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Bioprocess engineering of cell and tissue cultures for marine seaweeds

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Abstract

Seaweeds are a rich source of valuable compounds including food additives and biomedicinals. The bioprocess engineering of marine macroalgae or "seaweeds" for the production of these compounds is an emerging area of marine biotechnology. Bioprocess technology for marine macroalgae has three elements: cell and tissue culture development, photobioreactor design, and identification of strategies for eliciting secondary metabolite biosynthesis. In this paper, the first two elements are presented. Firstly, the development of phototrophic cell and tissue culture systems for representative species within brown, green, and red macroalgae is described. In vitro culture platforms include microscopic gametophytes, undifferentiated callus filaments, and "microplantlets" regenerated from callus. Secondly, the controlled cultivation of these phototrophic culture systems in stirred tank, bubble-column, airlift, and tubular photobioreactors is described. Limiting factors on biomass production in photobioreactors including light delivery, CO_2 transfer, and macronutrient delivery are compared. Finally, a mathematical model that integrates light delivery, CO_2 delivery, and macronutrient delivery into the material balance equations for biomass production in a perfusion bubble-column photobioreactor is presented, and model predictions are compared to biomass production data for microplantlet suspension cultures of the model red alga *Agardhiella subulata*.

Keywords: Cell and tissue culture; Macroalgae; Photobioreactor

1. Introduction

Macrophytic marine algae, commonly known as seaweeds, are nonvascular, multicellular, photosynthetic "marine plants" that inhabit the coastal regions of ocean waters, commonly

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within rocky intertidal or submerged reef-like habitats. Unlike microscopic algae (microalgae), seaweeds generally live attached to rocky substrates on the ocean bottom and can assume considerable anatomical complexity and intricate life histories. The three major divisions of marine macroalgae are the Heterokontophyta (includes brown algae), Rhodophyta (red algae), and Chlorophyta (green algae), which together encompass over 7000 species.

In the rocky intertidal marine environment, competition for light, nutrients, and space is fierce and many marine seaweeds have evolved chemical defense mechanisms to ward off predators and enhance survival (Carte, 1996). Not surprisingly, many of these chemicals are biologically active and some possess potent pharmacological activity. For example, the tropical red alga *Portieria hornemannii* contains halomon, a halogenated monoterpene with potent anti-tumor activity (Fuller et al., 1992). Another example is the temperate brown alga *Laminaria saccharina*, which contains eicosanoid and oxylipin compounds involved in the mediation of inflammation (Gerwick et al., 1993; Rorrer et al., 1997).

A major bioprocess technology barrier for production of new compounds from macrophytic marine algae is the development of in vitro culture systems suitable for bioreactor cultivation (Rorrer et al., 1998). A generic bioprocess development scheme that uses cell and tissue cultures of marine seaweeds for process biotechnology, aquaculture, and environmental remediation applications is presented in Fig. 1. In this paper, two critical elements of this bioprocess development scheme are presented. The first element is the development of cell and tissue culture systems for marine seaweeds. Specifically, we describe a variety of methods to develop phototropic suspension cultures suitable for cultivation in photobioreactor systems. The second element is the development of photobioreactor systems for phototrophic macroalgal suspension cultures. Specifically, we compare three major photobioreactor configurations for macroalgal suspension culture (bubble-column/airlift, stirred tank, tubular recycle) and assess the factors that limit their process cultivation performance.

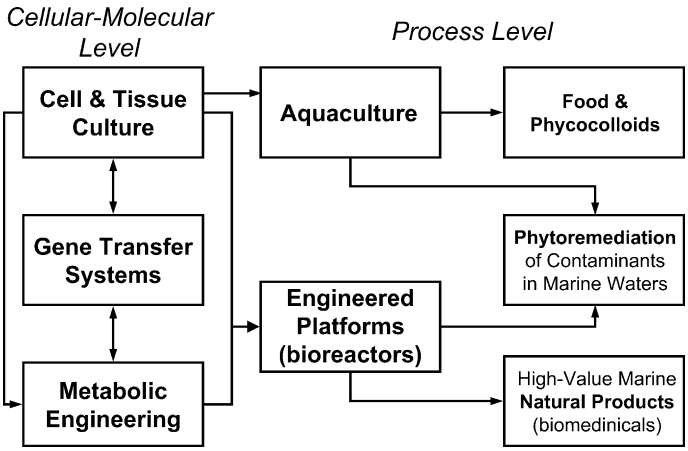

Fig. 1. Bioprocess development for marine seaweeds.

2. Development of cell and tissue culture systems for marine macroalgae

2.1. Unique features of macroalgal cell and tissue cultures

As stated in Section 1, engineered bioprocess systems for production of valuable compounds from macroalgae require axenic cell or tissue cultures suitable for cultivation in agitated bioreactors. In general, methods to establish cell and tissue cultures from non-vascular, anatomically complex macroalgae make use of the tools developed for vascular land plants. Unfortunately, specific techniques for the development of cell and tissue cultures of seaweeds are vastly underdeveloped relative to those for land plants (Butler and Evans, 1990; Aguirre-Lipperheide et al., 1995). Furthermore, the traditional rationale for establishing cell and tissue cultures from marine seaweeds has been strain improvement and micropropagation for mariculture, and not the development of culture platforms suitable for agitated bioreactors. However, in the past 10 years, our laboratory has made considerable progress in the development of cell and tissue cultures of marine macroalgae.

Table 1 summarizes the features of the macroalgal cell and tissue cultures we have developed for use in agitated bioreactor systems. Culture development and nutrient medium formations are detailed by Rorrer (2000). All three major classifications of macrophytic marine algae (brown, green, and red) are represented. The specific species selected for culture development represent model systems for biosynthesis of novel oxylipins and halogenated terpenoids (Table 1). These macroalgal cell and tissue cultures systems share common characteristics. For cell culture systems, the cell mass is generally filamentous and multicellular. For tissue culture systems, the thallus tissue consists of shoot tissues or uniserate filaments symmetrically emanating from a common center. Partial reduction in the anatomical complexity of the marine plant is sufficient for the development of a liquid suspension culture suitable for cultivation in agitated photobioreactor systems.

None of the culture systems described in Table 1 have been fully reduced to the friable, single cells typical of many land plant cell culture systems. Multicellular clumps or tissues are cultivated as an axenic liquid suspension and vegetatively subcultured in vitro. The nonfriable cell clumps or tissues are mechanically dissociated into smaller pieces prior to subculture using methods described by Rorrer (2000). The suspension cultures are phototrophic and thus require light as an energy source and CO_2 as the carbon source for photosynthesis. To date, no heterotrophic suspension cultures capable of growth on an externally supplied organic carbon source have been established from macrophytic marine algae.

2.2. Gametophyte isolation

Filamentous cell suspension cultures of the brown kelp *L. saccharina* (Laminariales and Phaeophyceae) were established by microscopic gametophyte isolation techniques. In essence, the female gametophytic life phase of this kelp (Fig. 2) was isolated and cultured in artificial medium under conditions that suppressed gametogensis and promoted vegetative growth (Qi and Rorrer, 1995; Rorrer, 2000).

The gametophyte cell mass consists of branched filaments, where individual filaments consist of cells of 5–20 μm diameter and 10–30 μm length that are joined end-on-end

Table 1
Summary of cell and tissue suspension culture development for macrophytic marine algae (Rorrer et al., 1998; Barahona and Rorrer, 2003)

Classification	Marine plant	Culture development strategy	Culture morphology	Bioactive compounds
Heterokontophyta (brown)	*Laminaria saccharina*	Isolation of microscopic gametophytic life phase	Clumped, branched filaments, 0.5–2 mm diameter	C_{18} and C_{20} hydroxy fatty acids (derived from ω-6 lipoxygenase oxidation of linoleic and arachidonic acids)
Chlorophyta (green)	*Acrosiphonia coalita*	Induction of linear filaments from apical tip explants	Uniserate linear or lightly branched filaments, 2–10 mm length	Coalital (C_{10} oxylipin derived from linolenic acid)
Rhodophyta (red)	*Agardhiella subulata*	Induction of un-differentiated cells from thallus explants	Undifferentiated clumps, 2–8 mm diameter; rounded cells and uniserate filaments	8-HETE (C_{20} eicosanoid) Agardhilactone (cyclopental eicosanoid)
	Ochtodes secundiramea	Microplantlets regenerated from callus-like tissue	Multiple thallus shoots from a common branch point, 2–15 mm shoot length	Acyclic and cyclic halogenated monoterpenes derived from myrcene precursor
	Portieria hornemannii	Microplantlets regenerated from callus-like tissue	Multiple thallus shoots from a common branch point, 2–15 mm shoot length, additional branching at shoot tip	

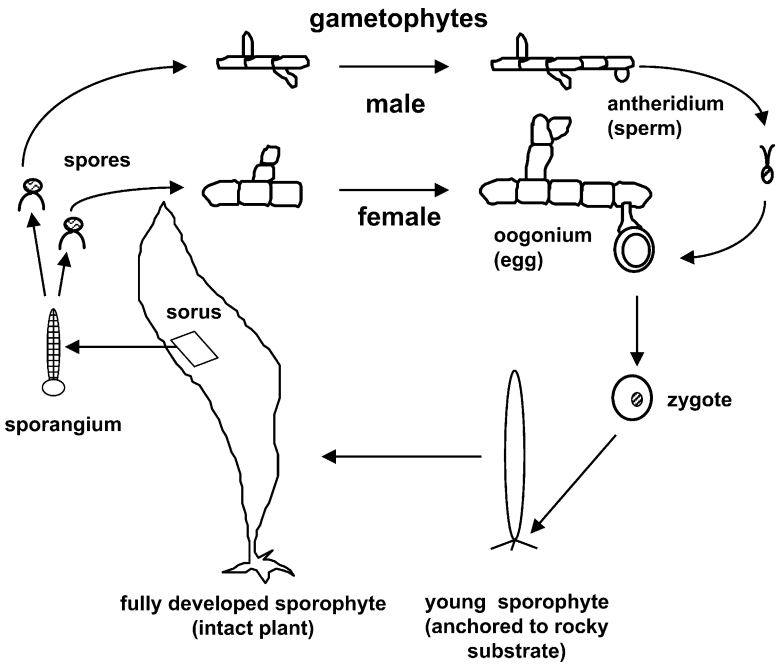

Fig. 2. Life cycle of the brown kelp *Laminaria saccharina*. Organisms are not drawn to scale, young sporophyte is initially microscopic.

with one another to form a multicellular linear array of cells, i.e., the filament possesses a uniserate morphology. Branched filaments pack together into yellowish-brown clumps ranging from 0.2 to 1.0 mm in diameter (Fig. 3).

