Photobioreactor scale-up for a shear-sensitive dinoflagellate microalga

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Abstract

Large scale culture of marine dinoflagellate microalgae has proved difficult owing to their extreme sensitivity to hydrodynamic forces in photobioreactors. This work discusses the scale up of Protoceratium reticulatum dinoflagellate culture from a successful 2 L stirred-tank photobioreactor operation to a 15 L stirred photobioreactor. Both bioreactors were equipped with internal spin filters for cell retention. A semicontinuous perfusion culture in the 15 L photobioreactor proved to be more productive than fed-batch perfusion culture. Under the best operational conditions, the average cell productivity in the 15 L photobioreactor was 5228 cell mL\(^{-1}\) day\(^{-1}\), or nearly 3.7-fold greater than the best attainable value in static flask cultures, but similar to the results obtained in the 2 L stirred tank. At 9.16 \(\mu\)g L\(^{-1}\) day\(^{-1}\) the average volumetric productivity of yessotoxin in the scaled up operation in the semicontinuous perfusion mode was comparable to the results obtained in the small bioreactor. Protoceratium reticulatum has a cell damage threshold shear rate of as low as 0.1 s\(^{-1}\). Notwithstanding this extreme shear sensitivity, the results suggest a good potential for mass scale culture of this dinoflagellate in suitably designed photobioreactors.

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1. Introduction

Dinoflagellates are one of the several divisions of microalgae. Dinoflagellates occur both in freshwater and in the seas. Although microalgae have an established history of commercial use and new uses are emerging [1], this is not the case for dinoflagellates. Dinoflagellates produce many structurally complex bioactive compounds of potential commercial interest [2], but development of marketable products from them has proved difficult. Small quantities of bioactives derived from dinoflagellates can be purchased, but generally at a prohibitively high price and in low purity. Unlike the other microalgae that are commonly grown in large-scale photobioreactors [3–6], many dinoflagellates appear to be overly sensitive to the myriad of hydrodynamic forces that are encountered in the turbulent environment of a typical photobioreactor.

Photosynthetic marine dinoflagellates respond to hydrodynamic shear forces in diverse ways [7]. Turbulence sensitivity has been studied for several dinoflagellate species, but invariably from an ecological perspective that may be relevant in the natural habitat, but not in photobioreactor culture. Small scale studies that are potentially relevant to photobioreactor culture have been reported using the highly sensitive dinoflagellate Protoceratium reticulatum. This microorganism is damaged by shear rate values as low as 0.1 s\(^{-1}\) [8]. It is therefore a good model system for studies of photobioreactors that are intended for culturing highly shear sensitive microalgae. At operational scales of 2 L or less, the use of modified turbulence regimes and protective additives in the culture medium have been shown to reduce turbulence-associated damage to Protoceratium reticulatum [8–10]. How this successful operational capability at small scale might translate to a larger photobioreactor has not been previously addressed for dinoflagellates.

Scale-up of photobioreactors is complicated by a nonhomogeneous distribution of light within the culture as a consequence of the self-shading by cells. For microalgae that are physically robust, increased intensity of agitation can be used to limit the time that a cell spends uninterrupted in the relatively dark interior of the bioreactor. This strategy is not workable with highly fragile dinoflagellates. Therefore, photobioreactor design and scale up methods that have proved successful for many relatively robust microalgae [3] cannot be directly translated to culturing the fragile dinoflagellates. This work reports on scale up of Protoceratium reticulatum culture from a previously reported 2 L stirred-tank photobioreactor [7,9] to a 15 L photobioreactor.

2. Materials and methods

2.1. Species and culture medium

Nonaxenic monocultures of the red-tide dinoflagellate Protoceratium reticulatum (CG14M) were used. This yessotoxins (YTXs)-producer was obtained from the Culture Collection of Harmful Microalgae of IEO, Vigo, Spain. Inocula were grown in filter sterilized (0.22 \(\mu\)m Millipore filter) L1 medium prepared in Mediterranean
Sea water [11]. The alga was grown at 18 ± 1 °C under a 12:12 h light–dark cycle. During the photoperiod, the average irradiance at the surface of the cultures was 100 µEm−2 s−1. Four overhead Philips TLD 36W/54 fluorescent lamps were used for illumination. Inocula were prepared in surface aerated 2 L Erlenmeyer flasks.

