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CULTIVATION OF AMPHIDINIUM CARTERAE IN A BUBBLE COLUMN PHOTOBIOREACTOR: ENERGY DISSIPATION RATE CHARACTERIZATION

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ABSTRACT

Amphidinium species belong to a genus of dinoflagellates that produce outstanding compounds with antitumor activity. However, the development of a stable producing system has not been conducted yet; perhaps because of the low biomass concentration and the possible difficulties in the scale-up, which are the result of shear stress limitations. The present work evaluates the energy dissipation rates generated in a one-litter bubble column photo bioreactor by using computational fluid dynamics for different superficial gas velocities. Energy dissipation rates were linked to the growth of Amphidinium carterae and this work shows that A. carterae can stand energy dissipation rates of 0.12 m2/s3 with a good growth rate and not visible shear damage. In addition, it was found that in cultivations without CO_2 additions is compulsory to use high superficial gas velocities in order to avoid pH increments out of the optimal one.

Keywords: amphidinium, CFD, energy dissipation rates.

INTRODUCTION

Marine dinoflagellates of the species Amphidinium are widespread species found in temperate and tropical marine water, both free-living and in endosymbiotic states. They include approximately 20 known species that produce a variety of cytotoxic macrolides (amphidinolides, carbenolide I, etc.) and/or long-chain polyketides (colopsinols, luteophanols, amphidinols, etc.) (Kobayashi and Tsuda 2004). Amphidinolide N, as well as caribenolide I, exhibit remarkable potent cytotoxicity against human tumor cell lines. Caribenolide I shows in vivo antitumor activity against the P388 mouse leukaemia cell line (T:C 150 at a dose of 0.03 mg/kg) and IC 50 of 0.001 µg/mL in vitro against the human colon carcinoma cell line HCT116 (Bauer et al. 1995). Another high value product produced by Amphidinium sp is peridinin-chlorophyll-protein (PCP) that is a light-harvesting carotenoid commonly used in immunoassays, such as fluorescence-activated cell sorting (FACS) and flow cytometry (Haxo et al. 1976).

Other dinoflagellates with important compounds are Prorocentrum lima, Protoceratium reticulatum and Alexandrium minutum producing okadic acid, yessotoxins and saxitoxins, respectively. Okadic acid is already used in tumor studies and saxitoxins have been proved as analgesic (Parker et al. 2002). However, the production of these biomolecules have proven to be extremely difficult due to slow growth rates and low cell densities achievable when scaled up. Consequently, extremely large volumes of culture broth are needed to produce small amounts of biocompounds (Beuzenberg et al. 2012). Consequently, evaluations of mode-of-action and pharmacological potential of these new compounds have not yet been attainable because of insufficient quantities for pre-clinical trials and lack of knowledge in the cultivation of these organisms on a large scale. (García Camacho *et al.* 2007) Culture of microalgae in a photo bioreactor is typically carried out at a relative high cell density as this maximizes the biomass productivity and ensures efficient use of the available light and CO2 source (Torzillo *et al.* 2010). High cell density requires a turbulence level that assures a proper mixing level. However, turbulence is known to affect the growth rate and morphology of dinoflagellates and consequently, thus being one of the major obstacles in cultures of dinoflagellates in conventional photobioreactors (García Camacho et al. 2007). The energy dissipation rate (EDR) is a variable that is used to quantify the turbulence in a vessel. Dinoflagellates can tolerate only low EDR compared to the majority of green algae. Moreover, there is evidence that being the cause of cell damage, the hydrodynamic shear stress mediates the production of reactive oxygen species (ROS) and lipid oxidation within cells (García Camacho et al. 2007). For example the shear sensitivity Protoceratium reticulatum shows agitation-associated damage threshold lower than 0.0087 m2/s3 (equivalent shear stress 0.16mN/m2) (Camacho et al. 2011). However, no details about the shear stress tolerance of Amphidinium species are available.