2.3. Single cell isolation

The macrophytic green alga *Acrosiphonia coalita* (Acrosiphoniales, Chlorophyta) has thallus tissue that consists of a branched network of uniserate filaments tightly wound into rope-like tissues that anchor to rocky substrates. A freely suspended tissue culture of *A. coalita* was established by a single cell filament isolation technique, where single cells were excised from field-collected tissue and then re-cultured under neutral gravity in a culture wheel (Rorrer et al., 1996; Rorrer, 2000). The tissues consist of dark green, linear or lightly branched linear uniseriate filaments of 10–50 μm diameter and 2–5 mm length (Fig. 4). Often, several of these filaments extend from a common core.

2.4. Callus induction and shoot tissue regeneration

Cell and tissue culture development efforts for macrophytic red algae have focused on plants that possess terete thallus morphology consisting of branched shoots with the most active cell division at the apical meristem of each shoot. Specific species include the

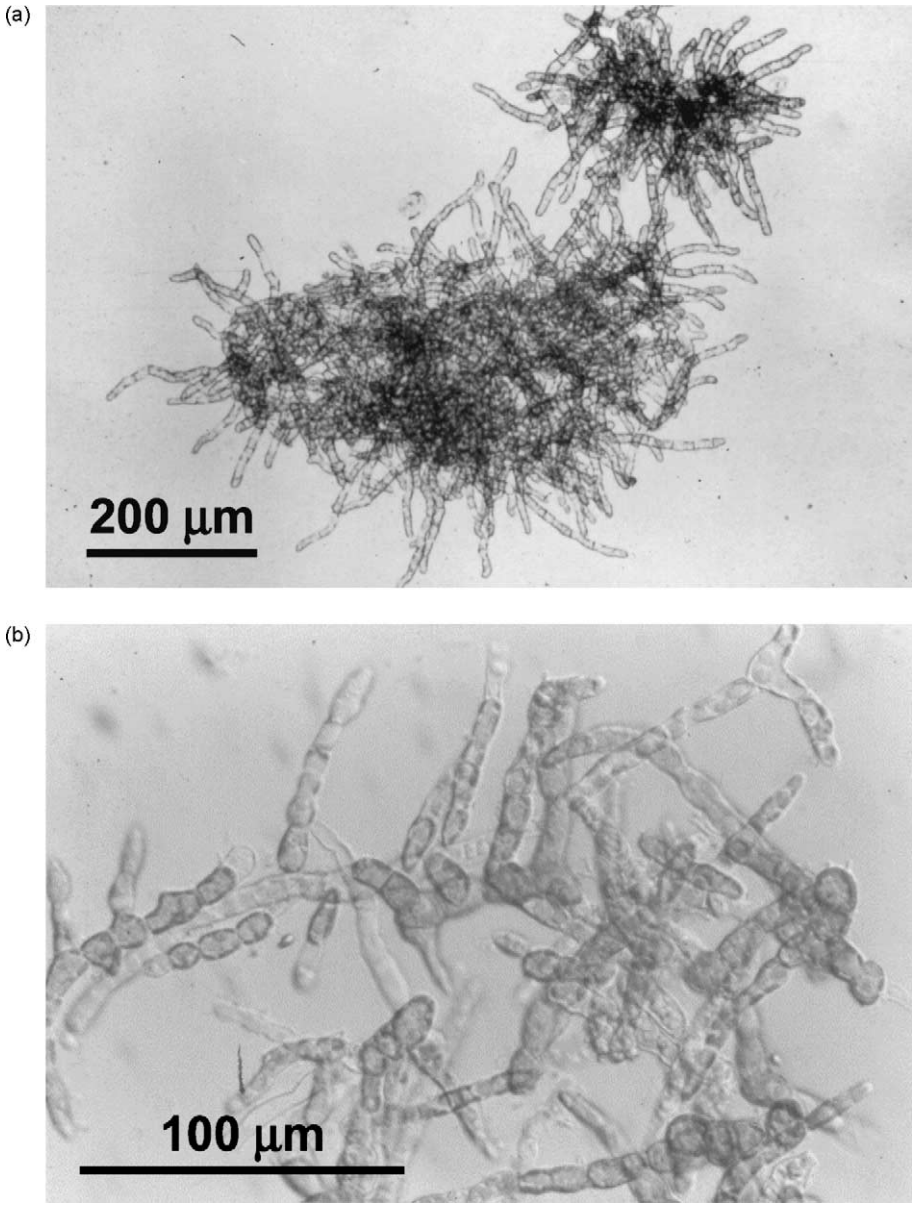

Fig. 3. Photomicrographs of *Laminaria saccharina* female gameotophytes: (a) filamentous cell clump and (b) dispersed filamentous cells.

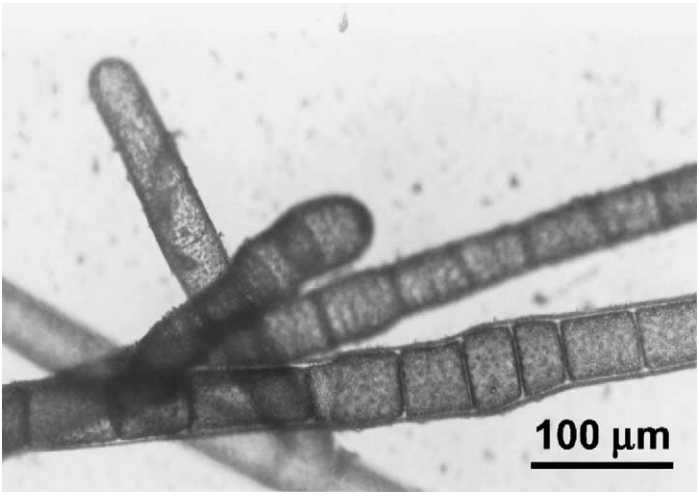

Fig. 4. *Acrosiphonia coalita* tissue culture, photomicrograph of uniserate filaments.

temperate red alga *Agardhiella subulata* (Gigartinales, Rhodophyceae) and the tropical red algae *Ochtodes secundiramea* (Cryptonamiales, Rhizophyllidaceae) and *P. hornemannii* (Cryptonemiales, Rhizophyllidaceae). For all of these plants, a common cell and tissue culture development platform is used (Fig. 5). This culture development platform has two major components: (1) callus induction from explants of field-collected plants and (2) partial regeneration of shoot tissues from callus to form "microplantlets".

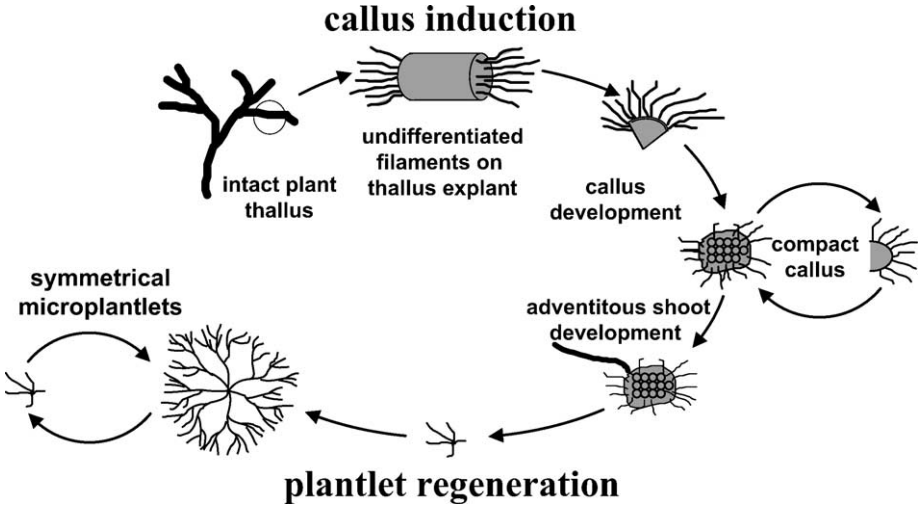

Fig. 5. Cell and tissue culture development scheme for the macrophytic red algae *Agardhiella subulata*, *Ochtodes secundiramea*, and *Portieria hornemannii*. The parent plant for each of these red algae possess terete thallus morphology, consisting of branched shoots with the most active cell division at the apical meristem of each shoot.

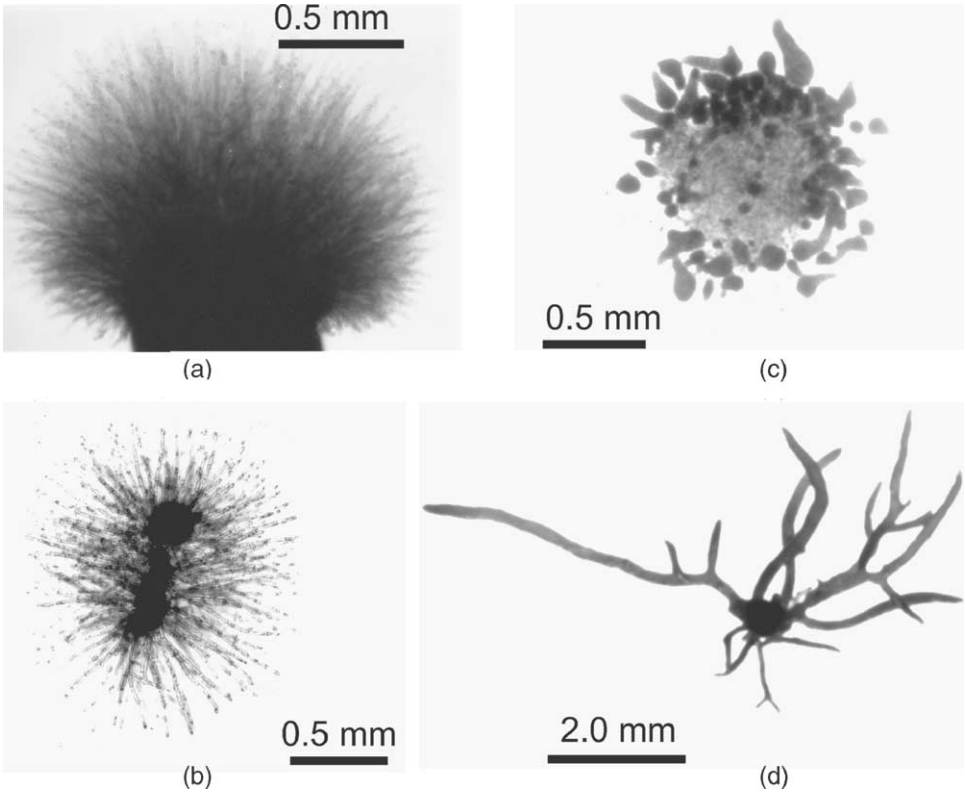

Fig. 6. Cell and tissue culture development for the macrophytic red alga *Agardhiella subulata*: (a) filamentous callus cells emanating from cut face of thallus explant tissue; (b) callus filament clump; (c) adventitious shoot tissues regenerating from terminal cells on filaments of the callus clump; and (d) final microplantlet.