2.2. Photobioreactors

Optimal production of P. reticulatum biomass in a 2 L stirred tank photobioreactor (2L-STB) [Braun Biotech, Germany] has been previously reported [7,9]. The current study focussed on scaling up the previously reported successful process to 15 L in a stirred photobioreactor (15L-STB) equipped with an internal spinfilter for cell retention.

The smaller bioreactor (2L-STB) was non-baffled and had the following relevant dimensions: 0.13 m culture depth; 0.13 m vessel diameter; 0.055 m impeller diameter (a 3-bladed marine propeller; 20°–blade pitch; 0.013 m blade width; 0.055 m blade length); and 0.02 m clearance of the impeller from the bottom of the vessel. An internal nylon (10 µm mesh) spinfilter (diameter = 5.5 cm; height = 11.5 cm) was installed on the impeller shaft for perfusion culture runs. The temperature, pH and dissolved oxygen (DO) concentration were measured online (Biostat B-DCU control unit; Braun Biotech, Germany) and logged by a computer. The culture temperature was controlled at 18 ± 1 °C by a thermostated glass jacket that surrounded the bioreactor vessel.

The larger 15 L stirred photobioreactor (15L-STB) was custom built to a design that was based on experience with the 2 L bioreactor. The 15 L bioreactor vessel was made of 5 mm thick borosilicate glass and had an internal diameter of 0.193 m. The aspect ratio of the vessel was 4.5. The internal spinfilter was made of the same material as described above for the small reactor. The spinfilter had a diameter of 7 cm and a height of 22 cm. The gas-free liquid height was about 0.61 m. The working volume was 15 L. A 3-bladed marine propeller was used for mixing. The propeller was located 0.05 m above the bottom of the photobioreactor vessel. As direct sparging with air damages P. reticulatum [8], a perforated pipe air sparger was located within the spinfilter to prevent direct contact between the algal cells and air bubbles. The air flow rate was 4.5 L min−1, or 0.3, v/v min. The complete geometric details of the culture vessel appear in Fig. 1.

The large photobioreactor was illuminated with up to 5 Phillips TLD 36W/54 fluorescent lamps placed around its periphery. The incident irradiance at the surface of the photobioreactor and in the center of the vessel was measured using a QSL-100 quantum scalar irradiance sensor (Biospherical Instruments, San Diego, USA). The incident irradiance (photosynthetically active) ranged from 242 to 766 µEm−2 s−1. During culture, the light level was progressively increased to prevent limitation of growth as a consequence of the increasing cell concentration. The dissolved oxygen concentration (DO), measured as a percentage of the oxygen concentration in air-saturated seawater, was monitored continuously as an indicator of the photosynthetic activity. The monitoring electrode was calibrated before each run. The culture temperature was controlled at 19 ± 1 °C by circulating thermostated water through the glass jacket that surrounded a portion of the photobioreactor vessel. The pH was controlled at pH 8.4 by automatic injection of carbon dioxide within the spinfilter, as needed. The sparger used for injecting carbon dioxide was separate from the earlier mentioned air sparger. This prevented mixing of CO2 with the air and enhanced the mass transfer of CO2.

Prior to each experimental run, the photobioreactor was thoroughly cleaned and any salt deposits were removed by an acid wash. For this, the bioreactor vessel was filled with tap water and concentrated hydrochloric acid (40 mL, 35%, w/w HCl) was added. The vessel was then sparged with air for at least 2 h. The bioreactor was then washed with tap water. The photobioreactor and associated pipework were sterilized by circulating filtered seawater (0.02 µm) mixed with 10 mL of sodium hypochlorite (10% solution) for 2 h. The bioreactor was then rinsed with filter sterilized seawater until the pH of the rinse became pH 8.6. The reactor was then filled with the L1 medium made in filter sterilized Mediterranean seawater. The medium (15 L) was inoculated using 0.003 m3 of an inoculum that was in the late exponential growth phase. The cell concentration in the freshly inoculated bioreactor was about 15,000 cells mL−1.