Scale-up of photobioreactors is complicated because of CO2 supply and the non-homogenous distribution of light within the culture, which is a consequence of the self-shading by cells (Olivieri et al. 2013). This problem can be overcome by increasing the intensity of agitation for physically robust microalgae (García Camacho et al. 2007). This strategy does not seem to be feasible for highly fragile dinoflagellates because of bulk average EDR, either in bubble columns or in stirred tanks, vary commonly within the range 0.1 to 0.4 m2/s3. Furthermore, local EDR are higher than the aforementioned ones by several magnitude orders (e.g. behind a rupturing bubble, in the vicinity of impellers, etc.) (García Camacho *et al*. 2007). It is important to highlight that EDR in bubble columns mainly depends of the superficial gas velocities (SGVs) and the reactor configuration (Lobatón, Suárez, and Molina 2011).

Therefore, photobioreactor design and scale-up methods having been proved successfully for many relatively robust microalgae cannot be directly translated to the culture of the fragile dinoflagellates.

MATERIALS AND METHODS

Computational model for the estimation of energy dissipation rates

A bubble column with 0.05 m diameter and 0.675 m height and clear liquid height of 0.45 m was simulated using an Eulerian model included in the ANSYS software (Student v.19.2). The model and all simulation conditions were validated against the experimental mixing time and gas hold-up (Lobatón, Suárez, and Molina 2011). As air was blown into the column through a sparger, the superficial gas velocity (SGV) was varied in the range of 0.004 to 0.011 m/s. This fluid dynamics information and particularly the EDR was linked to the Amphidinium carterae growth.

Amphidinium carterae cultures

Non-axenic A. carterae SAG 38.01 were obtained from the Culture Collection of Algae at Göttingen University. Cultures were started in 250 mL Erlenmeyer flasks containing 80 mL of medium and 20 mL of culture. The cultures were maintained during seven days at 70 µmol/m2×s of irradiance and a temperature of 25 ± 1 °C.

Experimental setting

Four bubble column photobioreactors with the same dimensions already mentioned in the computational model were filled with 800 mL of medium and 100 mL of culture. The photobioreactors were running in parallel with one as control (static culture) and the others three aerated with just air, controlled by sparger. The volumetric flows were 25 L/h, 55 L/h and 80 L/h, respectively, that represent superficial gas velocities (SGV) of 0.004 m/s, 0.007 m/s and 0.011 m/s, respectively (Figure-1). In the control, 25 L/h of air passed through the bubble column head space in order not to disturb the flow.

Temperature and pH were measured online by the control unit (Sixfors) and transmitted to a computer, where data were recorded. The light conditions (100 µmol/m2×s), medium f/2 and temperature 25 °C were the same in the four photobioreactors. Growth was determined using cell counts. Thus, 1 mL samples of the broth were collected daily, fixed with Lugol solution, and the cells were counted in a Fuchs-Rosennthal chamber. The cell yields were calculated using the following equation. Cf: final cell density, Co: initial cell density and t is time in days.

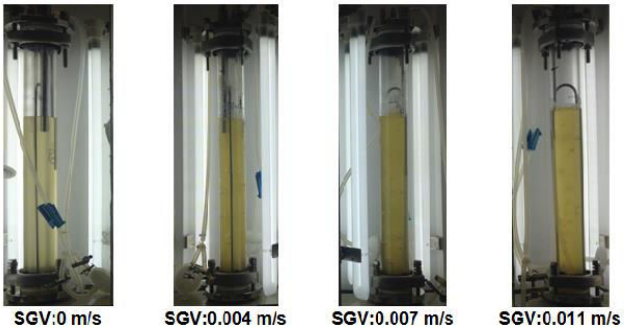

Figure-1. Experimental set up. Light conditions (100 µmol/m^2×s), medium f/2 and temperature 25°.

RESULTS

Photo bioreactor energy dissipation rates

Figure-2 shows the average EDR and the liquid velocities for different SGVs. The average EDR has slightly increased from the starting point of 0.01 to 0.04 m^2/s^3 because of the increment of SGV to 0.008 m/s. Subsequently, the EDR shows a drastically rise to 0.12 m^2/s^3 for SGV of 0.011 m/s. From the least SGV (0.004 m/s) until the maximum (0.011 m/s), the average liquid velocities ranged from 0.05 to 0.15 m/s. Although the EDR shows a big rise after the increase of the SGVs from 0.008 to 0.011 m/s, the average liquid velocities do not change dramatically.