The cell and tissue culture development platform for *A. subulata* (Huang et al., 1998; Rorrer, 2000) is overviewed below. Undifferentiated, filamentous cell clumps are established from *A. subulata* by a callus induction technique, where undifferentiated cell filaments grow away from the cut face of a thallus tissue explant (Fig. 6a). The filament clumps range from 2 to 8 mm in diameter, and consist of round, dark red cells at the center core, with clear or lightly pigmented uniseriate filaments emanating from the center as a thick bushy mass (Fig. 6b). Microplantlets of *A. subulata* are established by regeneration shoot tissues from the filament clumps. Shoots tissues regenerating from the callus filament clump are dark red with cortical and medullary cells (Fig. 6c). This tissue does not fully regenerate into the intact plant with asymmetic thallus stem and holdfast structures. Instead, in liquid culture, the shoots symmetrically emanate from a common center (Fig. 6d). These microplantlets are subcultured by mechanically chopping the tissues into small (ca. 2 mm) fragments. Individual shoots branch out to ultimately assume a symmetrical, ball-like overall shape that reaches about 10 mm diameter after 40–60 days in culture.

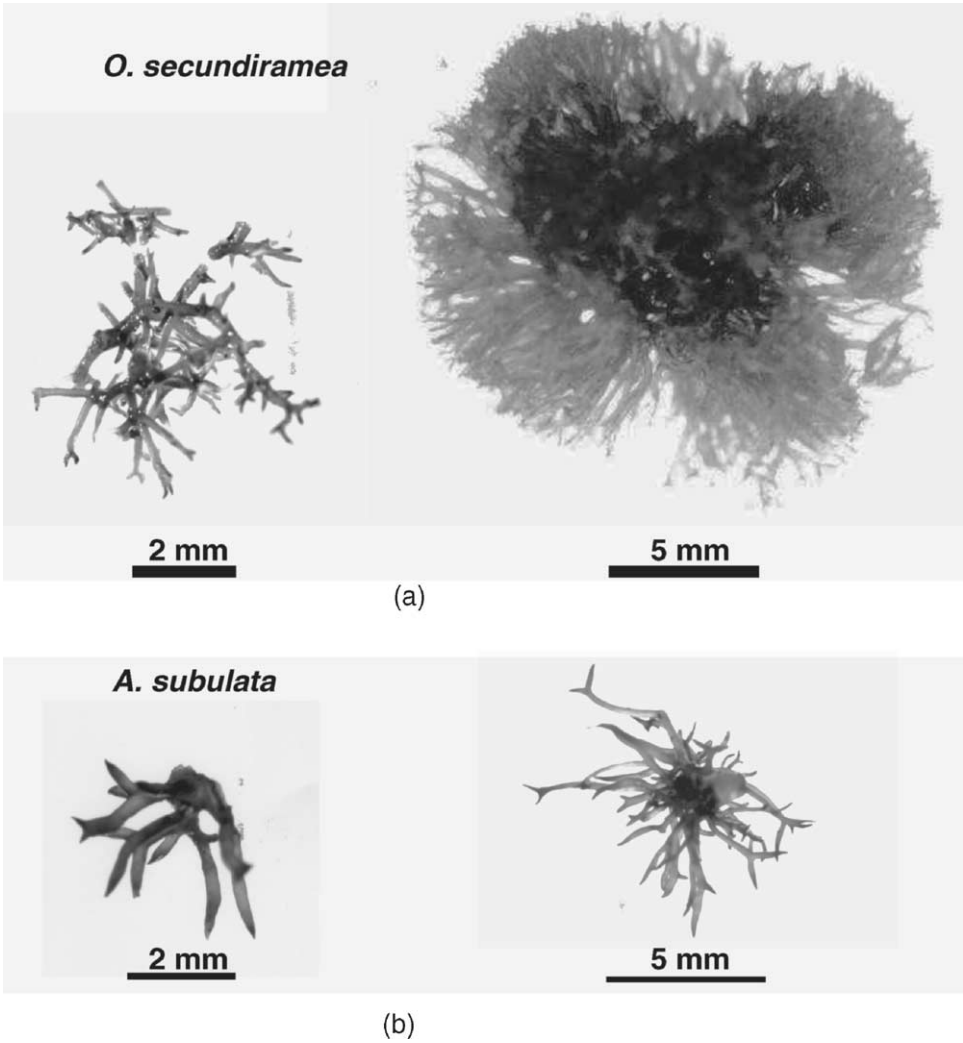

Fig. 7. Comparison of morphology of *A. subulata* and *O. secundiramea* microplantlets.

The cell and tissue culture development platform developed for *A. subulata* was successfully extended to *O. secundiramea* (Maliakal et al., 2001) and *P. hornemannii* (Barahona and Rorrer, 2003). The microplantlet morphologies of *Agardhiella* and *Ochtodes* are compared in Fig. 7. The *Ochtodes* and *Portieria* microplantlet shoots bifurcate at the shoot tip, whereas *Agardhiella* microplantlet shoots branch out from the thallus tissue. However, both *Ochtodes* and *Portieria* microplantlets tend to assume a bushy, ball-like shape in long-term culture.

Microplantlets are ideally suited for cultivation in agitated photobioreactor systems. In the microplantlet morphology, the biomass is compacted into ball-like tissues that grow

freely in liquid medium. The microplantlets are easily suspended but also easily separate from the culture liquid to facilitate biomass harvesting. Since the biomass is compacted into multicellular tissue and not dispersed in the liquid medium as single cells, the light attenuation through the culture suspension is low relative to microalgal suspension cultures at the same cell mass density. Finally, the microplantlets are nonfriable and do not break apart when subjected to hydrodynamic forces created by aeration and agitation of the culture liquid (Huang and Rorrer, 2002a, 2003).

3. Photobioreactors for macroalgal cell and tissue culture

3.1. Basic features

All of the macroalgal culture systems listed in Table 1 are photolithotrophic. Therefore, they require light, CO_2, and dissolved nutrients (N, P, trace metals, and vitamins) for growth. Typical environmental conditions are provided in Table 2. Furthermore, in cell or tissue culture, the macroalgal cell mass grows freely suspended in liquid medium. Consequently, bioreactors for macroalgal suspension culture must provide illumination, gas exchange (CO_2 addition and O_2 removal), nutrient delivery, mixing, and temperature control.

The types of photobioreactors suitable for macroalgal cell and tissue cultures are compared in Table 3. Schematics and photographs of representative bench-scale photobioreactor systems are presented in Figs. 8–10. All of these photobioreactor configurations—the bubble-column/airlift (Fig. 8), the stirred tank (Fig. 9), and the tubular recycle (Fig. 10) require four common elements. Firstly, for controlled production of high-value and bioactive secondary metabolites, cultivation of axenic tissues within an enclosed and sterilizable bioreactor vessel is required. Secondly, the bioreactor control volume is externally illuminated by artificial light, and so a portion of the bioreactor vessel must be transparent. Suitable vessel materials include glass or polycarbonate for bubble-column/airlift photobioreactors, and translucent silicone tubing for tubular photobioreactors. Third, inorganic carbon for photosynthetic growth is supplied by CO_2 in the aeration gas. When the aeration gas is bubbled into the liquid medium, the CO_2 dissolves into the liquid by an interphase mass transfer process and is then consumed by the photolithotropic cell. Fourth, since the macroalgal cell clumps and tissues are nonfriable, a continuous cultivation process is not possible, as detailed next.

3.2. Batch versus continuous cultivation

Growth of nonfriable cell and tissue cultures of marine macroalgae is inherently an unsteady state process. For all macroalgal cell and tissue suspension cultures, we observe that the tissues do not break apart during cultivation; the cell clumps or tissues simply grow in size relative to their initial size at inoculation as the cells divide and grow (Fig. 7). For example, in perfusion bubble-column photobioreactor culture of *A. subulata* microplantlets, the biomass density of the suspension increased from 1.0 to 14 g FW L^{-1}, but the number of plantlets in the suspension remained constant at about 1100 plantlets per liter (Huang and Rorrer, 2002a). Since the macroalgal cell clumps or tissues do not break apart upon

Table 2
Typical environmental conditions and growth rates for the macroalgal cell and tissue culture systems summarized in Table 1

Process condition	Culture system				
	Laminaria saccharina	*Acrosiphonia Coalita*	*Agardhiella subulata*		*Ochtodes secundiramea*/ *Portieria hornemanni*
	Gametophyte cells	Tissue filaments	Filament clumps	Microplantets	Microplantets
Seawater nutrient medium	GP2	Instant ocean + PES	ASP12	ASP12	Natural seawater + ESS
pH range	8.0–8.8	8.0–8.8	8.0–8.8	8.0–8.8	8.0–8.8
Tested temperature range (typical) (°C)	10–15 (12)	10–18 (12)	24 (24)	12–28 (24)	24–29 (26)
Tested incident light intensity range (typical) ($\mu E\,m^{-2}\,s^{-1}$)	5–80 (30)	10–80 (30)	10–40 (10)	80 (40)	140 (100)
Specific growth rate (typical) (% per day)	10	15	3	6	15

Table 3
Comparison of enclosed photobioreactor configurations for macroalgal suspension cultures

Photobioreactor configuration	Mixing and biomass suspension	Aeration and gas exchange	Light transfer	Shear damage potential	Macroalgal suspension cultures validated
Bubble-column	Adequate	Very good	Adequate	Low	*L. saccharina* gametophyte cell clumps; *A. coalita* tissue filaments; *A. subulata* microplantlets; and *O. secundiramea* microplantlets
Airlift (internal draft tube)	Very good	Very good	Good	Low	*A. subulata* microplantlets and *O. secundiramea* microplantlets
Externally illuminated stirred tank	Excellent	Good–excellent	Poor	High	*L. saccharina* gametophyte cell clumps; *A. coalita* tissue filaments; *A. subulata* microplantlets; *O. secundiramea* microplantlets; and *P. hornemannii* microplantlets
Tubular recycle (helical array)	Tube-poor, tank-excellent	Adequate	Excellent	Tube-moderate Tank-high Pump-high	*L. saccharina* gametophyte cell clumps

