1.992	4.048	16	6.61	6.44	645.1	710.5
				10	1.109			5.411	20	6.27	6.47		168.9
1.092				12	1.321	1.363	1.563	7.233					
				14	1.723			9.669					
1.300				16	1.901	2.268	1.887	12.925					
				18	1.600								
1.135				20	1.810	1.712	1.846						
1.214													

Table A-11. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Run 11

BIOR#1

mg chl a/L vs.time,0 to 8 days	
Regression Output:	
Constant	0.237
Std Err of Y Est	0.174
R Squared	0.903
No. of Observations	5.000
Degrees of Freedom	3.000
X Coefficient(s)	0.145
Std Err of Coef.	0.028

BIOR#2

mg DCW/L vs.time, 0 to 16 days	
Regression Output:	
Constant	4.266
Std Err of Y Est	0.172
R Squared	0.972
No. of Observations	5.000
Degrees of Freedom	3.000
X Coefficient(s)	0.138
Std Err of Coef.	0.014

BIOR#3

mg DCW/L vs. time,0 to 16 days	
Regression Output:	
Constant	4.694
Std Err of Y Est	0.317
R Squared	0.879
No. of Observations	5.000
Degrees of Freedom	3.000
X Coefficient(s)	0.117
Std Err of Coef.	0.025

Table 12. Laminarina saccharina Gametophyte Cell Cultivation Dry Cell Density

Bubble Column Bioreactor Cultivation
Compilation and Analysis of Dry Cell Density Data

Run # Bioreactor	Sample # Trial#	Sample Volume (mL)	Wt. Dried FF (g)	Wt. Dried FF + Cells + Residue (g)	Washing Techniqu	Corrected Dr Cell Density X (mg DCW/L)	Ave. Corrected Dry Cell Density X (mg DCW/L)	Ave. Corrected Dry Cell Density 1s for X (mg DCW/L)
R3-B1	Xi-1	20.0	0.0855	0.0944	DD H2O	614.8	596.0	26.6
	Xi-2	20.0	0.0855	0.0934	DD H2O	558.3		
	Xi-3	20.0	0.0853	0.0942	DD H2O	614.9		
	Xf-1	20.0	0.0857	0.0938	DD H2O	569.6	565.9	2.7
	Xf-2	20.0	0.0850	0.0930	DD H2O	564.0		
	Xf-3	20.0	0.0841	0.0921	DD H2O	564.0		
R3-B2	Xi-1	20.0	0.0855	0.0989	DD H2O	869.1	859.7	17.5
	Xi-2	20.0	0.0855	0.0990	DD H2O	874.7		
	Xi-3	20.0	0.0861	0.0989	DD H2O	835.2		
	Xf-1	20.0	0.0848	0.0945	DD H2O	660.1	652.5	10.7
	Xf-2	20.0	0.0839	0.0932	DD H2O	637.5		
	Xf-3	20.0	0.0835	0.0932	DD H2O	660.1		
R3-B3	Xi-1	20.0	0.0855	0.0960	DD H2O	705.2	692.1	11.6
	Xi-2	20.0	0.0853	0.0956	DD H2O	694.0		
	Xi-3	20.0	0.0855	0.0955	DD H2O	677.0		
	Xf-1	20.0	0.0835	0.0923	DD H2O	609.2	618.6	9.6
	Xf-2	20.0	0.0839	0.0931	DD H2O	631.8		
	Xf-3	20.0	0.0834	0.0923	DD H2O	614.8		
R4-B1	Xi-1	20.0	0.0896	0.1005	DD H2O	727.9	739.2	10.6
	Xi-2	20.0	0.0896	0.1007	DD H2O	739.1		
	Xi-3	20.0	0.0895	0.1005	DD H2O	733.5		
	Xi-4	20.0	0.0895	0.1009	DD H2O	756.1		
	Xf-1	20.0	0.0839	0.0922	DD H2O	581.0	595.1	14.1
	Xf-2	20.0	0.0840	0.0928	DD H2O	609.2		
R4-B2	Xi-1	20.0	0.0896	0.1005	DD H2O	727.9	739.2	10.6
	Xi-2	20.0	0.0896	0.1007	DD H2O	739.1		
	Xi-3	20.0	0.0895	0.1005	DD H2O	733.5		
	Xi-4	20.0	0.0895	0.1009	DD H2O	756.1		
	Xf-1	20.0	0.0838	0.0918	DD H2O	564.0	572.5	8.5
	Xf-2	20.0	0.0848	0.0931	DD H2O	581.0		
R4-B3	Xi-1	20.0	0.0896	0.1005	DD H2O	727.9	739.2	10.6
	Xi-2	20.0	0.0896	0.1007	DD H2O	739.1		
	Xi-3	20.0	0.0895	0.1005	DD H2O	733.5		
	Xi-4	20.0	0.0895	0.1009	DD H2O	756.1		
	Xf-1	20.0	0.0849	0.0987	DD H2O	891.7	730.7	161.0
	Xf-2	20.0	0.0848	0.0929	DD H2O	569.6		