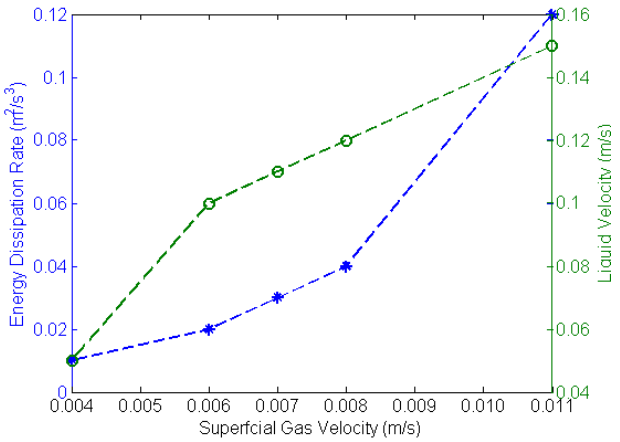

Figure-2. Average EDR and the liquid velocities for different SGVs.

Figure-3 displays the local EDR in a front as well as a top view for two different photobioreactor heights. The SGV in Figures 3 (a) and (b) were 0.008 m/s and 0.011 m/s, respectively. Figure-3 shows that the bulk of the reactor had an EDR (<0.08 m^2/s^3) and a small zone with EDR values that range from 0.08 to 0.4 m^2/s^3. In other words, the higher EDR seems to be associated with the bubble plume. The width of the bubble plume zone was enlarged as well as the range of EDR from 0.32 to 0.4 m^2/s^3 (Figure-3), with the increase of the SGV from 0.008 to 0.011m/s.

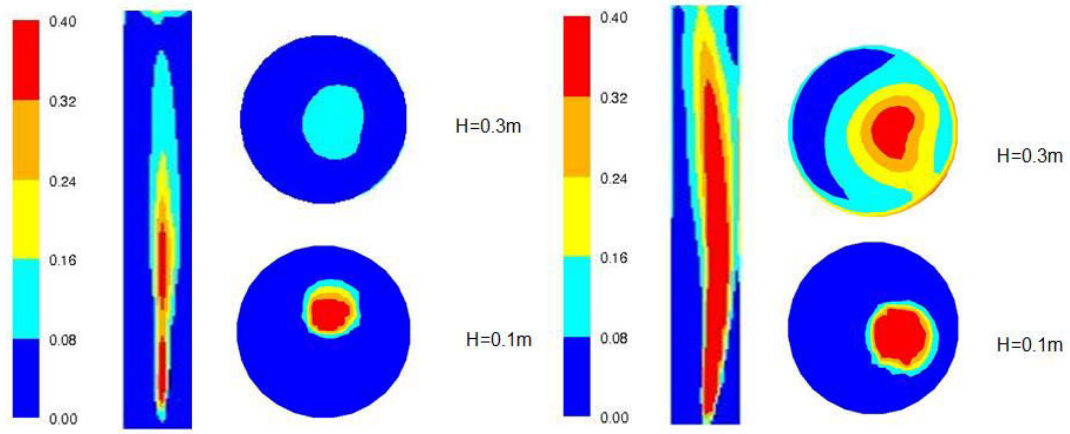

Figure-3. Local energy dissipation rate (m^2/s^3) for a superficial gas velocity of 0.008 m/s; (a) Local energy dissipation rate (m^2/s^3) for a superficial gas velocity of 0.011m/s; time step 100 s (b)