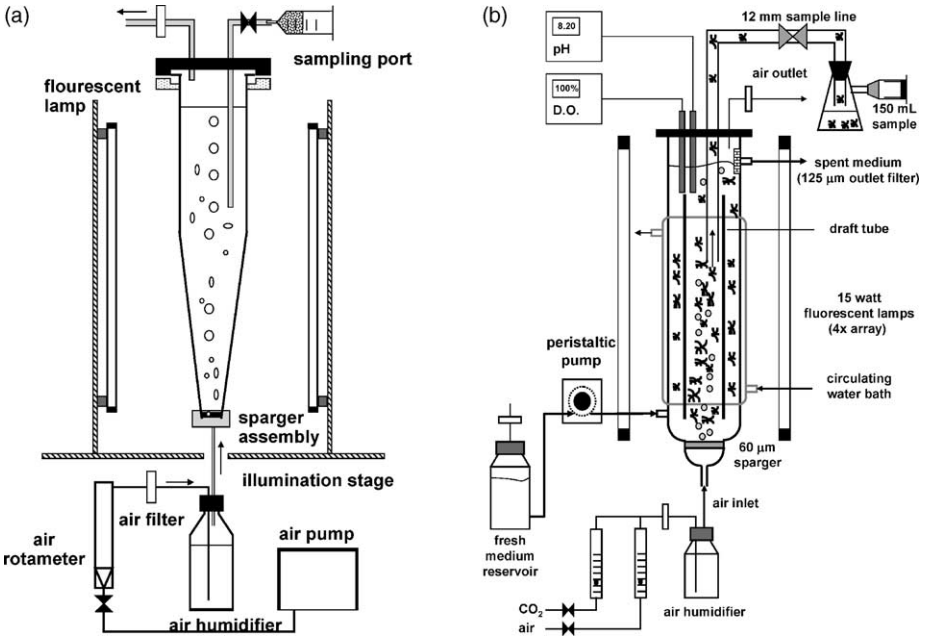

Fig. 8. Representative bench-scale bubble-column and airlift photobioreactors for macroalgal cell and tissue culture: (a) 250 mL bubble-column photobioreactor and (b) 3.0 L airlift photobioreactor equipped for continuous medium perfusion.

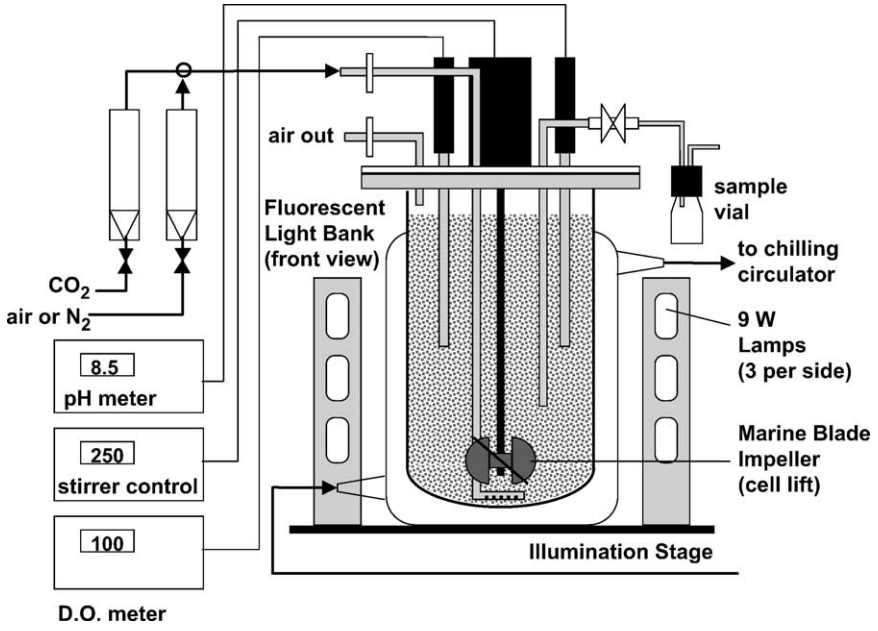

Fig. 9. Stirred tank photobioreactor (3.0 L) for macroalgal cell and tissue culture.

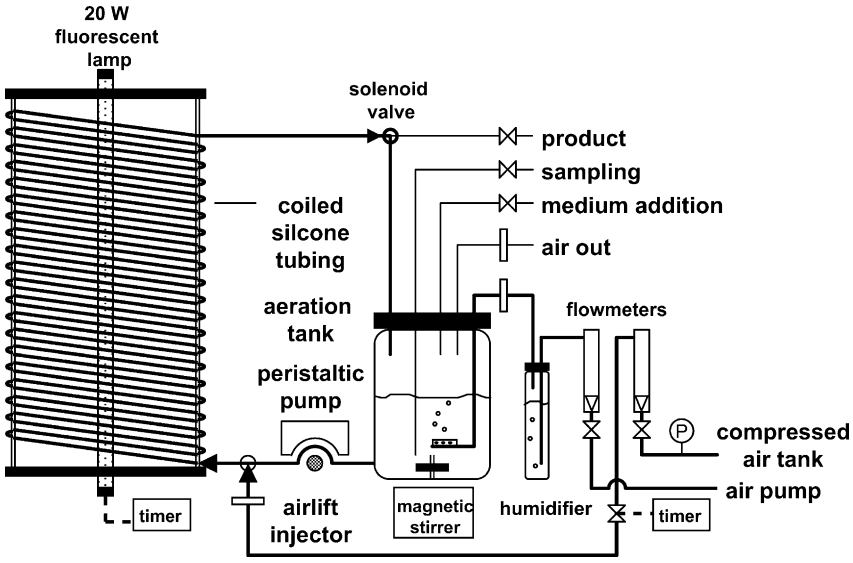

Fig. 10. Tubular recycle photobioreactor (3.0 L) for *L. saccharina* female gametophyte cell culture.

cell division, the steady state cultivation within a well mixed continuous flow bioreactor is not possible because dilution of the culture suspension by fresh medium addition will dilute out these cell clumps or tissue. Consequently, the biomass must be retained within the vessel. However, if the macroalgal cell or tissues are mechanically dissociated (e.g., cut into smaller pieces), then continuous cultivation might be possible. In this scenario, effluent microplantlets from the continuous flow bioreactor would be cut into smaller pieces, and a portion of these pieces would be inoculated into the bioreactor with the nutrient feed medium.

3.3. Limiting factors on biomass production in photobioreactors

The photobioreactor configurations shown in Figs. 8–10 are designed to provide mixing, illumination, gas exchange, and dissolved macronutrients to the macroalgal suspension culture under controlled conditions. Table 3 compares the relative ability of each photobioreactor to provide mixing, illumination, and gas exchange.

3.3.1. Mixing

Macroalgal suspension cultures sediment easily due to their large clump size and so must be well agitated to maintain the cell biomass as a uniform suspension in the liquid medium. In a bubble-column bioreactor, the culture is mixed and suspended by the rising air bubbles. In an airlift bioreactor, biomass suspension is improved by a “draft tube” of diameter d_i which fits within a cylindrical vessel of width d. The draft tube provides a defined liquid circulation pattern where the suspension moves upward within the draft tube (riser) and downward outside of the draft tube (downcomer). Draft tube airlift bioreactors

also improve light transfer to the culture suspension because the culture suspension is kept close to the illuminated bioreactor wall and confined within a short light path ($d - d_i$) as it moves through the downcomer.

Aerated stirred tank bioreactors improve mixing and biomass suspension. Cell-lift and marine-blade impellers provide axial circulation patterns (Doran, 1999) which help to suspend macroalgal cell clumps and tissues. Furthermore, the rotating impeller helps to break up and disperse the air bubbles to improve interphase mass transfer for CO_2 delivery. In general, cell cultures derived from terrestrial plants are prone to damage by hydrodynamic shear forces generated by the stirred tank impeller rotation (Doran, 1999). However, the nonfriable cell and tissue cultures of marine macroalgal plants are not prone to shear damage in stirred tank photobioreactors; these include the *Laminaria* gametophyte cell culture (Qi and Rorrer, 1995), the *Acrosiphonia* tissue culture (Rorrer et al., 1996), *Portieria*/*Ochtodes* microplantlet cultures (Barahona and Rorrer, 2003), and the *A. subulata* microplantlet culture (Huang and Rorrer, 2003). For example, growth of *A. subulata* microplantlets was not affected at impeller tip speeds ranging from 17 to 72 s^{-1} (Huang and Rorrer, 2003).

The mode of mixing has an effect on the final morphology of bioreactor cultured microplantets. Photographs of *Agardhiella* microplantlets cultivated in the stirred tank photobioreactor versus a bubble-column bioreactor are presented in Fig. 11. For microplantlets cultivated within the bubble-column photobioreactor, primary shoots and their secondary

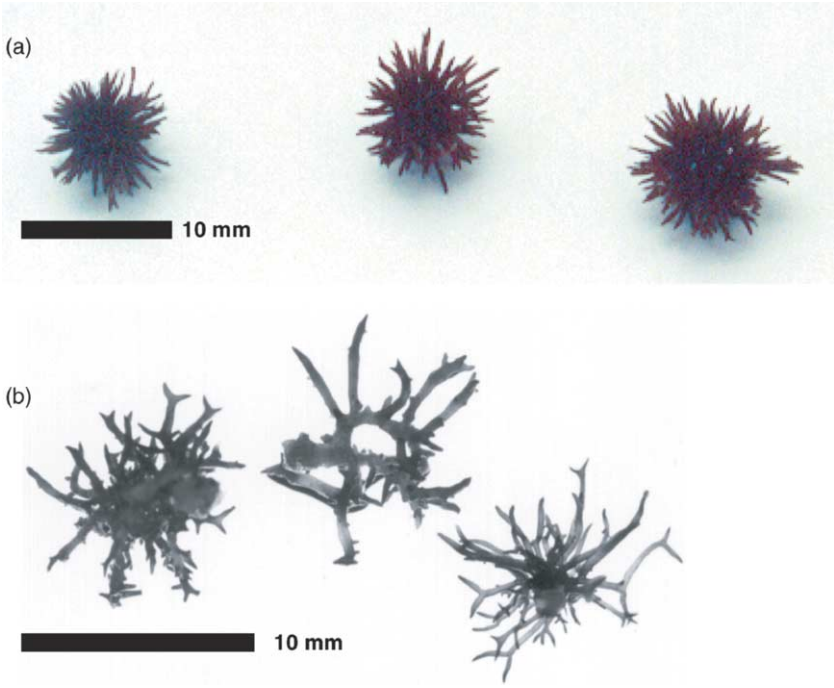

Fig. 11. Photographs of photobioreactor cultured *A. subulata* microplantlets 43 days after inoculation: (a) 500 mL stirred tank and (b) 250 mL bubble-column. Cultivation conditions are detailed in Table 6.