Table 12. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Dry Cell Density

Run # Bioreactor	Sample # Trial#	Sample Volume (mL)	Wt. Dried FF (g)	Wt. Dried FF + Cells + Residue (g)	Washing Techniqu	Corrected Dry Cell Dens X (mg DCW/L)	Ave.Corrected Dry Cell Density X (mg DCW/L)	Ave. Corrected Dry Cell Density 1s for X (mg DCW/L)
R4-B4	Xi-1	20.0	0.0896	0.1005	DD H2O	727.9	739.2	10.6
	Xi-2	20.0	0.0896	0.1007	DD H2O	739.1		
	Xi-3	20.0	0.0895	0.1005	DD H2O	733.5		
	Xi-4	20.0	0.0895	0.1009	DD H2O	756.1		
	Xf-1	20.0	0.0850	0.0965	DD H2O	761.7	742.0	19.8
	Xf-2	20.0	0.0850	0.0958	DD H2O	722.2		
R5-B1	Xi-1	20.0	0.0709	0.0768	DD H2O	445.3	448.2	2.8
	Xi-2	20.0	0.0713	0.0773	DD H20	451.0		
	Xf-1	20.0	0.0736	0.0960	GP2-1	850.0	762.5	87.5
	Xf-2	20.0	0.0734	0.0923	GP2-1	675.0		
R5-B2	Xi-1	20.0	0.0709	0.0768	DD H20	445.3	448.2	2.8
	Xi-2	20.0	0.0713	0.0773	DD H20	451.0		
	Xf-1	20.0	0.0748	0.0893	GP2-1	455.0	457.5	2.5
	Xf-2	20.0	0.0726	0.0872	GP2-1	460.0		
R5-B3	Xi-1	20.0	0.0709	0.0768	DD H20	445.3	448.2	2.8
	Xi-2	20.0	0.0713	0.0773	DD H20	451.0		
	Xf-1	20.0	0.0734	0.1022	GP2-1	1170.0	1180.0	10.0
	Xf-2	20.0	0.0736	0.1028	GP2-1	1190.0		
R5-B4	Xi-1	20.0	0.0709	0.0768	DD H20	445.3	448.2	2.8
	Xi-2	20.0	0.0713	0.0773	DD H20	451.0		
	Xf-1	20.0	0.0879	0.1119	GP2-1	930.0	900.0	30.0
	Xf-2	20.0	0.0705	0.0933	GP2-1	870.0		
R6-B1	Xi-1	20.0	0.0731	0.0898	GP2-1	565.0	610.0	45.0
	Xi-2	20.0	0.0723	0.0908	GP2-1	655.0		
	Xf-1	20.0	0.0755	0.0895	GP2-1	430.0	417.5	12.5
	Xf-2	20.0	0.0759	0.0894	GP2-1	405.0		
R6-B2	Xf-1	20.0	0.0712	0.0860	GP2-1	470.0	470.0	0.0
	Xf-2	20.0	0.0709	0.0857	GP2-1	470.0		
R6-B3	Xf-1	20.0	0.0757	0.0971	GP2-1	800.0	785.0	15.0
	Xf-2	20.0	0.0711	0.0919	GP2-1	770.0		
R6-B4	Xf-1	20.0	0.0713	0.0860	GP2-1	465.0	455.0	10.0
	Xf-2	20.0	0.0711	0.0854	GP2-1	445.0		
R7-B1	Xi-1	20.0	0.0728	0.1113	GP2-1	1655.0	1482.5	172.5
	Xi-2	20.0	0.0734	0.1050	GP2-1	1310.0		
	Xi-3	20.0	0.0730	0.0911	DD H2O	1134.7	1058.4	76.3
	Xi-4	20.0	0.0715	0.0869	DD H2O	982.1		
	Xf-1	20.0	0.0726	0.0895	GP2-1	575.0	590.0	15.0
	Xf-2	20.0	0.0730	0.0905	GP2-1	605.0		

Table 12. (cont.) Laminarina saccharina Gametophyte Cell Cultivation Dry Cell Density

Run # Bioreactor	Sample # Trial#	Sample Volume (mL)	Wt. Dried FF (g)	Wt. Dried FF + Cells + Residue (g)	Washing Techniqu	Corrected Dry Cell Dens X (mg DCW/L)	Average Correct Dry Cell Density X (mg DCW/L)	Average Correct Dry Cell Density 1s for X (mg DCW/L)
R7-B2	Xf-1	20.0	0.0730	0.0931	GP2-1	735.0	765.0	30.0
	Xf-2	20.0	0.0730	0.0943	GP2-1	795.0		
R7-B3	Xf-1	20.0	0.0728	0.0864	GP2-1	410.0	430.0	20.0
	Xf-2	20.0	0.0725	0.0869	GP2-1	450.0		
R7-B4	Xf-1	20.0	0.0709	0.0929	GP2-1	830.0	870.0	40.0
	Xf-2	20.0	0.0738	0.0974	GP2-1	910.0		
R8-B1	Xi-1	20.0	0.0734	0.0945	GP2-1	785.0	752.5	32.5
	Xi-2	20.0	0.0732	0.0930	GP2-1	720.0		
	Xf-1	20.0	0.0752	0.0932	DD H20	1129.0	1123.4	5.7
	Xf-2	20.0	0.0755	0.0933	DD H20	1117.7		
R8-B2	Xi-1	20.0	0.0734	0.0945	GP2-1	785.0	752.5	32.5
	Xi-2	20.0	0.0732	0.0930	GP2-1	720.0		
	Xf-1	20.0	0.0753	0.0798	DD H2O	366.2	369.1	2.8
	Xf-2	20.0	0.0753	0.0799	DD H2O	371.9		
R8-B3	Xi-1	20.0	0.0734	0.0945	GP2-1	785.0	752.5	32.5
	Xi-2	20.0	0.0732	0.0930	GP2-1	720.0		
	Xf-1	20.0	0.0756	0.0864	DD H2O	722.2	736.3	14.1
	Xf-2	20.0	0.0747	0.0860	DD H2O	750.4		
R8-B4	Xi-1	20.0	0.0734	0.0945	GP2-1	785.0	752.5	32.5
	Xi-2	20.0	0.0732	0.0930	GP2-1	720.0		
	Xf-1	20.0	0.0761	0.0969	DD H2O	1287.2	1312.6	25.4
	Xf-2	20.0	0.0757	0.0974	DD H2O	1338.0		
R9-B1	Xi-1	20	0.0701	0.0929	GP2-2	870.0	865.0	5.0
	Xi-2	20	0.0704	0.0930	GP2-2	860.0		
	Xf-1	20	0.0802	0.0949	GP2-2	465.0	432.5	32.5
	Xf-2	20	0.0700	0.0834	GP2-2	400.0		
R9-B3	Xi-1	20	0.0701	0.0929	GP2-2	870.0	865.0	5.0
	Xi-2	20	0.0704	0.0930	GP2-2	860.0		
	Xf-1	20	0.0804	0.1027	GP2-2	845.0	880.0	35.0
	Xf-2	20	0.0774	0.1011	GP2-2	915.0		
R10-B1	Xi-1	20	0.0776	0.1041	GP2-2	1055.0	1050.0	5.0
	Xi-2	20	0.0778	0.1041	GP2-2	1045.0		
	Xf-1	20	0.0705	0.0787	GP2-2	140.0	127.5	12.5
	Xf-2	20	0.0704	0.0781	GP2-2	115.0		
R10-B3	Xi-1	20	0.0776	0.1041	GP2-2	1055.0	1050.0	5.0
	Xf-2	20	0.0778	0.1041	GP2-2	1045.0		
	Xf-1	20	0.0703	0.0868	GP2-2	555.0	550.0	5.0
	Xf-2	20	0.0703	0.0866	GP2-2	545.0		