The photobioreactor was operated as a perfusion culture (i.e. the cells remained in the bioreactor) in the fed-batch and semi-continuous modes in different experiments. In semi-continuous experiments, the volume of the culture medium removed was replenished with an equal volume of the fresh medium. When cell growth ceased, small volumes of highly concentrated aliquots of the L1 medium were added in order to increase the nutrient concentration in the culture medium to higher than the values in the normal L1 medium. The culture setup is shown in Fig. 2.

2.3. Hydrodynamic characterization of photobioreactors

The acid tracer method [12] was used to measure the mixing time (τm), a direct measure of the mixing capability of a reactor. Mixing time was defined as the time required to attain a 5% deviation from complete homogeneity from the instance of tracer addition. For tracer response measurements, the reactor was filled with seawater and the pH was reduced to 2 by adding hydrochloric acid (35%, w/v). The vessel was then bubbled with air for 20 min to remove any dissolved carbonates in the form of carbon dioxide. The stirrer speed was then set and the pH was raised to pH 4.5 by adding 12 M sodium hydroxide. The acid tracer (2 mL of 35% hydrochloric acid) was injected into the bioreactor, the pH of the vessel was monitored continuously, and the mixing time was calculated as the time required to attain a 95% deviation from complete homogeneity from the instance of tracer addition.
Fig. 2. Flow setup for the scaled-up photobioreactor: (1) seawater reservoir; (2) medium tank; (3) air compressor; (4) chiller for cooling water; (5) carbon dioxide cylinder; (6) water filters; (7) gas filters; (8) flow meter; (9) ultraviolet lamp; (10) dissolved oxygen electrode; (11) pH electrode; (12) temperature probe; (13) pH indicator and controller; (14) dissolved oxygen indicator; (15) data acquisition card; (16) carbon dioxide mass flow meter; (17) stirrer motor; (18) spinfilter.

Fig. 3. Average shear rate \( \gamma_p \) in the small bioreactor (2L-STB) versus the impeller rotational speed \( N_R \).
tor 2L-STBs. The value of $k_i$ for the impeller used was therefore 1.71. For different combinations of stirred tanks and axial-type impellers, the $k_i$ has been previously shown to depend on the Flow number ($N_R$) [21]. Using the experimentally determined value of $k_i$, a $N_R$ value of 0.24 could be read from the published correlation (Fig. 4 of ref. 21) [21].

2.4. Kinetic parameters

Growth was determined by cell counts. Broth samples (1 mL) were collected daily, fixed with Lugol’s solution, and the cells were counted on a Sedgewick–Rafter counting slide. The cell-specific growth rate $μ$ (days$^{-1}$) was calculated using the following equation:

$$μ = \frac{ln N_{t_i} - ln N_{t_{i+1}}}{t_{i+1} - t_i}$$  \hspace{1cm} (10)

where $N_{t_i}$ and $N_i$ are the cell concentrations (cells mL$^{-1}$) at times $t_{i+1}$ and $t_i$ (days), respectively.

2.5. Flow cytometric measurements

Flow cytometry was used for measuring the relative mean cell size and the concentration of reactive oxygen species (ROS) in the cells [15]. All flow cytometric measurements were carried out in a Coulter Epics XL-MCL (Beckman Coulter, Inc.) flow cytometer as described previously [15].

2.6. Determination of YTXs concentration

Yessotoxins (YTXs) were measured separately in the biomass as well as in the culture supernatant following the method of Paz et al. [22]. A HPLC system (Shimadzu AV10; Shimadzu Corporation, Kyoto, Japan) with a fluorescence detector (Shimadzu RF-10AX) and an autosampler (Shimadzu SIL-10ADVP) was used.

3. Results and discussion

Scale-up of mechanically mixed photobioreactors for culturing dinoflagellates was the focus of this work. The scale up study was informed by prior experience of a successful culture in a 2 L photobioreactor, the 2L-STB [7,9], and the experimentally established limits on shear stress tolerance of $P$. reticulatum in shake flasks [8,15]. First, the fluid-dynamics in the 2L-STB were characterized under different operational regimes that had previously proved successful for $P$. reticulatum culture. This informed the design of the larger bioreactor 15L-STB and selection of the suitable operational regimes for evaluating the dinoflagellate culture in it.

