Aquaculture 326-329 (2012) 106–115

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Optimizing conditions for the continuous culture of *Isochrysis* affinis *galbana* relevant to commercial hatcheries

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ARTICLE INFO

Article history:
Received 23 March 2011
Received in revised form 4 November 2011
Accepted 8 November 2011
Available online 19 November 2011

Keywords:
Isochrysis affinis *galbana*
Irradiance efficiency
Factor interaction
Nutrient limitation
Microalgae cultures

ABSTRACT

Optimal culture conditions were investigated for the continuous culture of *Isochrysis* affinis *galbana* (T-Iso) by assessing the effects of irradiance, temperature, pH and nitrogen and phosphorus requirements.
Assessment of the growth rate-irradiance relationship in a turbidostat experiment highlighted that an optimum irradiance efficiency for growth (E_μ) occurred at irradiance much lower than the saturating level. Furthermore, our modeling approach for productivity pointed out that an optimal set of irradiance and dilution rate could be achieved in order to maximize irradiance efficiency.
Optimal conditions for growth were assessed for temperature, pH and irradiance by means of a factorial design experiment. The resulting model predicted that optimal culture conditions occurred at pH = 6.8, irradiance = 780 µmol photons.m^{-2}.s^{-1} and temperature = 30 °C and emphasized significant pH–temperature and pH–irradiance interactions on growth.
The critical N:P ratio was shown to be a decreasing function of the growth rate and revealed high requirements of T-Iso for nitrogen relative to phosphorus. Finally, we propose an integrative optimization procedure for the continuous culture of *I.* affinis *galbana* and outcomes are discussed.

1. Introduction

Microalgae are photosynthetic microorganisms that constitute the first link of the aquatic food chain. Due to their biodiversity, microalgae have many potential application, particularly in the areas of pharmaceuticals, cosmetics and energy (Pulz and Gross, 2004; Rosenberg et al., 2008; Spolaore et al., 2006). However, their use as food in aquaculture remains the major reason for their production (Benemann, 1992; Brown, 2002; Pulz and Gross, 2004). Microalgae are mainly used as food for mollusks and zooplankton, but are also used in the nutrition of early-life-stage crustaceans and small fishes (Borowitzka, 1997; Brown, 2002; Duerr et al., 1998). Despite various alternative diets tested, such as concentrated microalgae (McCausland et al., 1999; Robert et al., 2001), yeast (Nell, 2002), bacteria (Douillet and Langdon, 1994) or even lipid-substitution compounds (Coutteau et al., 1996; Knauer and Southgate, 1997a, 1997b), living microalgae remain necessary for production of larval and juvenile bivalves (Robert and Trintignac, 1997). In France, shellfish production is dominated by the Pacific oyster *Crassostrea gigas*. Because of high inter-annual variations in spatfall (Robert and Gerard, 1999) and an increasing demand for triploid oysters (Nell, 2002), French hatchery spat production of *C. gigas* has regularly increased from 10% to 20–25% over the last decade (Rico-Villa et al., 2008). Currently, batch production of microalgae (<1 m^3) is the most widely used method of algal production in hatcheries, but this is expensive and leads to variable microalgal biochemical composition: 30% of total production costs are related to larval feeding and thus to microalgae cultivation (Coutteau and Sorgeloos, 1992). In addition, live microalgae production is considered to be the major bottleneck in mollusk hatcheries (Fabregas et al., 1986). Optimization of microalgal culture conditions is, therefore, necessary to reduce production costs and meet the demand of hatchery managers for an easy, cheap, reliable method of producing microalgae of high nutritional value (Coutteau and Sorgeloos, 1992).

Continuous culture is an attractive alternative to batch production (Bougaran et al., 2003) as it allows full automation of the microalgae production process, thereby reducing labor costs. Also, it provides a stable quantity and quality of microalgal biomass due to a better control of the growing environment (Borowitzka, 1997). However, appropriate equipment is required (Loubière et al., 2009) as well as specific knowledge of the microalgae species and their optimal culture conditions.

Numerous studies have shown that the biochemical composition and physiological status of microalgae cells are altered by different

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doi:10.1016/j.aquaculture.2011.11.020

environmental factors. According to Tzovenis et al. (2003) and Sukenik and Wahnon (1991), irradiance increases the growth rate of *Isochrysis* affinis *galbana* as well as its levels of carbohydrates, DHA and saturated fatty acids, while it reduces lipid content. Increased temperature was reported to reduce carbohydrates in T-Iso and *Chaetoceros sp* (Renaud et al., 2002) and to increase the carbon quota of *Thalassiosira pseudonana* (Berges et al., 2002). It is also reported that high pH leads to increased growth, CO_2 uptake and amino acid content in *Skeletonema costatum* (Taraldsvik and Myklestad, 2000). The factors affecting microalgae cultures include also dilution rate (Alonso et al., 2000; Sukenik and Wahnon, 1991) and culture medium (Fabregas et al., 1995; Sanchez et al., 2000). In the present study, three experiments were designed to find ways of improving *I.* affinis *galbana* (T-Iso) growth and reducing the production costs associated with labor, energy and chemical supplements, which are considered as the greatest expenses for hatcheries (Boeing, 2000). In a first experiment, the relationship between growth rate and irradiance was assessed; irradiance efficiency for T-Iso growth could be computed for an optically clear culture (A_{680}<0.1). A modeling approach was then used to assess the irradiance efficiency for productivity in a light-limited photobioreactor. We then developed a new experimental device, based on a factorial design approach in order to not only assess the main effects of temperature, irradiance and pH on growth rate, but also the putative interactions between these factors. To complete our optimization procedure for the culture conditions, chemostat experiments were used to further characterize the requirements of T-Iso for nitrogen and phosphorus over a range of growth rate.