Appendix B

Dry Cell Density

Dry Cell Density Measurement for *L. Saccharina* Female Gametophytes

Procedure

1. Dry three 47 mm, 0.45 μ m pore size, Millipore filters in oven at 70 °C overnight. Dry in covered 100 x 15 mm, marked, glass petri dishes.
2. Remove a filter from the oven in a hot dry glass covered petri dish and transport quickly to analytical balance.
3. Immediately remove filter from petri dish with forceps an place on balance.
4. Wait for balance reading to stabilize on one reading for five seconds. Record initial weight.
5. Repeat for all three filters.
6. Place filter on vacuum filtration stage and rinse with deionized water.
7. Pipette 20 mL of culture sample onto filter.
8. Vacuum filter the cells.
9. Obtain two blank dry filters and pipette 20 mL of GP2 solution onto filter to get the salt content of filter. Dry the filters at 70 °C in oven.
10. Remove filter from stage with forceps and transfer to marked glass petri dish.
11. Place in 70 °C oven. Watch carefully for first 30 minutes of drying, lifting filter from petri dish occasionally to prevent sticking. If filter does stick, re-wet with small amount of deionized water and lift filter.
12. Repeat for each sample.
13. After drying for 12 hrs, cover petri dish and transfer from oven to analytical balance.
14. Quickly remove filter from petri dish and place on balance. Record final weight after balance reading has stabilized for 5 seconds.
15. Calculate weight of cells by subtraction.
16. Divide final weight by mL filtered to obtain dry cell weight per liter.

Table B-1. Determination of correction factor for GP2 washing method.

Filter: Millipore 47 mm x 0.45 μ m (catalog # HWAP04700)

Washing: 20 mL GP2 medium

Trial #	Wt. Dried FP (g)	Wt. Dried FP + GP2 Salt (g)	GP2 Salt, m_{GP2} (g)	GP2 salt Dried FP
1	0.0778	0.0834	0.0056	0.0720
2	0.0779	0.0831	0.0052	0.0668
3	0.0777	0.0824	0.0047	0.0605
4	0.0772	0.0830	0.0058	0.0751
5	0.0805	0.0870	0.0065	0.0807
6	0.0686	0.0735	0.0049	0.0714
7	0.0770	0.0810	0.0040	0.0519
8	0.0772	0.0828	0.0056	0.0725
9	0.0777	0.0830	0.0053	0.0682
10	0.0774	0.0828	0.0054	0.0698
11	0.0774	0.0827	0.0053	0.0685
12	0.0774	0.0828	0.0054	0.0698
13	0.0771	0.0828	0.0057	0.0739
14	0.0774	0.0831	0.0057	0.0736
15	0.0706	0.0752	0.0046	0.0652
16	0.0703	0.0749	0.0046	0.0654
17	0.0803	0.0863	0.0060	0.0747
18	0.0809	0.0864	0.0055	0.0680
Avg	0.0767	0.0820	0.0053	0.0693
1 s	0.0034	0.0038	0.0006	0.0063
1s/Avg*100	4.41	4.58	11.02	9.06

Table B-2. Calibration curve of DD water washing versus GP2 washing.

Dry Cell Density (mg DCW/L)	
DD H_2O Washing $X_{(DD\ H2O)}$	GP2 Washing $X_{(GP2\text{-}1,2)}$
180	283
360	578
565	763
570	710
770	1005
1130	1385

$$X_{(GP2\text{-}1,2)} = X_{(DD\ H2O)} \times 1.13 + 112 \quad (\text{mg DCW/L})$$

Regression Output

Constant	111.6 (mg DCW/L)
Std Err of Y Est	42.84
R Squared	0.990
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	1.134 (mg DCW/L/mg DCW/L)
Std Err of Coef.	0.058