3.1. Fluid-dynamics in 2L-STB

The 2L stirred bioreactor (2L-STB) used an axial flow impeller and a baffles-free design because such reactors have been successfully operated at quite large scales for growing fragile animal cells in suspension [17]. In bioreactors for animal cells, a relatively large pitched-bladed impeller is used as it can provide good mixing at a relatively low agitation speed. In keeping with this strategy, the impeller used was relatively large compared to the tank diameter, i.e. $T/D$ ratio was 2.36 instead of a value of 3 that is typically used in stirred bioreactors intended for culturing microorganisms.

Mixing time in 2L-STB was characterized as an important index of the ability to attain homogeneity in the culture. The mixing time values are shown in Fig. 4 for various rotational speeds of the impeller. Consistent with a characteristic pattern, mixing improved rapidly, i.e. the mixing time declined, as the impeller speed increased up to a value of around 200 rpm. This was the transitional flow regime in which the bulk flow produced by the impeller had a significant influence on mixing time. Once the agitation speed exceeded about 200 rpm, the mixing time was barely affected by impeller speed (Fig. 4). This was the turbulent flow regime in which the eddies in the fluid had a greater influence on the mixing characteristics compared to the bulk flow produced by the impeller. In the transitional flow regime, the installation of the spinfilter actually improved the mixing somewhat compared to operation without the spinfilter. This was due to turbulence produced by the spinfilter which rotated at the same speed as the impeller. In the turbulent regime, the spinfilter barely affected the mixing, as the turbulent eddies within the fluid were largely responsible for the observed mixing effect.

The average energy dissipation rate $ε_p$ (Eq. (2)) and the maximum energy dissipation rate $ε_{max}$ (Eq. (5)) are shown in Fig. 5 for various values of the impeller speed. The ratio $ε_{max}/ε_p$ is also shown (Fig. 5) as an indicator of homogeneity of turbulence. The maximum energy dissipation rate, a measure of energy dissipation in the vicinity of the impeller, increased linearly with increasing agitation rate as expected. In contrast, $ε_{max}/ε_p$ increased exponentially with increasing agitation speed of the impeller (Fig. 5). This implied an increased spatial nonuniformity in the intensity of turbulence in the bioreactor. Notwithstanding this increased heterogeneity of turbulence, at the highest impeller speed, the $ε_{max}/ε_p$ value was well below 80. For comparison, $ε_{max}/ε_p$ values of nearly 100 are encountered in highly agitated bioreactors [23]. The presence of spinfilter considerably increased $ε_p$ in the transitional flow region ($2500 ≤ Re ≤ 10,000$), to reduce the heterogeneity of turbulence (see Fig. 5).

Fig. 4. Mixing time ($θ_p$) in the small bioreactor (2L-STB) versus the rotational speed ($N_R$) and the Reynolds number ($Re$) of the impeller. Data are shown with and without the spinfilter installed.

Fig. 5. Effects of impeller rotational speed ($N_R$) on the average energy dissipation rate ($ε_p$), the theoretical maximum energy dissipation rate ($ε_{max}$) and the homogeneity of turbulence (i.e. $ε_p/ε_{max}$). Data are for 2L-STB with and without the spinfilter.
calculated using the maximum energy dissipation rate \( \varepsilon_{\text{max}} \). The value of \( \varepsilon_{\text{max}} \) was 120 mW kg\(^{-1}\) at this speed the value of the maximum energy dissipation rate was found to damage \( P. \) reticulatum measured for \( P. \) reticulatum corresponded to an average energy dissipation rate of approximately 8.8 mW kg\(^{-1}\) [8]. In the 2L-STB this level of average energy dissipation occurred at an impeller speed of 100 rpm (Fig. 5), but at this speed the value of the maximum energy dissipation rate was 120 mW kg\(^{-1}\) (Fig. 5), or 14-fold greater than the damaging threshold in the shake flasks. Furthermore, at an agitation speed of 100 rpm, the spintfilter also dissipated energy at an estimated level of 48 mW kg\(^{-1}\), that was substantially above the damaging threshold in shake flasks [8].