2. Materials and methods

2.1. Microalgal strain

The prymnesiophyceae *I.* affinis *galbana* was obtained from the Culture Collection of Algae and Protozoa (CCAP 927/14, Scotland). The strain was maintained in the laboratory in a volume of 250 mL. Transfer was made into 2 L-flasks, 5 to 7 days later, to culture a microalgal population sufficient for the subsequent experiments.

2.2. Culture medium

For the experiments and strain maintenance, cultures were grown in 0.22 μm filtered sterilized seawater, enriched with the Walne (1966) medium, unless otherwise specified.

2.3. Assessment of cell population and check for steady-state

Cellular concentration was assessed daily with absorption A_{680} measurements and by image analysis on Malassez slides using specific image analysis software (Samba Technologies, Meylan, France) after Lugol dying. Steady state was considered to have been achieved for continuous cultures when cell concentration and A_{680} did not vary by more than 10% for three consecutive days.

2.4. Assessment of the growth rate-irradiance relationship

The growth of T-Iso as a function of irradiance was assessed in turbidostat mode in a planar-torus photobioreactor. A detailed description of the experimental apparatus is given in Loubière et al. (2009). Optically clear cultures were run at low cell density (10^6 cell.mL^{-1}) to avoid self-shading; therefore, incident and averaged irradiance inside the photobioreactor were considered similar. Temperature was set at 27 °C, and pH maintained at 7.2 by automated injection of CO_2. Growth rate was measured as dilution rate (D) at steady state for different levels of irradiance ranging from 10 to 450 μmol photon.m^{-2}.s^{-1}, as measured at the surface of the photobioreactor using a spherical Li-Cor LI193 quantum scalar meter.

A modified Michaelis–Menten model was used to fit the experimental data (Eq. 1):

$$\mu = \mu_m^*(I-Ic)/(Ki+I-Ic) \tag{1}$$

where μ is the growth rate (d^{-1}), μ_m the maximal growth rate (d^{-1}), I the averaged irradiance inside the culture (μmol photon.m^{-2}.s^{-1}), I_c the irradiance at the compensation point (μmol photon.m^{-2}.s^{-1}) and K_i the half-saturation irradiance (μmol photon.m^{-2}.s^{-1}).

Irradiance efficiency for growth rate E_μ (μm².μmol photon^{-1}) was calculated as the ratio of growth rate to irradiance, i.e. the growth rate sustained per irradiance unit. When modeling the growth rate on irradiance curve with Eq. (1), E_μ is then given by Eq. (2). It can be demonstrated that the maximum E_μ^* can be computed from Eq. (3).

$$E_\mu = \mu_m^*\left(1-\frac{Ic}{I}\right)\Big/(Ki+I-Ic) \tag{2}$$

$$E_\mu^* = \mu_m^*\sqrt{Ki-Ic}\Big/\left(\left(Ki+\sqrt{Ki-Ic}\right)\left(Ic+\sqrt{Ki-Ic}\right)\right), \text{for } I = Ic+\sqrt{Ki*Ic} \tag{3}$$

We further used the same concept for productivity in a light-limited photobioreactor, with a dilution rate D, and denoted E_P (kg.m^{-1}.μmol photon^{-1}) the irradiance efficiency for the volumetric productivity (i.e. the ratio of volumetric productivity (D*X) to incident irradiance). The maximum E_P is hereafter denoted E_P^*.

2.5. Modeling of productivity in a light-limited photobioreactor

Our modeling approach was based on that previously described in Loubière et al. (2009) except that, in the present work, we refer to a planar photobioreactor with a 60 mm optical path length, rather than a tubular photobioreactor. The two-flux formulation of the general theory of radiative transfer was used to compute the irradiance field inside the photobioreactor, according to Eqs. (4) and (5):

$$I(z) = 2\cdot I_0(1+\alpha)\cdot\exp(\delta\cdot(L-z))-(1-\alpha)\cdot\exp(-\delta\cdot(L-z))/ (1+\alpha)^2\cdot\exp(\delta\cdot L)-(1-\alpha)^2\cdot\exp(-\delta\cdot L) \tag{4}$$

with

$$\alpha = \sqrt{E_a/(E_a+2\cdot b\cdot E_s)}, \delta = X\cdot\sqrt{E_a\cdot(E_a+2\cdot b\cdot E_s)} \tag{5}$$

where E_a, E_s and b are T-Iso mass absorption and light scattering coefficients, b the backward scattering fraction, L the optical path length in the photobioreactor, z the depth along the light direction inside the photobioreactor and I_0 the irradiance at the surface of the photobioreactor. The optical properties for T-Iso were taken as in Loubière et al. (2009), with E_a = 293 m^2 kg^{-1}, E_s = 757 m^2 kg^{-1} and b = 0.01162.

The irradiance field, i.e. the distribution of irradiance inside the photobioreactor, was computed along the optical path length and, together with the relationship of growth rate with irradiance, this allowed the local growth rate to be determined for all the positions used for the irradiance field computation. The growth rate was then integrated along the optical path length of the photobioreactor in order to compute the mean specific growth rate that is used to solve Eq. (6):

$$\dot{X} = (\bar{\mu}-D)*X \tag{6}$$

where $\bar{\mu}$ is the mean specific growth rate (d^{-1}), D is dilution rate (d^{-1}), X is biomass (g.L^{-1}).

The ordinary differential equation is solved by Matlab ODE function with input parameters previously calculated by user m-files Matlab functions. Finally, the photobioreactor productivity was computed at steady state for a set of irradiance and dilution rates.