Amphidinium carterae growth

Figure-4 (a) shows an increase of growth and final cell density of A. carterae with the rise of the superficial gas velocities whereas Figure-5 shows the increase of the cell yield on A. carterae with the increase of the average EDR. Although A. carterae is a shear sensitive microalga, this research states that it can tolerate values of energy dissipation rates up to 0.12 m^2/s^3 which corresponds with a SGV of 0.011 m/s without any lethal effects. However, the inhibition due to the turbulence seems to start already at a value 0.01 m^2/s^3 since there was a twofold reduction in cell yield compared with the static cultivation in 100 mL Erlenmeyer flasks ($7e^4$ cell/ml×day). Yeung *et al.* found that the growth inhibition in dinoflagellates by turbulence can be due to a transient cell cycle arrest, while not irreparably altering the cell metabolism (Yeung and Wong, 2003). In addition, figure 3 shows that there are two zones with different EDR values, one that range from 0 to 0.08 m^2/s^3 and the bubble plume zone with higher values. Therefore, microalgae are exposed to an intermittent EDR due to the transition between these zones. Since Molina et al. have found better growth of Protoceratium reticulatum in intermittent EDR exposure *(Camacho et al.,* 2011), this might be advantageous for A. carterae, too. To sum up, at a values of EDR of 0.12 m^2/s^3 in cultivations of A. carterae, the biochemical process in the cells seems to be affected by turbulence but without any serious damage, whearas mass transfer and available light are of higher importance.

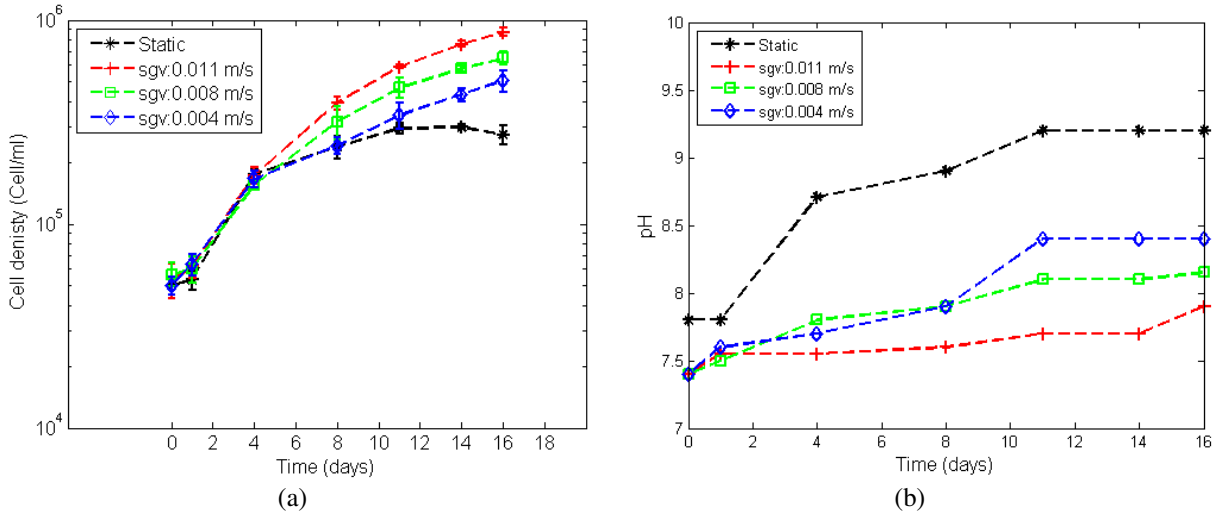

Figure-4. A. carterae cell density for different superficial gas velocities (a) pH changes during the cultivation at different SGVs (b).

Figure-4(b) shows that after day 6, the growth curve starts to present differences in the cell densities as result of the SGV increase. This could mean that the algae require either more carbon or light, so that the mixing process would start to control the growth of A. carterae. In Figure-4(a). It becomes obvious that pH increased during the cultivation but this was less pronounced with the SGVs rise. This increase in pH can be related with the CO_2

diminution; however, the SGV increase raises the gas hold-up and consequently, the mass transfer rate, which generates more CO_2 available to the algae.

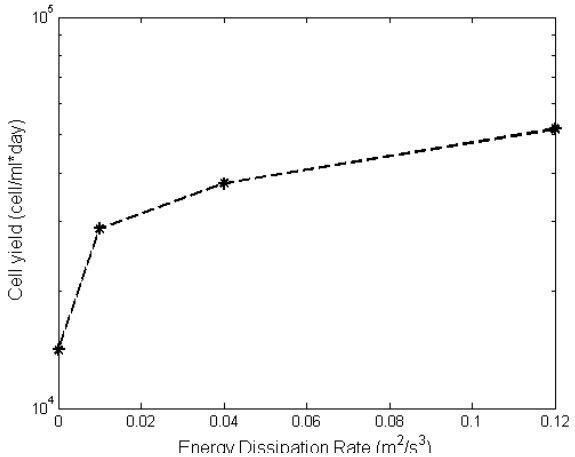

Figure 5. Energy dissipation rate against A. carterae cell yield.