In shake flasks, the shear damage threshold for \( P. \) reticulatum corresponded to an average energy dissipation rate of approximately 8.8 mW kg\(^{-1}\) [8]. In the 2L-STB this level of average energy dissipation occurred at an impeller speed of 100 rpm (Fig. 5), but at this speed the value of the maximum energy dissipation rate was 120 mW kg\(^{-1}\) (Fig. 5), or 14-fold greater than the damaging threshold in the shake flasks. Furthermore, at an agitation speed of 100 rpm, the spintfilter also dissipated energy at an estimated level of 48 mW kg\(^{-1}\), that was substantially above the damaging threshold in shake flasks [8].

The shear stress at the surface of a spintfilter could be conservatively estimated using the analysis of Bird et al. [24] for a Stormer viscometer. In a Stormer viscometer an inner cylinder of dimensionless radius \( f \) rotates with an angular speed in rads\(^{-1}\) \( (N_{\text{visc}}) \) inside a stationary outer cylinder of radius \( R \). At the surface of the inner cylinder the shear stress \( \tau_i \) can be estimated as follows:

\[
\tau_i = 2 \mu_i N_{\text{visc}} \left( \frac{1}{1-f^2} \right) \tag{11}
\]

For the bioreactor 2L-STB, the diameters of the spintfilter and tank were 5.5 and 13 cm, respectively; therefore the \( f \)-value was 0.42.

The shear stress at the surface of the spintfilter varied with the impeller agitation speed and the Reynolds number, as shown in Fig. 7. Clearly, the shear stress at the surface (Fig. 7) was much greater than the damaging shear stress threshold reported [8], but this was fully tolerated by the cells at least up to a rotational speed of 50 rpm [9]. This apparent discrepancy was explained by the cells having been kept out of the boundary layer of the spintfilter by the local hydrodynamic forces, as reported by others. For example, Figueredo-Cardero et al. [25] showed that the concentration of the cells near the surface of a rotating cylindrical filter mesh was low compared to the concentration in the bulk fluid. Therefore, for all practical purposes, the cells did not experience the shear stress associated with the spintfilter. As observed with certain cells [26], dinoflagellate cells could potentially be made to adapt to higher levels of shear stress than they would normally tolerate.

Based on the above considerations, a conservative impeller/spintfilter rotation speed value of 50 rpm appeared to be acceptable for a successful culture of \( P. \) reticulatum in the 2L-STB equipped with a spintfilter [7,9]. At this value of the rotation speed, the average energy dissipation (3.0 mW kg\(^{-1}\) including the spintfilter) was far below the damaging threshold and the maximum energy dissipation value was 14.9 mW kg\(^{-1}\), or a little higher than the damaging threshold.

### 3.3. Scale-up considerations

Two scaling-up criteria were considered in designing the 15 L stirred photobioreactor (15L-STB). The first criterion related with the frequency of passage of the cells through the high-shear zone of the impeller. Frequency of the cell passage through the impeller zone is directly related to the circulation time \( (t_c) \) in a stirred reactor. Therefore, a possible basis for scale up of a shear sensitive culture is to attempt to keep the circulation times at the different scales the same. The circulation times and the volume flow rate generated by the impeller were calculated for the small bioreactor (2L-STB) and a hypothetical 15 L bioreactor using Eqs. (2)–(9). The calculated values are shown in Table 1. Two different impeller sizes were evaluated for the larger bioreactor. At the agitation rate of 50 rpm that had given good results in the small bioreactor, the circulation time was estimated to be 54 s (Table 1). For attaining approximately the same circulation time in the larger bioreactor at
Table 1

Circulation time ($t_R$) and impeller flow rate ($Q$) values in photobioreactors 2L-STB and 15L-STB for various combinations of the impeller diameter ($D$) and rotational speed ($N_R$).