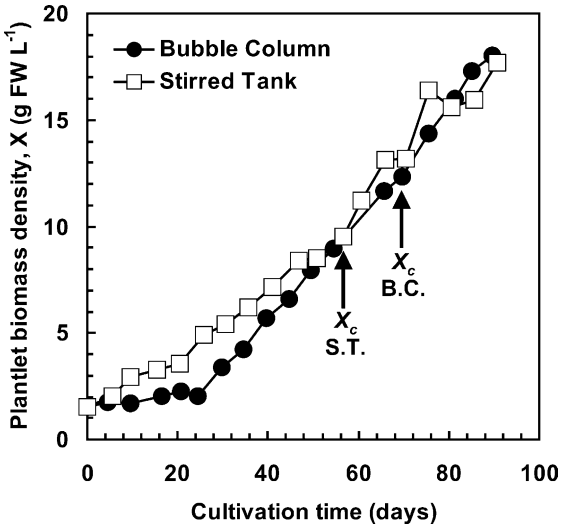

Fig. 12. Comparison of plantlet biomass density vs. time curve for *A. subulata* microplantlets cultivated in a 500 mL stirred tank photobioreactor and a 250 mL bubble-column photobioreactor. Cultivation conditions are detailed in Table 6. The term X_c is the biomass density as which biomass production becomes limited by CO_2 input to the culture suspension. S.T. is stirred tank, B.C. is bubble column.

branches tend to elongate freely, resulting in an asymmetric shape. However, for microplantlets cultivated within the stirred tank bioreactor, linear non-branched shoots emanate from the central core, and the overall shape of the plantlets is spherical and compact. The fluid mixing pattern and physical contact of impeller with the plantlets within the stirred tank may have forced microplantlets to grow into this compact and spherical shape. However, there is not much difference in the growth curves for cultivation of *A. subulata* microplantlets in a bubble-column versus stirred tank photobioreactor (Fig. 12).

3.3.2. Light delivery

In microalgal suspension cultures, light delivery is commonly viewed as the limiting variable in photobioreactor design. Light delivery is also an important parameter in the design of photobioreactors for macroalgal cell and tissue suspension culture. However, in macroalgal suspension culture, growth is light saturated at relatively low light intensities compared to microalgae because in the rocky intertidal, benthic macoalgae experience relatively low light intensity. Therefore, external illumination of the bioreactor with cool-white fluorescent lamps is generally sufficient for bench or pilot-scale reactors provided the light path through the culture is not too long and the bioreactor vessel is transparent to light.

The specific growth rate (μ, day^{-1}) and net photosynthetic oxygen evolution rate (P_o, $\mathrm{mmol\,O_2\,g^{-1}\,DW\,h^{-1}}$) are dependent on the light intensity delivered to the culture (I, $\mu\mathrm{E\,m^{-2}\,s^{-1}}$). This relationship is described by a decaying exponential saturation relationship of the form

$$\mu = \mu^{o}_{max}(1 - e^{-I/I_k}) \quad (1)$$

$$P_o = -Q_o + P_{o,max}(1 - e^{-I/I_k}) \quad (2)$$

where μ^o_{max} is the specific growth rate at light saturation under conditions where culture growth is not limited by CO_2 or macronutrient supply, Q_o is the specific O_2 respiration rate (mmol O_2 g^{-1} DW h^{-1}), $P_{o,max}$ is the specific photosynthetic oxygen evolution rate at light saturation (mmol O_2 g^{-1} DW h^{-1}), and I_k is the light intensity where μ or P_o are 63.2% of μ^o_{max} or $P_{o,max}$ respectively, i.e., at $I = I_k$, $1 - e^{-1} = 0.632$. Typical values of I_k for macroalgal suspension cultures are compared in Table 4. Eqs. (1) and (2) do not include a photoinhibition term. The "*P–I* curves" defined by Eqs. (1) and (2) predict that light saturated growth on oxygen evolution rate is achieved and sustained as the light intensity increases. However, if photoinhibition is present, then the specific oxygen evolution rate will initially increase towards saturation and then decrease with further increase in the light intensity, a reversible process often associated with PSII damage by UV portion of the spectrum and its subsequent repair by the cell. Photoinhibition is observed in many macroalgae but only at light intensities exceeding 500 μE m^{-2} s^{-1} (Coutinho and Zingmark, 1987). In contrast, macroalgal suspensions are cultivated in photobioreactors at incident light intensities below 200 μE m^{-2} s^{-1}, far below the photoinhibition threshold.

The attenuation of light by the macroalgal culture suspension is described by the Beer–Lambert law, given by

$$I(z) = I_o\, e^{-k'z} \quad (3)$$

where I_o is the light intensity incident to the vessel surface (μE m^{-2} s^{-1}), k' is the apparent light attenuation constant (cm^{-1}) for the culture suspension, and z is the position normal to the illuminated vessel surface. The attenuation of light through the culture is the result of scattering and shading of light by the suspended biomass clumps, the absorption of photons by the photosynthetic apparatus of the cell, and the absorption of light by the liquid medium. Thus, the apparent light attenuation constant (k') is a linear function of the cell density:

$$k' = k_o + k_c X \quad (4)$$

where k_o is the light attenuation constant of the cell-free liquid medium, k_c is the intrinsic light attenuation constant (L g^{-1} DW cm^{-1}), and X is the biomass density of the culture suspension (g DW L^{-1}).

Methods for estimation of k_c for macroalgal suspension cultures are described by Mullikin and Rorrer (1998) and Huang and Rorrer (2002a). Values of k_c for representative macroalgal culture systems are compared in Table 4. In macroalgal cell and tissue suspension cultures, biomass is concentrated into visible (1–10 mm) clumps, which have low values of k_c relative to microalgal suspension cultures. For example, as shown in Table 4, k_c values for macroalgal suspension cultures range from 0.06 to 0.15 L g^{-1} DW cm^{-1}, whereas for microalgal suspension cultures, k_c ranges from 0.38 to 0.82 L g^{-1} DW cm^{-1} (Molina-Grima et al., 1994), almost a factor of 10 or higher. As shown in Table 4, k_c increases as the clump size (d_p) decreases. In microalgal suspension cultures, the biomass is dispersed as single microscopic cells which scatter and shade light to a greater extent than clumped cell mass.

In photobioreactor vessel design and scaleup, the "mean light intensity" must be specified. For one-dimensional light transfer, the mean light intensity (I_m) is defined by

Table 4
Light attenuation parameters for macroalgal suspension cultures

Marine plant	Class	Culture system	Intrinsic light attenuation constant, k_c (L g^{-1} DW cm^{-1})[a]	Range of light saturation constant, I_k ($\mu E\ m^{-2}\ s^{-1}$)	Reference
L. saccharina	Brown	Microscopic gametophyte suspension	0.153 ± 0.014 ($d_p < 1$ mm)[b]	4–6	Mullikin and Rorrer (1998)
A. coalita	Green	Tissue suspension	0.108 ± 0.0050 ($d_p < 3$ mm)	N/A	Unpublished Data
A. subulata	Red	Microplantlet suspension	0.0612 ± 0.0050 ($d_p = 8.4 \pm 1.6$ mm); 0.0887 ± 0.0070 ($d_p = 4.6 \pm 1.0$ mm); 0.120 ± 0.010 ($d_p = 2.4 \pm 1.1$ mm)	13–18	Huang and Rorrer (2002a); Unpublished Data
O. secundiramea	Red	Microplantlet suspension	0.0593 ± 0.0030 ($d_p = 8$–10 mm); 0.153 ± 0.017 ($d_p < 3$ mm)	58–64	Polzin and Rorrer (2003); Unpublished Data

[a] DW, dry cell mass.
[b] d_p, diameter of cell clump or microplantlet.

$$I_m(X) = \frac{\alpha I_o}{(k_o + k_c X)d}[1 - e^{-(k_o + k_c X)d}] \quad (5)$$

where d is the path length for light transfer within the vessel, α is the number of planes of illumination associated with the bioreactor vessel, and I_o is the light intensity incident to one plane of illumination. For a vessel uniformly illuminated from two opposing sides, $\alpha = 2$, because even though the light intensity incident to each vessel surface is I_o, the photon input from both sides superimposes.

In photobioreactor vessel design and scaleup, it is often desired to let $I_m(X) > I_k$ at all points during the cultivation process to insure that the specific growth rate is at least one-half of its light saturation value. Based on the discussion above, cultivation to high cell density (large X) requires a small light path length d. Consequently, the illuminated surface area to culture volume ratio (S/V) must be high. For a cylindrical vessel, $S/V = d^{-1}$, where d is the vessel diameter. Furthermore, the total light power input to the reactor per unit volume is $P = SI_o/V$. Hence, I_o and d are chosen in a way to meet practical constraints to achieve the target I_m at desired X. For example, consider the following design constraints: (1) final cell density of 3.0 g DW L^{-1} is desired; (2) the minimum practical vessel diameter is 20 cm; and (3) the light source can provide up to $I_o = 150\ \mu E\ m^{-2}\ s^{-1}$ with two-sided illumination ($\alpha = 2$). For the *L. saccharina* gametophyte suspension culture, assume I_k is 20 $\mu E\ m^{-2}\ s^{-1}$, and k_c is 0.15 L g^{-1} DW cm^{-1}. Therefore, for a 20 cm diameter vessel, the required I_o is 90 $\mu E\ m^{-2}\ s^{-1}$, whereas for a 150 $\mu E\ m^{-2}\ s^{-1}$ light source, $d = 33$ cm. Both calculations yield a power input per unit culture volume (P) of 4500 $\mu E\ m^{-3}\ s^{-1}$.