Some authors have concluded that dinoflagellates have the same chlorophyll A activity but higher carbon/chlorophyll a ratio than diatoms. This idea is supported by the fact that dinoflagellates have an organic cell wall, which represents more than 5 to 12% of the dry weight in contrast to diatoms, which have a siliceous wall (Guillard and Keller, 1984). Previous experiments made with the diatom Chlamydomonas reinhardtii at different SGVs with a mix of air and 3% CO_2 did not show significant growth differences. So, the CO_2 in the C. reinhardtii cultivation was sufficient to support the growth, contrary to what had happened in the A. carterae cultivations.

Moreover, a recent report (Dason and Colman, 2004) suggests that A. carterae has an unusual poor internal pH control and consequently, it shows poor growth when the external pH departs from its "optimal" pH of 8. Thus A. carterae may have stopped growing because the pH dropped from 8 to 7, as a result of the increase of the CO_2 conditions from 0.035% to 3%. When the external pH was reduced from 8 to 7, the internal pH of A. carterae dropped from 7.92 to 7.04. Different ways to buffer the medium were used without positive results (Dason and Colman, 2004). However, the same author had reported that a normal pH 8 for A. carterae is CO_2 limited because its CO_2 uptake rate is faster than the uptake rate for HCO_3^-, but that the CO_2 concentration is lower than that of HCO_3^-. In addition, the author proved the lack of external carbonic anhydrase (CA) in A. Carterae, which may have limited the conversion of the species from HCO_3^- to CO_2^- and its subsequent faster transport (Dason, Emma Huertas and Colman, 2004). These findings support the idea that this system may be carbon-limited and that increasing the SGV may raise the available CO_2 in the medium and therefore, may support a better growth.

When light-limitation starts as a result of increasing cell density, higher liquid velocities can help the microalgae moving through the light gradients. Many authors have proved that increases in the fluid velocities facilitate a faster transition between the dark and the light zone. Therefore, better light usage can be another reason for the increase in cell yield, as a result of the SGV increase.

To sum up, the increase in SGV displays good mass transfer and light availability but also high average and local EDR that can induce some changes of biochemical processes in the cell. (Fan *et al.*, 2008)found that the performance of Chlorella vulgaris cultivation was improved by using a membrane-sparged gas supply due to larger values of the overall mass transfer coefficient compared to other photobioreactor configurations. The usage of horizontal internals in bubble columns has been reported as a possibility to increase the overall mass transfer rate, also as a consequence of a specific augmentation of the interfacialarea. These options could be possibilities to improve the A. carterae cultivation without increasing the SGV. In case that a SGV reduction is not possible, cell protection e.g. via cell encapsulation can be another alternative. The use of a higher CO_2 concentration than present in the air can also enhance the cultivation of A. carterae but attention must be taken not to decrease pH from its normal value. Besides, the deoxygenation so as to prevent a possible inhibition by oxygen has to be also studied.

CONCLUSIONS

The EDR value 0.12 m^2/s^3 generated the highest growth rate. This phenomenon probably was caused by equilibrium in shear damage, mass transfer and light limitations. Since the inhibition by turbulence in Amphidinium Carterae seems to be overtaken by other phenomena like carbon limitations or oxygen inhibition under the conditions in this research. This idea is support in the fact that the increased of the SGV supported a better growth. However, the combination of a low EDR value (<0.01 m^2/s^3) and a high overall mass transfer coefficient are suggested as the main criterion in the successful design and scale-up of photobioreactors in order to cultivate this promising dinoflagellate.. In addition, this approach can be used to study other promising dinoflagellates. The advantage of regarding the combination of CFD and photobioreactor cultivation in the study of shear stress tolerance with dinoflagellates is that other phenomena like mass transfer and light limitations can be taken into account in the global growth analysis

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