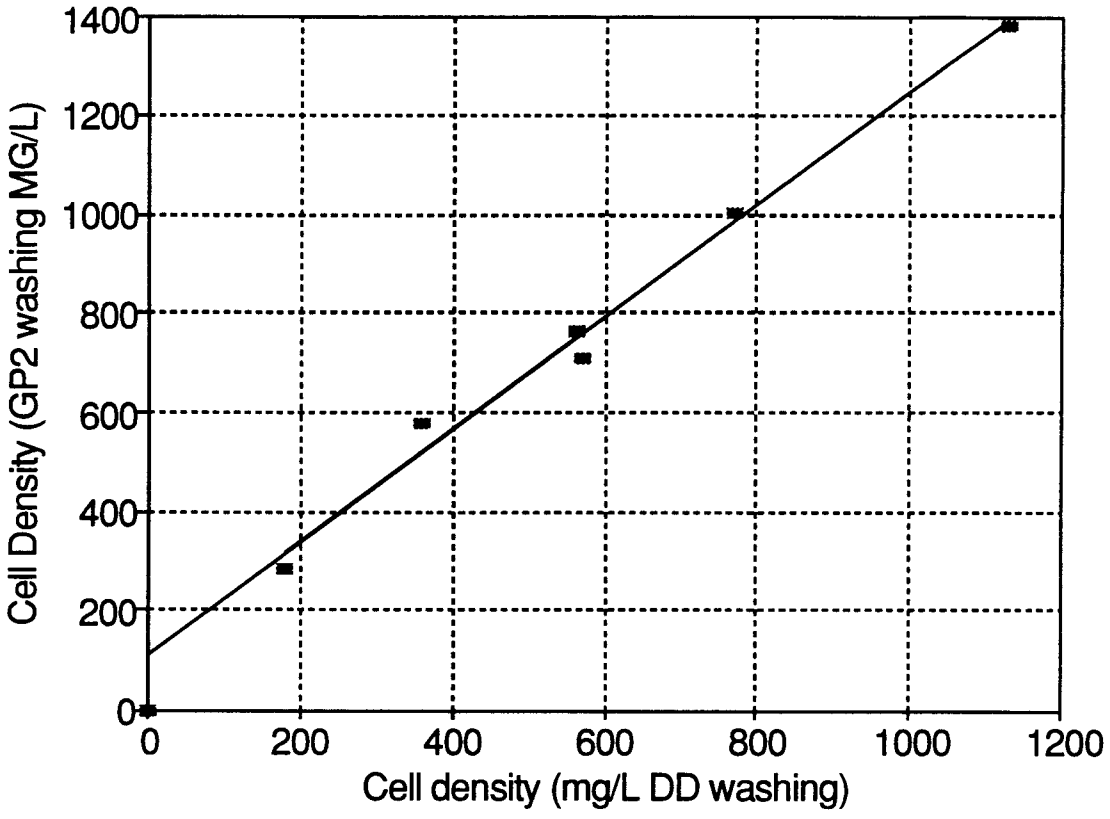

Figure B-1. DD water eashing versus GP2 washing.

Appendix C

Chlorophyll a Analysis

Dry Cell Density vs. Chl a Absorbance Calibration Curve

Procedure

1. Select 5 two week or four week old flask cultures of *L. saacharina* female gametophyte.
2. Blend cultures for 15-30 seconds at "liquefy" speed.
3. Filter the cultures through a 60 μ m nylon mesh filter.
4. Add the filtered gametophytes to 1 L of fresh GP2 medium.
5. Add stir bar to the 1 L flask.
6. Agitate culture continuously while sampling.
7. Take two samples, each in 5 mL, 10 mL, 20 mL, and 30 mL aliquots for absorbance measurement.
8. Filter samples through a 20 μ m nylon mesh filter. Measure chl a absorbance by spectrophotometric assay.
9. Take two samples of 20 mL, 30 mL, 50 mL, 70 mL, 100 mL for DCW measurement.
10. Filter through 0.45 μ m Millipore filter that has been pre-dried and weighed (dry cell weight procedure).
11. From dry cell weight measurements find average mg/L.
12. Plot chl a absorbance versus dry cell density.

Table C-1. Calibration Curve of mg DCW/L vs. mg chl a/L(15 days).

Culture: Laminaria saccharina female gametophytes
Cell Line: L-3
Flask#: 15,16,17,18,20,21,22,13
Date: 8/15/93
Age: 15 days
Cell Densit 69.7 mg DCW/L DDH20 washing
190.8 mg DCW/L GP2 washing

Sample# Trial#	Culture Volume (mL)	mg DCW/ 5 mL MeO	AU/ 5 mL MeO (665 nm)	mg chl a/ 5 mL MeO	mg chl a/ 5 mL MeOH (predicted)
0	0.0	0.00			0.0001
1-1	5.0	0.95	0.062	0.0050	0.0051
1-2	5.0	0.95	0.061	0.0050	0.0051
2-1	10.0	1.91	0.126	0.0103	0.0102
2-2	10.0	1.91	0.128	0.0104	0.0102
3-1	20.0	3.82	0.247	0.0201	0.0202
3-2	20.0	3.82	0.248	0.0202	0.0202
4-1	30.0	5.72	0.370	0.0301	0.0302
4-2	30.0	5.72	0.372	0.0303	0.0302
5-1	40.0	7.63	0.494	0.0402	0.0403

mg DCW/L	mg chl a/L	mg chl a/L predicted
0.0		0.026
190.8	1.010	1.029
190.8	0.994	1.029
381.5	2.053	2.032
381.5	2.085	2.032
763.0	4.024	4.038
763.0	4.040	4.038
1144.6	6.027	6.045
1144.6	6.060	6.045
1526.1	8.047	8.051

Regression Output:		
Constant		0.000
Std Err of Y Est		0.000
R Squared		1.000
No. of Observations		9.000
Degrees of Freedom		7.000
X Coefficient(s)	0.005	mg chl a/m
Std Err of Coef.	0.000	
Wt% chl a =	0.526	