For macroalgal culture suspensions that can be pumped, the tubular recycle photobioreactor (Fig. 10) offers excellent light transfer and low light attenuation. This is because the path length for light transfer is reduced down to diameter of the coiled tubing, and the lamp is placed within the center of the coil. The tubular recycle photobioreactor can successfullly cultivate *L. saccharina* gametophyte cell clump suspensions (Mullikin and Rorrer, 1998). The tubular recycle photobioreactor is not suitable for the microplantlet cultures as their large tissue size makes it difficult for them to pass through the tubing without settling. A mathematical model for the tubular recycle photobioreactor is described by Rorrer and Mullikin (1999).

Macroalgal cell suspension cultures require diurnal photoperiod defined as times the culture is exposed to light and dark within a 24 h time period. Although photoperiod is important to the development of macrophytic marine algae in the natural environment (Dring, 1984), the effects of photoperiod on biomass production still remain unclear. Photoperiod had a marked effect on cumulative biomass production of *A. subulata* microplantlets in a perfusion bubble-column photobioreactor, as shown in Fig. 13. Here, biomass production was optimal at a 16:8 LD photoperiod, but was completely shut down at 20:4 LD. A mathematical model for biomass growth based on cumulative photodamage of the photosynthetic apparatus of the cell mass as continuous illumination of the culture is approached is proposed by Huang and Rorrer (2002b).

3.3.3. CO_2 delivery

Continuous bubbling aeration of the culture with CO_2 in air accomplishes four processes: (1) transfer of CO_2 to the culture; (2) maintenance of the dissolved inorganic carbon level in

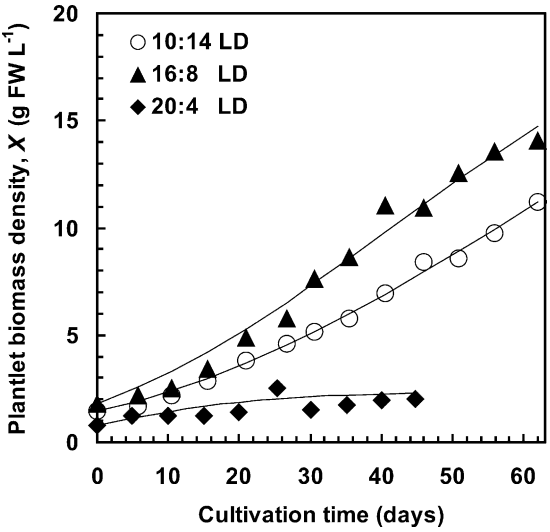

Fig. 13. Effect of illumination photoperiod on plantlet biomass density vs. time curve for *A. subulata* microplantlets cultivated a 250 mL bubble-column photobioreactor. Cultivation conditions are detailed in Table 6.

the culture medium; (3) pH control; and (4) removal of dissolved O_2 produced by photosynthesis. Carbon dioxide is supplied to the culture in accordance with the gas–liquid interphase mass transfer principles. The volumetric CO_2 transfer rate (n_{CO_2}, mmol CO_2 L^{-1} h^{-1}) is defined as

$$n_{CO_2} = k_L a(C^*_{CO_2} - C_{CO_2}) = k_L a\left(\frac{p_{CO_2}}{H_{CO_2}} - C_{CO_2}\right) \tag{6a}$$

$$n_{CO_2} \approx CO_2 - TR = k_L a \frac{p_{CO_2}}{H_{CO_2}} \tag{6b}$$

where C_{CO_2} is the dissolved CO_2 concentration (mmol L^{-1}) in the liquid medium, $C^*_{CO_2}$ is the dissolved CO_2 concentration in the liquid medium in equilibrium with the CO_2 partial pressure of the aeration gas (p_{CO_2}), H_{CO_2} is the Henry's law constant for CO_2 dissolved in seawater (0.034 atm mmol^{-1} L at 25 °C, Raven, 1984), and $k_L a$ is the volumetric mass transfer coefficient for CO_2 gas transfer (h^{-1}) to the liquid.

The CO_2 mass transfer process includes the speciation of inorganic carbon in seawater, which affects the culture pH. Bicarbonate buffering is used to maintain a setpoint pH at a desired CO_2 gas partial pressure (Rorrer et al., 1996; Huang and Rorrer, 2003). The basic principles are highlighted below. Macroalgal cell and tissue cultures grow best in seawater-based medium at pH 8–9, where CO_2 speciates to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-})

$$CO_2(g) \leftrightarrow CO_2(aq) \tag{7a}$$

$$CO_2(aq) + H_2O \overset{k_1}{\leftrightarrow} HCO_3^- + H^+ \tag{7b}$$

$$CO_2 + OH^- \overset{k_2}{\leftrightarrow} HCO_3^- \tag{7c}$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \tag{7d}$$

At 25 °C, the forward rate constants are $k_1 = 0.037\,s^{-1}$ and $k_2 = 8500\,mol\,(L\,s)^{-1}$ (Raven, 1984). The subsequent mass action expressions for dissolved CO_2 speciation in liquid medium are

$$K_1 = \frac{[H^+][HCO_3^-]}{C^*_{CO_2}} \tag{8a}$$

$$K_2 = \frac{[CO_3^{2-}][H^+]}{[HCO_3^-]}. \tag{8b}$$

From the mass action expressions and Henry's law for absorption of CO_2, it can be shown that the predicted equilibrium concentrations of bicarbonate $[HCO_3^-]$ and carbonate $[CO_3^{2-}]$ ions are

$$[HCO_3^-] = 10^{-(+pK_{a,1}-pH)}\frac{p_{CO_2}}{H_{CO_2}} \quad [CO_3^{2-}] = 10^{(+pK_{a,2}-pH)}[HCO_3^-] \tag{9}$$

The pK_a values for dissociation of HCO_3^- and CO_3^{2-} are $pK_{a,1} = 6.00$ and $pK_{a,2} = 9.10$, respectively, in seawater of 35 ppt salinity at 25 °C (Raven, 1984). For example, at CO_2 partial pressure of 35 Pa, temperature of 25 °C, and pH of 8.0, the dissolved CO_2 species concentrations are 0.010 mM CO_2, 1.02 mM HCO_3^-, and 0.081 mM CO_3^{2-}.

Calculations show that the chemical speciation Eqs. (7a)–(7d) do not affect the mass transfer coefficient. Specifically, the enhancement factor E is used to describe the relationship between the liquid-phase mass transfer coefficient with chemical reaction (k_L) relative to the mass transfer coefficient without chemical reaction (k_L^o). For the system described by Eq. (7), the enhancement factor is defined by Molina-Grima et al. (1993) as

$$\frac{(k_L a)}{(k_L a)^o} = E = \left[\left(\frac{(k_1 + k_2 C_{OH}) D_{CO_2}}{(k_L^o)^2}\right) + 1\right]^{1/2} \tag{10}$$

where C_{OH} is the OH^- concentration, D_{CO_2} is the molecular diffusion coefficient of CO_2 dissolved in seawater, equal to $1.94 \times 10^{-5}\,cm^2\,s^{-1}$ at 25 °C (Raven, 1984), and k_L is liquid-phase mass transfer coefficient around the aeration bubble, which is estimated by the following well-known mass transfer correlation for bubble swarms rising in liquid (Blanch and Clark, 1997)

$$\frac{k_L^o d_b}{D_{CO_2}} = 0.132\left(\frac{d_b^3(\rho_L - \rho_G)g}{\rho_L \nu_L^2}\right)^{1/2}\left(\frac{\nu_L}{D_{CO_2}}\right)^{1/2}. \tag{11}$$

In Eq. (11), d_b is the bubble diameter (nominally, 1.0 mm), g is the gravitational acceleration constant (981 cm s^{-2}), ρ_G is the density of the aeration gas (0.0012 g cm^{-3}), ρ_L is the density of the liquid (1.0 g cm^{-3}), and ν_L is the kinematic viscosity of the liquid (0.01 $cm^2\,s^{-1}$). Therefore, from Eqs. (10) and (11), at pH 8.5 and 25 °C, $C_{OH} = 3.16 \times 10^{-6}\,mol\,L^{-1}$ and $E = 1.0001$, demonstrating the mass transfer process is not enhanced by the CO_2 speciation

reactions. At pH 8 and higher, the $k_L a$ for CO_2 interphase mass transfer is scaled to the $k_L a$ for O_2 interphase mass transfer using Penetration Theory, to account for the chemical speciation of dissolved CO_2:

$$(k_L a)_{CO_2} = (k_L a)_{O_2} \left(\frac{D_{CO_2}}{D_{O_2}} \right)^{1/2} \tag{12}$$

where $D_{CO_2}/D_{O_2} = 0.93$ (Molina-Grima et al., 1993). The $k_L a$ for O_2 is easily measured by the dynamic "gassing in" method. In the dynamic gassing in method, liquid medium in the bioreactor is stripped of dissolved oxygen by nitrogen sparging. Then, air is bubbled in, and $k_L a$ is estimated by fitting the dissolved oxygen versus time data to dynamic model equation

$$C_{O_2}(t) = \frac{p_{O_2}}{H_{O_2}} - \left(\frac{p_{O_2}}{H_{O_2}} - C_{O_2,o} \right) e^{-k_L a(t-t_o)} \tag{13}$$

where H_{O_2} is the Henry's law constant for O_2 dissolved in seawater (1.07 atm mmol^{-1} L, Raven, 1984). Typical values of $k_L a$ for O_2 in bubble-column and stirred tank photobioreactor systems are presented in Table 5. Although decreasing the bubble diameter, the aeration rate, and the impeller tip speed all increase $k_L a$ value, bubble diameter has the dominant effect.

Alternatively, the CO_2 delivery rate per unit volume of culture (mmol CO_2 (L h)$^{-1}$) can be defined as

$$n_{CO_2} = \frac{v_g}{V} \frac{p_{CO_2,in} - p_{CO_2,out}}{RT} \tag{14}$$

where $p_{CO_2,out}$ is the partial pressure of CO_2 in the outlet aeration gas, R is the gas constant (8.206×10^{-5} L atm (mmol K)$^{-1}$), T is the temperature of the aeration gas (K), v_g is the volumetric flowrate of the aeration gas (L h^{-1}), and V is the liquid culture volume (L). If $p_{CO_2,out}$ goes to zero, then

$$n_{CO_2} \cong \frac{v_g}{V} \frac{p_{CO_2,in}}{RT} \tag{15}$$

If $k_L a$ is relatively high and v_g is relatively low, then Eq. (15) will limit the maximum possible rate of CO_2 delivery.