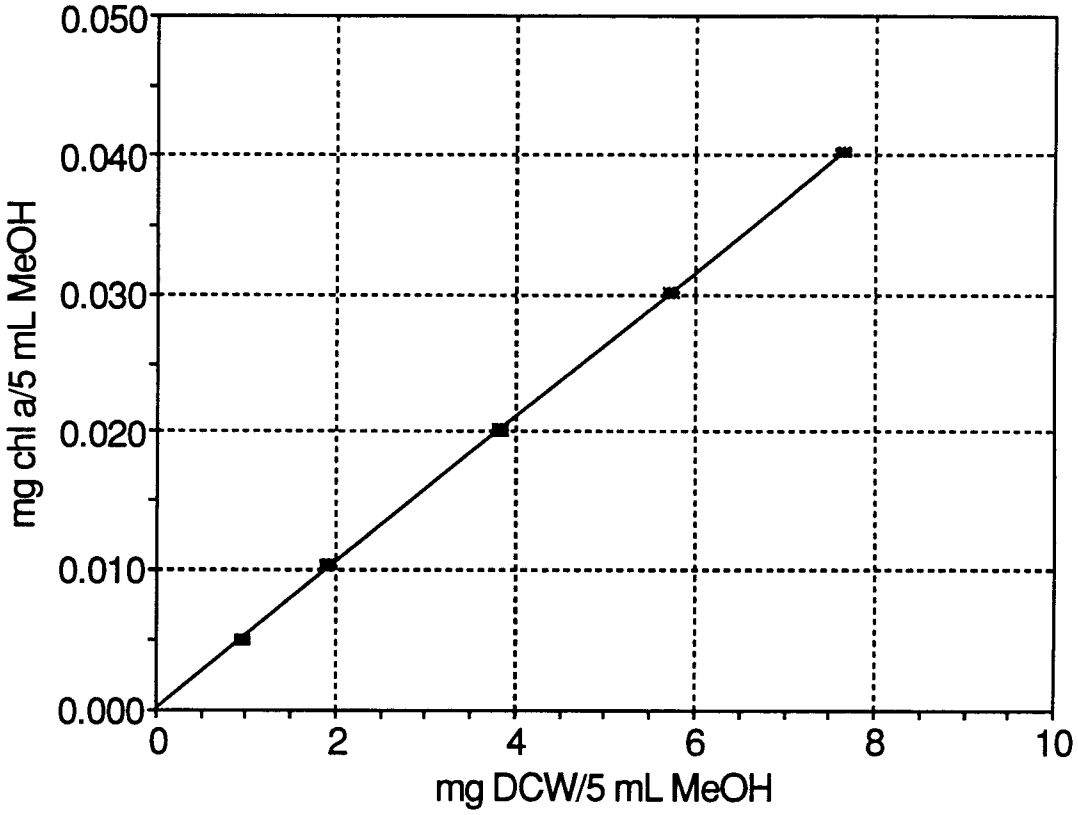

Figure C-1. 15 day chl a content calibration curve.

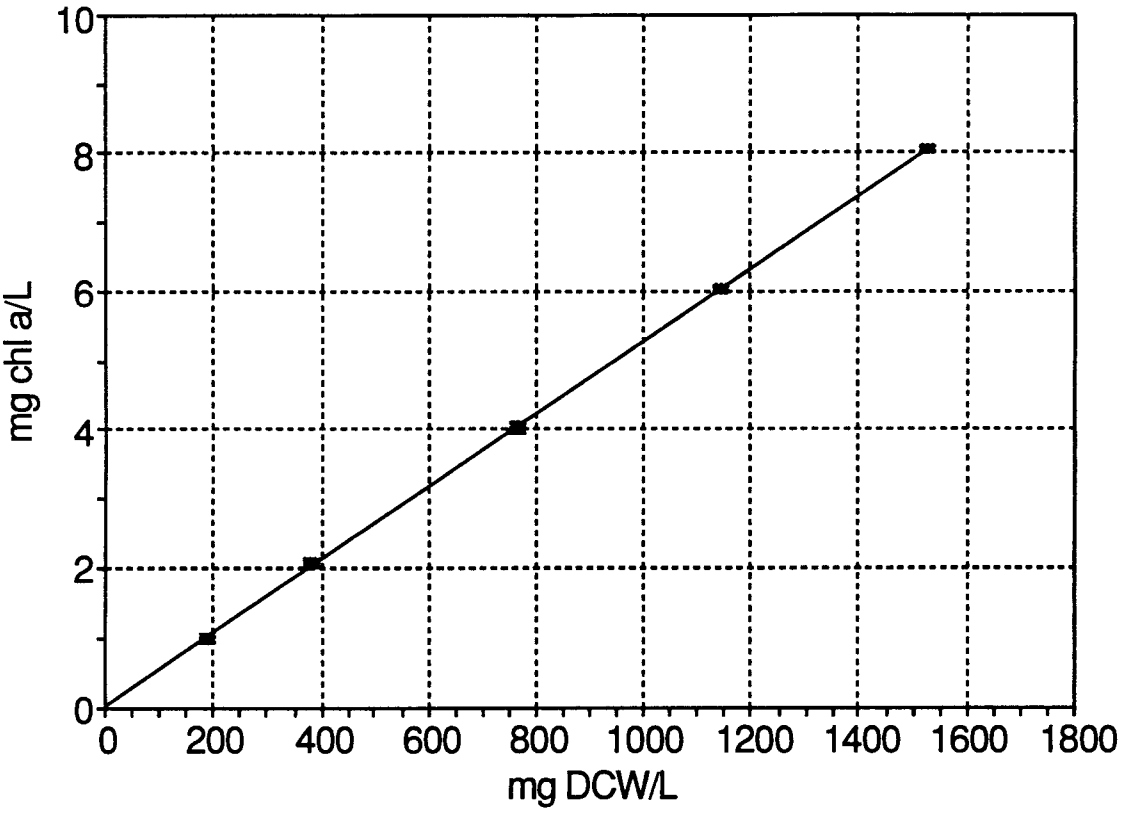

Figure C-2. 15 day chl a calibration curve with 0.53wt% chl a content.

Table C-2. Calibration Curve of mg DCW/L vs. mg chl a/L(30 days).