The phototrophic culture consumes dissolved CO_2 for photosynthetic growth. The volumetric CO_2 consumption rate (q_{CO_2}, mmol CO_2 (L h)$^{-1}$) can be estimated by two approaches. First, q_{CO_2} is can be estimated from the biomass production rate (μX) by

$$q_{CO_2} = \frac{\mu X}{Y_{X/CO_2}} \tag{16}$$

where Y_{X/CO_2} is the biomass yield coefficient for CO_2 consumption (g DW mmol^{-1} CO_2 consumed). Secondly, q_{CO_2} can be estimated from the specific oxygen evolution rate by

$$q_{CO_2} = X(P_o + Q_o) \frac{\nu_{CO_2}}{\nu_{O_2}} \tag{17}$$

Table 5
Ranges of $k_L a$ for bench-scale photobioreactors using in macroalgal cell and tissue culture studies

Photobioreactor	Vessel diameter (cm)	Aeration rate (L air L^{-1} culture min^{-1})	Bubble diameter (mm)	Agitation mode (tip speed)	Range of $k_L a$ (h^{-1})	Reference
3.0 L bubble-column	8.5	0.04–1.35	<1	Rising air bubbles	24–640	Huang and Rorrer (2003); Unpublished Data
0.5 L stirred tank	10.5		<1	Two-blade paddle impeller, $d_I = 5.5$ cm		Huang and Rorrer (2003)
		0.10–0.50		$N = 120$ rpm (34.5 cm s^{-1})	45–120	
		0.30		$N = 60$–250 rpm (17.3–72 cm s^{-1})	70–250	
3.0 L stirred tank	13.0	0.43	>5	Marine impeller, $d_I = 4.5$ cm; $N = 100$–325 rpm (23.6–76.6 cm s^{-1})	5–19	Rorrer et al. (1996)

where ν_{CO_2} and ν_{O_2} are the stoichiometric coefficients for CO_2 consumption and O_2 evolution, as defined by the overall photosynthetic biomass stoichiometry. As a first approximation, if the biomass production is described by Calvin photosynthesis stoichiometry given by

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \tag{18}$$

then, $\nu_{CO_2} = 1$ mol CO_2, $\nu_{O_2} = 1.0$ mol O_2, and $Y_{X/CO_2} = 30$ mg DCW produced per mol CO_2 consumed.

To avoid CO_2 mass transfer limited growth, $n_{CO_2} > q_{CO_2}$. However, as the biomass density of the suspension increases, there is ultimately a point where continued biomass production is limited by CO_2 input. We define X_c as the "critical biomass density" where the biomass production rate becomes limited by the CO_2 input:

$$X_c = \frac{n_{CO_2} Y_{X/CO_2} f}{\mu} \tag{19}$$

In Eq. (19), f is the fractional illumination photoperiod (e.g., $f = 0.42$ for a 10:14 LD photoperiod) of the cultivation process, as it is assumed that biomass production does not occur during the dark phase of the photoperiod. Biomass density past X_c increases linearly with time if n_{CO_2} is constant:

$$X(t) = X_c(t_c) + n_{CO_2} Y_{X/CO_2} f(t - t_c) \tag{20}$$

where t_c is the cultivation time at which $X = X_c$. For the data presented in Fig. 12, the point in the cultivation where $X = X_c$ is indicated; detailed parameters used in this calculation are provided in Table 6. Although macroalgal suspension cultures grow rather slowly with specific growth rates generally below 0.20 day^{-1}, and $k_L a$ values in aerated photobioreactors are high, it is difficult to provide sufficient CO_2 delivery rates that avoid the CO_2 limited growth rate condition at high cell density unless CO_2 is added to the aeration gas to increase n_{CO_2}. Typically, about 10× ambient CO_2 concentration (i.e., 3500 ppm CO_2) must be provided in the aeration gas in order for the biomass production rate to not be limited by the rate of CO_2 input.

3.3.4. Macronutrient delivery

If the culture suspension is continuously supplied with light and CO_2, then the cumulative biomass production will be limited ultimately by available macronutrients dissolved in the liquid medium, principally N (nitrogen) in the form of nitrate (e.g., $NaNO_3$), and P (phosphorus) in the form of phosphate (e.g., Na_2HPO_4). Biomass stoichiometry is used to quantify the nutrient demands on the culture. For example, based on our previous studies, the overall stoichiometry for biomass production by *A. subulata* microplantlet suspension cultures is given by

$$\begin{aligned} &848\,CO_2 + 872\,H_2O + 24\,NaNO_3 + Na_2HPO_4 + 26\,HCl \\ &\quad \rightarrow (CH_2O)_{848}(NH_3)_{24}(H_3PO_4) + 896\,O_2 + 26\,NaCl \end{aligned} \tag{21}$$

The biomass yield coefficient based on nitrate-limited growth ($Y_{X/N}$) is 1.08 g DW $mmol^{-1}$ N, biomass yield coefficient based on phosphate-limited growth ($Y_{X/P}$) is 25.8 g DW $mmol^{-1}$ P

Table 6
Process conditions for photobioreactor cultivation of *Agardhiella subulata* microplantlets in liquid suspension

Process variable	Stirred tank (500 mL, 140 rpm, $d_I = 5.5$ cm)	Bubble-column (250 mL)
Cultivation volume, V (mL)	500	250
Vessel inner diameter (cm)	10.5	4.5
ASP12 medium replacement rate (mL every 5 days (20% per day))	500	250
Temperature (°C)	24	24
Incident light intensity, I_o (μmol photons m^{-2} s^{-1})	80	38
Planes of illumination, α	2	2
Photoperiod (LD, $f = 0.42$)	10 h:14 h	10 h:14 h
Aeration rate (mL min^{-1}, L air (L $min)^{-1}$)	150 (0.3)	100 (0.4)
Nominal bubble diameter, d_b (mm)	1	1
Measured $k_L a$ for O_2 (h^{-1})	100.7	89.5 ± 2.3
CO_2 partial pressure (atm)	0.00035	0.00035
n_{CO_2} (as CO_2–TR, mmol CO_2 (L $h)^{-1}$)	0.982	0.873
n_{CO_2} (as CO_2 delivery in aeration gas, mmol CO_2 (L $h)^{-1}$)	0.258	0.344
Average specific growth rate, μ (s^{-1}) (days)	0.035 ± 0.002 (0–47)	0.034 ± 0.004 (0–45)
Critical biomass density, X_c (g FW L^{-1})	8.9	12.3
Average cultivation pH	8.83 ± 0.09	8.73 ± 0.07

FW, wet cell mass.

(Huang and Rorrer, 2003), and the biomass yield coefficient gas on CO_2 consumption is 30.6 mg DW $mmol^{-1}$ CO_2.

Macronutrient delivery is defined by the type of cultivation process. In batch cultivation, macronutrients are initially loaded into the liquid growth medium at culture inoculation. Nitrate and phosphate concentration in the liquid medium decreases with time until one (or both) goes to zero. At this point, the "limiting nutrient" is consumed, and biomass production ultimately ceases even if light and CO_2 are still being continuously supplied. For example, in nitrate-limited batch culture, the final biomass density (X_f) is estimated by

$$X_f = X_i + C_{N,i} Y_{X/N} \tag{22}$$

where X_i and $C_{N,i}$ are the cell density and nitrate concentration at inoculation.

In perfusion cultivation, liquid medium containing dissolved macronutrients is continuously added to the bioreactor culture suspension. The spent medium exits the bioreactor but the cell mass is retained within the bioreactor. Under these conditions, cumulative biomass production will continue, but the rate of biomass production will ultimately be limited by the rate of macronutrient delivery, the rate of CO_2 delivery, or the attenuation of light through the dense culture suspension. The ultimate limit to biomass production is space. If the tissues completely fill up the bioreactor control volume, e.g., form a packed bed, then biomass production will stop even in the presence of an infinite sink of nutrients (light, CO_2, and macronutrients). In perfusion culture of *O. secundiramea* microplantlets, cell densities exceeding 10 g of dry cell weight per liter with specific growth rates near 0.2 day^{-1} can

be obtained from the microplantlet suspension culture systems under optimal conditions of light delivery, nutrient delivery, aeration, and mixing (Polzin and Rorrer, 2003).

3.4. Sample model development and predictions: the perfusion photobioreactor

The photobioreactor modeling elements described in Section 3.3 are easily integrated into a single model. Below, we illustrate how these elements can be combined to predict biomass production, light transfer, and nutrient delivery within a perfusion photobioreactor shown in Fig. 8b. This previously unpublished model is developed under the following six assumptions: (1) the culture suspension is well mixed; (2) the total culture volume (V) is constant; (3) the input volumetric medium flowrate equals output effluent medium volumetric flowrate ($v_o = v$); (4) the biomass is retained within the vessel; (5) N and P consumption are growth associated; (5) the cultivation is not limited by CO_2 mass transfer rate; and (6) the growth rate assumes zero-order kinetics with respect to dissolved CO_2 concentration, which is valid at elevated levels of CO_2 in the aeration gas.

The material balances on biomass (X), nitrate (N), and phosphate (P) are given respectively by

$$\frac{dX}{dt} = \mu X \tag{23}$$

$$\frac{dC_N}{dt} = (C_{N,o} - C_N)\frac{v_o}{V} - \frac{\mu X}{Y_{X/N}} \tag{24}$$

$$\frac{dC_P}{dt} = (C_{P,o} - C_P)\frac{v_o}{V} - \frac{\mu X}{Y_{X/P}} \tag{25}$$

where v_o is the volumetric flowrate of the perfusion medium, and $C_{N,o}$ and $C_{P,o}$ are the concentrations of nitrate and phosphate, respectively, in the perfusion medium. All other variables were defined previously. Eqs. (23)–(25) are defined by initial conditions X_i, $C_{N,i}$, and $C_{P,i}$ at cultivation time $t = 0$.