Culture: Laminaria saccharina female gametophytes
Flask#: DS-2
Cell Line: 13,14,19,26,27
Date: 9/1/93
Age: 30 days
Cell Density: 90.6 mg DCW/L DD H20 washing
214.4 mg DCW/L GP2 washing

Sample# Trial#	Culture Volume (mL)	mg DCW/ 5 mL MeOH	AU/ 5 mL MeO (665 nm)	mg chl a/ 5 mL MeOH	mg chl a/ 5 mL MeOH (predicted)
0	0.0	0.00			0.0022
1-1	5.0	1.07	0.085	0.0069	0.0083
1-2	5.0	1.07	0.108	0.0088	0.0083
2-1	10.0	2.14	0.163	0.0133	0.0144
2-2	10.0	2.14	0.162	0.0132	0.0144
3-1	20.0	4.29	0.339	0.0276	0.0265
3-2	20.0	4.29	0.343	0.0279	0.0265
4-1	30.0	6.43	0.526	0.0428	0.0387
4-2	30.0	6.43	0.483	0.0393	0.0387
5-1	40.0	8.58	0.591	0.0481	0.0508
5-2	40.0	8.58	0.605	0.0493	0.0508

mg DCW/L	mg chl a/L	mg chl a/L predicted
0.0		0.445
214.4	1.385	1.659
214.4	1.759	1.659
428.8	2.655	2.874
428.8	2.639	2.874
857.5	5.522	5.304
857.5	5.587	5.304
1286.3	8.569	7.733
1286.3	7.868	7.733
1715.0	9.627	10.163
1715.0	9.855	10.163

Regression Output:		
Constant		0.002
Std Err of Y Est		0.002
R Squared		0.986
No. of Observations		10.000
Degrees of Freedom		8.000
X Coefficient(s)	0.006	mg chl a/mg
Std Err of Coef.	0.000	
Wt% chl a =	0.567	

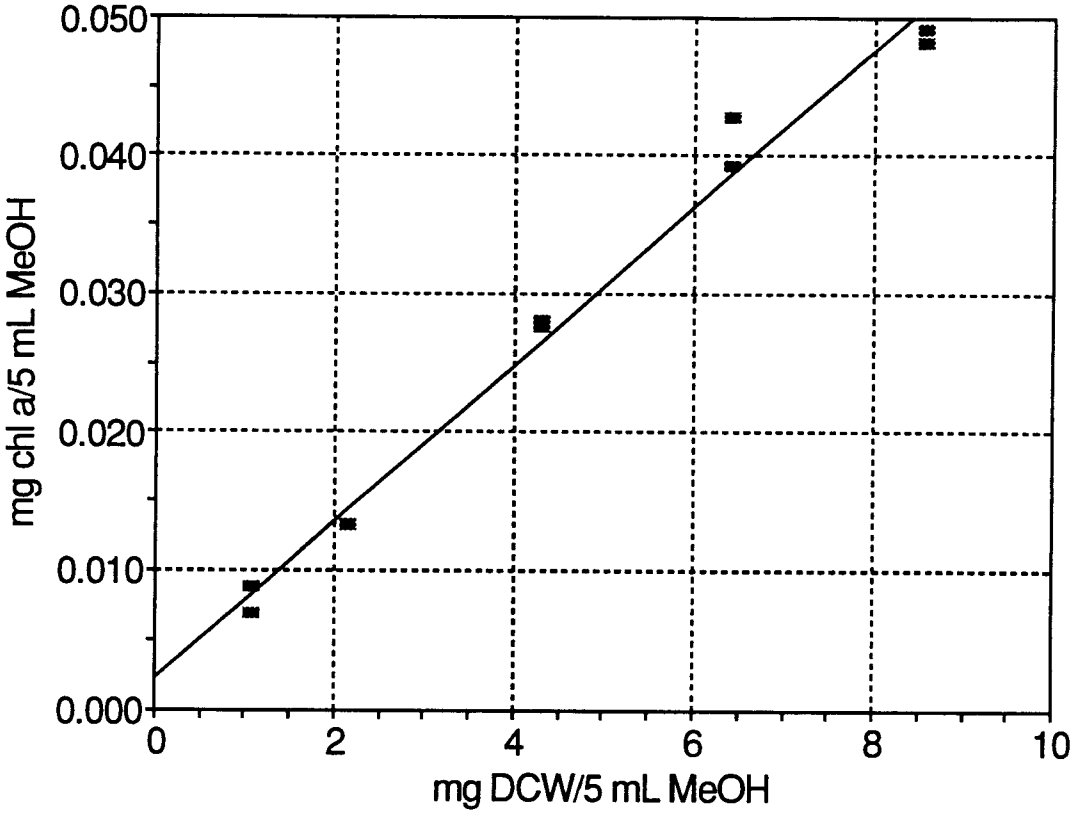

Figure C-3. 30 day chl a content calibration curve.