Macronutrient concentrations, dissolved CO_2 concentration, and mean light intensity simultaneously affect growth rate (μ). The simplest model assumes a multiplicative dependence on the effect of dissolved nitrate concentration (C_N), dissolved phosphate concentration (C_P), dissolved CO_2 concentration (C_{CO_2}), and mean light intensity (I_m) on the specific growth rate. Monod saturation kinetics are used to describe the dependence of nitrate, phosphate, and dissolved CO_2 concentration growth rate. Exponential saturation kinetics have been experimentally verified to describe the effect of light intensity on growth rate, as described by Eqs. (1) and (5). The final equation is

$$\mu = \mu^o_{max} f \frac{K_N}{K_N + C_N}\frac{K_P}{K_P + C_P}\frac{K_{CO_2}}{K_{CO_2} + C_{CO_2}}(1 - e^{-I_m(X)/I_k}) \tag{26}$$

where K_N, K_P, and K_{CO_2} are the half-saturation constants for nitrate, phosphate, and dissolved carbon dioxide, respectively. For macroalgae, K_N values are usually below 0.050 mM and K_P values are below 0.010 mM (Table 7). If dissolved macronutrients are not continuously added to the culture suspension, then they may ultimately limit biomass production. In contrast, illumination and CO_2 are continuously added to the culture suspension, as detailed

Table 7
Perfusion photobioreactor model input parameters for prediction of *A. subulata* microplantlet growth performance at 24 °C

Variable		Value and units	Reference/details
Definition	Symbol		
Illumination parameters			
Planes of illumination	α	2 (two-sided)	[a]
Incident light intensity	I_o	43 μE m^{-2} s^{-1}	[a]
Fractional photoperiod	f	10 h light/24 h (0.42)	[a]
Attenuation constant: liquid medium	k_o	0.24 cm^{-1}	[a]
Attenuation constant: biomass	k_c	0.107 L g^{-1} DW cm^{-1}	[a]
Vessel diameter	d	8.5 cm	[a]
Intrinsic culture growth parameters			
Biomass yield coefficient: nitrate	$Y_{X/N}$	1.08 g DW $mmol^{-1}$ N	[b]
Biomass yield coefficient: phosphate	$Y_{X/P}$	25.8 g DW $mmol^{-1}$ P	[b]
Biomass yield coefficient: CO_2	Y_{X/CO_2}	30.6 mg DW $mmol^{-1}$ CO_2	[b]
Maximum specific growth rate at saturation	μ^o_{max}	0.18 day^{-1}	[c]
Half-saturation constant, nitrate	K_N	2.2 μM	[d]
Half-saturation constant, phosphate	K_P	3 μM	[e]
Light intensity of 63.2% of saturation	I_k	18 μE m^{-2} s^{-1}	[a]
Initial plantlet density	X_i	0.5 g DW L^{-1}	
Medium delivery parameters			
Medium		ASP12 artificial seawater	[a]
Volumetric flowrate of perfusion medium	v_o	400 mL per day	[a]
Culture suspension volume	V	2000 mL	[a]
Nitrate concentration, perfusion medium	$C_{N,o}$	10 mmol N L^{-1}	[a]
Phosphate concentration, perfusion medium	$C_{P,o}$	0.037 mmol P L^{-1}	[a]
Initial nitrate concentration	$C_{N,I}$	10 mmol N L^{-1}	[a]
Initial phosphate concentration	$C_{P,I}$	0.037 mmol P L^{-1}	[a]
Aeration parameters			
Aeration rate	v_g	552 mL min^{-1}	[a]
CO_2 partial pressure (culture pH)	p_{CO_2}	3790 ppm (8.1)	[a]
	$C^*_{CO_2}$	0.11 mM	
n_{CO_2} (as CO_2–TR)		15.2 mmol CO_2 $(L\ h)^{-1}$	[a]
n_{CO_2} (as CO_2 delivery in aeration gas)		2.70 mmol CO_2 $(L\ h)^{-1}$	[a]

[a] Huang and Rorrer (2002a).
[b] Huang and Rorrer (2003).
[c] Huang and Rorrer (2002b).
[d] DeBoer et al. (1978).
[e] Chopin et al. (1990).

in Sections 3.3.2 and 3.3.3. The question of whether or not the growth rate is limited by dissolved CO_2 concentration is under debate (Lobban and Harrison, 1994). Half-saturation K_{CO_2} valves derived from photosynthesis rate versus dissolved CO_2 concentration data from several field-collected macroalgae (Surif and Raven, 1990) range from 0.0070 to 0.016 mM. At the ambient gas phase CO_2 partial pressure of 350 ppm (35 Pa), the dissolved CO_2 concentration is 0.010 mM; however, at 3790 ppm CO_2 used in our cultivation studies (Table 7), the dissolved CO_2 concentration is 0.11 mM. Furthermore, since CO_2 is

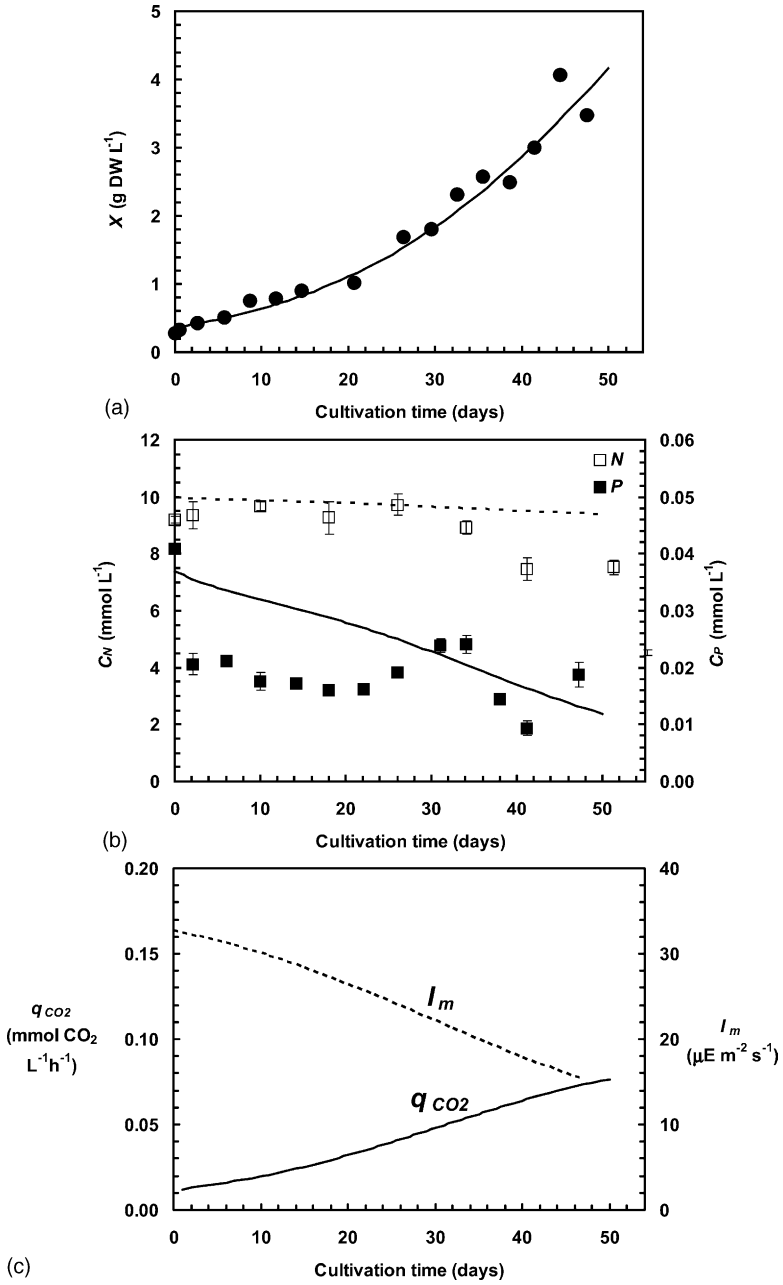

Fig. 14. Comparison of data and model predictions for cultivation of *A. subulata* microplantlets within a bubble-column photobioreactor equipped with continuous nutrient medium perfusion (Fig. 8b). (a) Growth curve, solid line is model prediction; (b) nitrate and phosphate concentration vs. time, solid lines are model predictions; (c) predicted volumetric CO_2 demand (q_{CO_2}) and mean light intensity (I_m) vs. time. Process model input parameters and cultivation conditions are detailed in Table 7.

delivered continuously to the culture, as an approximation, the specific growth rate assumes zero-order kinetics with respect to dissolved CO_2 concentration, and Eq. (26) reduces to

$$\mu = \mu^{\circ}_{\max} f \frac{K_N}{K_N + C_N} \frac{K_P}{K_P + C_P} (1 - e^{-I_m(X)/I_k}). \quad (27)$$

Model input parameters for cultivation of *A. subulata* microplantlet suspension cultures in a perfusion bubble-column photobioreactor are provided in Table 7. Simultaneous numerical integration of material balance Eqs. (23)–(25) and their constitutive Eqs. (4), (5), and (27) by the fourth-order Runge–Kutta method was used to predict the growth performance and nutrient consumption based solely on these intrinsic input parameters. Sample model predictions are compared with data in Fig. 14a and b. Additional model predictions for I_m and volumetric CO_2 demand (q_{CO_2}) during cultivation are presented in Fig. 14c. From Fig. 14c, it can be seen that the peak CO_2 demand is far below the CO_2 delivery and interphase mass transfer rates (Table 7). Therefore, the rate of biomass production is not limited by the rate of CO_2 input. However, the growth rate is decreasing over time as the result of light attenuation as the culture is moving to high biomass density. Furthermore, the model under-predicts macronutrient consumption, as *Agardhiella* species are known to undergo "luxury uptake" of macronutrients (DeBoer et al., 1978; Chopin et al., 1990) that exceeds N and P required by photosynthetic biomass stoichiometry.

4. Summary and conclusions

Bioprocess technology for marine macroalgae has three elements: cell and tissue culture development, photobioreactor design, and identification of strategies for eliciting secondary metabolite biosynthesis. This paper focused on the first two elements. First, cell and tissue culture systems for representative species within brown, green, and red macroalgae have been developed. In vitro culture platforms include microscopic gametophytes, undifferentiated callus filaments, uniserate tissue filaments, and symmetrical "microplantlets" regenerated from callus filaments. The controlled cultivation of these culture systems has been demonstrated in stirred tank, bubble-column, airlift, and tubular recycle photobioreactors in both batch and medium perfusion modes of macronutrient delivery. Mathematical models that integrate light delivery, CO_2 delivery, and macronutrient delivery into the material balance equations for biomass production can be used to predict growth performance in these photobioreactor systems.

Microalgal cultivation technology development is often driven by the need to intensify biomass production. In contrast, we envision that photobioreactor cultivation of cell and tissue cultures derived from marine seaweeds will be a future platform for the controlled biological production of unique, high value chemicals from these macrophytic marine organisms including pharmaceuticals and "nutraceuticals". Consequently, for macroalgal culture technology development providing a controlled growth environment suitable for secondary metabolite biosynthesis may outweigh the need for optimization of growth rate or minimization of the consumption of resources for biomass cultivation, e.g., CO_2, light, or macronutrients. Our ongoing efforts are correlating photobioreactor growth performance

to the production of potentially valued secondary metabolites, including terpenoids and oxylipins.

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