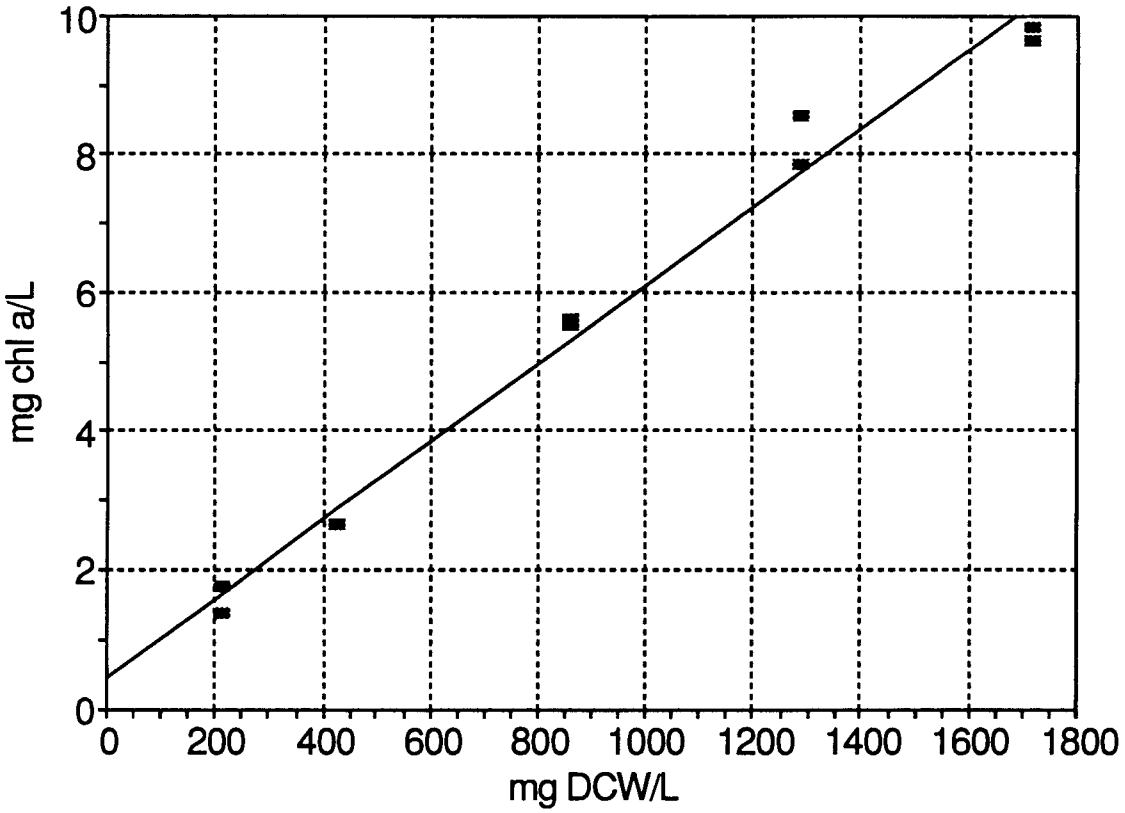

Figure C-4. 30 day chl a calibration curve with 0.57wt% chl a content.

Calibration Curve of Absorbance vs. Concentration for Chlorophyll a Standard Solution

Procedures

1. Add 1 mg chlorophyll a (SIGMA 6144, from *Aracystis nidulans* algae) to 100 mL HPLC grade MeOH and get a standard solution of 10 mg/L.
2. Dilute the 10 mg/L standard solution to 7.5 mg/L, 5 mg/L, 2.5 mg/L and 1.25 mg/L in HPLC grade MeOH.
3. Measure the absorbance of each solution with spectrophotometer at 665 nm.

Table C-3. Calibration curve of absorbance versus standard chl a soluation.

Chl a Concentration (mg/L) $C_{chl\ a}$	Absorbance (at 665 nm) AU_{665}	Chl a Concentration from Equation (2)	Calibrated Data For C_{chl} (mg/L)
0.00	0.000	0.00	1.37
1.25	0.176	2.87	3.63
2.50	0.288	4.69	5.06
5.00	0.425	6.92	6.82
7.50	0.567	9.24	8.63
10.00	0.884	14.4	12.69

$$C_{chl} = 12.810 \cdot AU_{665} - 0.745$$

Regression Output

Constant	-0.745 (mg chl a/L)
Std Err of Y Est	0.743
R Squared	0.968
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	12.810 (mg chl a/L/AU_{665})
Std Err of Coef.	1.350

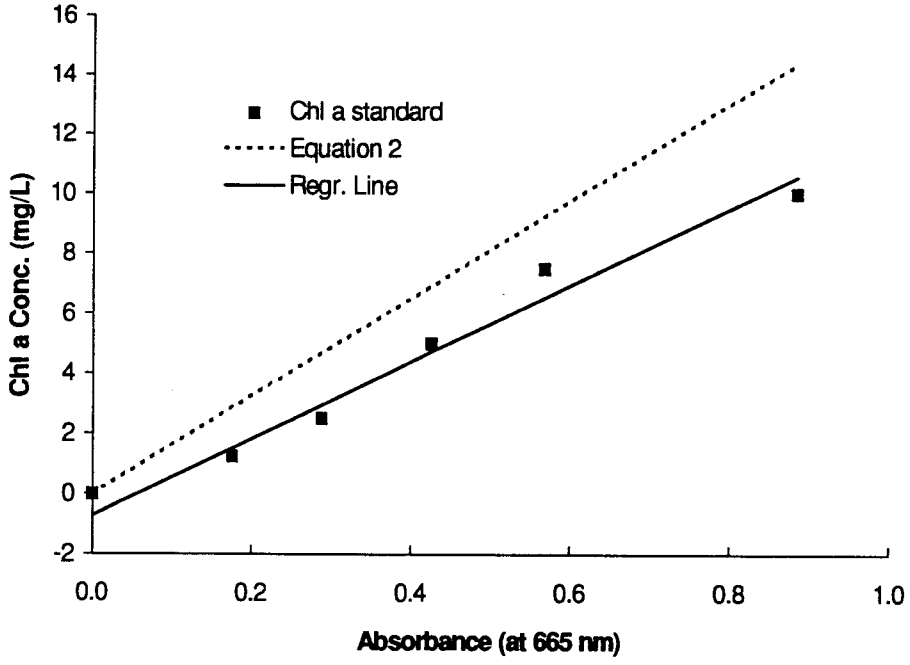

Figure C-5. Calibration curve of chl a standard versus absorbance at 665 nm..

Appendix D

Nitrate Analysis

Nitrate Concentration Measurement with Nitrate Electrode

(cited from the operating instruction of Nitrate Electrodes, Cole-Parmer 27502-30)

Procedure

1. By serial dilution of the 0.1 M or 1000 ppm standard, prepare 100 and 10 ppm nitrate standards. Add 2 mL of ISA per 100 mL of standard. Prepare standards with a composition similar to the samples if the samples have an ionic strength above 0.1 M.
2. Place the most dilute solution (10^{-4} M or 10 ppm) in a 150 mL beaker on the magnetic stirrer and stir at a constant rate. Make sure the meter is in the mV mode, lower the electrode tip into the solution. When the reading stabilizes, record the mV reading.
3. Place the mid-range solution (10^{-3} or 100 ppm) in a 150 mL beaker on the magnetic stirrer and stir. After rinsing the electrode in distilled water, blot dry and immerse the electrode tip in the solution. When the reading stabilizes, record the mV reading.
4. Place the most concentrated solution (10^{-2} M or 1000 ppm) in a 150 mL beaker on the magnetic stirrer and stir. After rinsing the electrode in distill water, blot dry, and immerse the electrode tip in the solution. When the reading stabilizes, record the mV reading.
5. Using the semi-logarithmic graph paper, plot the mV reading (linear axis) against the concentration (log axis). Extrapolate the calibration curve down to about $1.0 \cdot 10^{-5}$ M (1.0 ppm NO_3^{-1}).
6. Add 100 mL of your sample and 2 mL of ISA to a clean, dry 150 mL beaker. Place the beaker on the magnetic stirrer and stir at a constant rate. After rinsing the electrode tip with distilled water and blotting dry, lower the electrode tip into the solution. When the reading stabilizes, record the mV reading. Determine the concentration directly from the calibration curve.

7. Check the calibration every two hours. Assuming no change in ambient temperature, place the electrode tip in the mid-range standard. After the reading stabilizes, compare it to the original reading recorded in step 3 above. A reading differing by more than 0.5 mV or a change in the ambient temperature necessitates the repetition of Steps 2-5.
8. Using same procedure as above to measure the GP2 medium with nitrate concentrations of 63.5, 254, and 508 mg/L. Plot the mV reading (linear axis) against the concentration (log axis) to get a calibration curve for GP2 medium.

Table D-1. Calibration curve of mV reading versus nitrate concentration.

Nitrate Concentration in Standard Solutions (ppm)	mV reading (mv)
10	208.4
100	164.2
1000	111.0
Nitrate Concentration in GP2 Medium (ppm)	
0.1	145.2
16.0	145.2
32.0	144.7
63.5	143.7
254.0	135.8
508.0	126.5

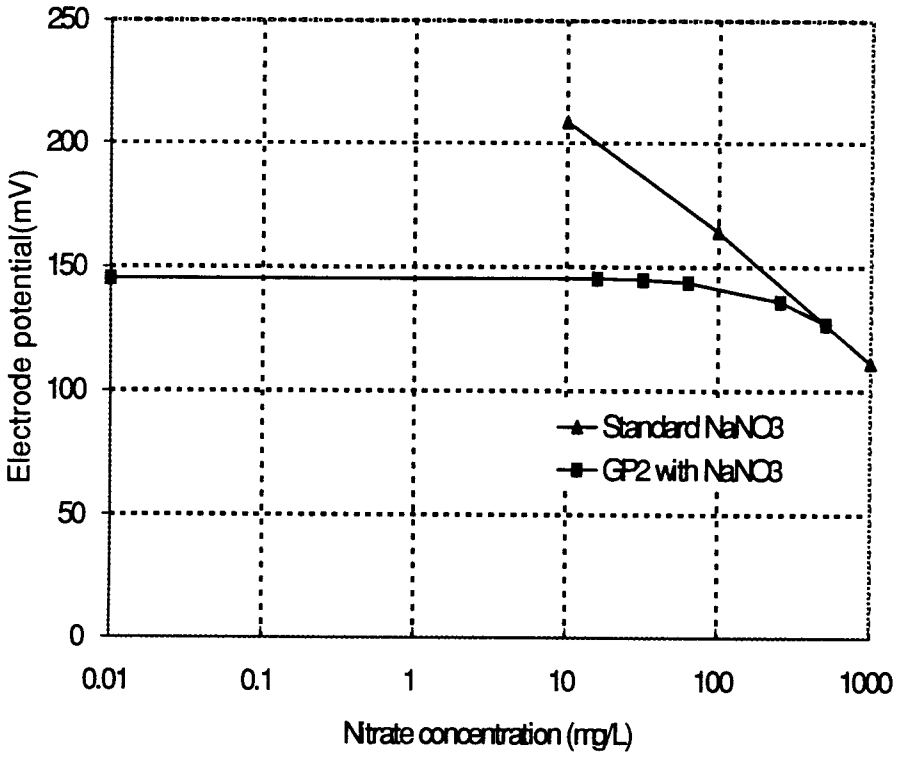

Figure D-1. Calibration curve of mV reading versus nitrate concentration.

Appendix E

Acrosiphonia coalita Tissue Culture

Acrosiphonia coalita Tissue Culture Isolation

1. Rinse a few good looking branches. Remove the heavily infected filaments, especially those at the holdfast area.
2. Sonicate gently in sterile sea water, twice for 10 sec each, and replace the sea water to rinse out the contamination.
3. Place in antibiotics mixture overnight, and not longer than 48 hours.
4. Rinse the antibiotics (all work is done in sterile sea water), and place the treated branches in the best culture conditions you know of.
5. Let the tissues grow for 5-7 days. Under the disecting scope cut out the best looking new growth, at least 5 cells long. The longer the better, but do not wait more than a week or so because contaminating algae may quickly produce spores and infect your new clean branches.
6. Transfer the new, relatively clean cuts, into clean medium and let them grow for another week.
7. If you want them totally bacteria free, treat the potentially clean filament with a 10% betadine for 1 min. Rinse well but gently, until the brown color of the iodine is gone.
8. Put your most clean branches in clean medium and good culture conditions for a week or longer as you desire.

Acrosiphonia coalita Tissue Culture Subculture

1. Pipette out half of the clear medium from the selected 250 mL culture flasks.
2. Transfer two culture flasks (50 mL culture left in 250 mL flask) to the 500 mL modified blending cup.
3. Blend the culture at "liquefy" setting for 15 seconds.
4. Transfer the blended culture to two 50 mL autoclaved centrifuge tube.
5. Centrifuge at 1000 rpm for 5 min.
6. Pipette out medium supernate.
7. Cut up the biomass in the centrifuge tube using sterile razor blade.
8. Poured 40 mL fresh PES medium to the centrifuge tube. Mix thoroughly for about 1 minute, then let stand for 5 minutes.
9. Centrifuge at 1000 rpm for 5 minutes. Remove supernate with pipette.
10. Re-suspend pellet in 50 mL fresh PES liquid medium.
11. Inoculate: 12.5 mL of suspended culture to 100 mL of fresh PES liquid medium in 250 mL flask, 4 flasks total (approximately 25% v/v inoculum).