<table>
<thead>
<tr>
<th>$N_R$ (rpm)</th>
<th>2L-STB</th>
<th>15L-STB</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D = 5.5 \text{ cm}$</td>
<td>$D = 8.1 \text{ cm}$</td>
<td>$D = 11 \text{ cm}$</td>
</tr>
<tr>
<td>$Q (\text{L s}^{-1})$</td>
<td>$t_R (\text{s})$</td>
<td>$Q (\text{L s}^{-1})$</td>
</tr>
<tr>
<td>30</td>
<td>0.020</td>
<td>90</td>
</tr>
<tr>
<td>40</td>
<td>0.027</td>
<td>68</td>
</tr>
<tr>
<td>50</td>
<td>0.033</td>
<td>54</td>
</tr>
<tr>
<td>80</td>
<td>0.053</td>
<td>34</td>
</tr>
<tr>
<td>100</td>
<td>0.067</td>
<td>27</td>
</tr>
<tr>
<td>150</td>
<td>0.100</td>
<td>18</td>
</tr>
</tbody>
</table>

An agitation speed of 50 rpm, the reactor was predicted to require an impeller that had nearly twice the diameter (i.e. 11 cm) of the impeller used in the small bioreactor (Table 1).

The second scaling-up criterion frequently used with fragile cells consists of keeping the tip speed ($u$) of the impeller identical at the different scales [27]. This is because the tip speed is directly correlated with the maximum shear rate in a stirred bioreactor. For the small bioreactor (2L-STB) operated at an impeller rotational speed of 50 rpm, the tip speed was calculated to be 0.144 m s$^{-1}$. If this same tip speed was to be used in the larger bioreactor with an 11 cm diameter impeller, the rotational speed of the impeller would be too low for most motor-controller systems used in commercial stirred bioreactors. On the other hand, an 11 cm impeller rotating at 50 rpm would have a tip speed of 0.288 m s$^{-1}$ which is probably too high for $P$. reticulatum as it would produce a relatively high maximum energy dissipation ($\varepsilon_{\text{max}}$) value. Therefore, a decision was made to use a smaller impeller (diameter = 8.1 cm).

A further consideration in scale up was the need to minimize light limitations. Therefore, too large an increase in the diameter of the vessel as a consequence of scale up was unacceptable, as previously pointed out in relation to scale up of tubular photobioreactors [28]. In any vessel with a relatively large diameter, a high concentration of biomass, and the light being supplied solely through the outer surface, an optically dark zone would exist in the center. Turbulence is essential to prevent the cells from residing in the dark zone continuously for a long period. The size of the dark zone can be kept to a minimum by limiting the vessel diameter. Consequently, the diameter of the larger bioreactor was fixed at 20 cm and the volume was increased by increasing the aspect ratio 4.5-fold compared to that of the smaller bioreactor. In this taller bioreactor, the impeller-to-tank diameter ratio ($D/T$) was kept at nearly the same value as in the smaller bioreactor. This led to an impeller diameter of 8.14 cm in the larger vessel. An impeller of this size rotating at 50 rpm would have an acceptable tip speed of 0.2 m s$^{-1}$, but the circulation time would be nearly three times as long (Table 1) as in the small reactor. Of course, this higher circulation time would reduce the frequency of passage of the cells through the high-shear zone of the impeller. This was felt to be an acceptable compromise. Therefore, the larger bioreactor (15L-STB) was constructed with the dimensions shown in Fig. 1.

3.4. Evaluation of 15L-STB

Attempts were made to culture $P$. reticulatum in the large photobioreactor 15L-STB (Fig. 1) in a batch operation initially with an impeller speed of 75 rpm. Profiles of the cell concentration ($N$), dissolved oxygen (DO), the average cell diameter ($d_p$) and the reactive oxygen species (ROS) for this batch run are shown in Fig. 8a. The
cell concentration peaked 8 days after inoculation and declined rapidly afterwards. The peak cell concentration was low at around 25,000 cell mL\(^{-1}\). During the short initial growth phase, the average cell diameter increased sharply to attain a maximum value of 29.5 μm. On day 5, the average cell diameter began to decline quite suddenly while the cells were still growing. The dissolved oxygen level rose initially because of photosynthesis, but after day 1 the level continually declined even though the cells grew until day 9 (Fig. 8a). This suggests that oxygen removal by aeration generally exceeded oxygen production by photosynthesis.

The rapid increase in cell size immediately after inoculation (Fig. 8a) suggests oxidative cell damage triggered by the inhibitory shear forces as the primary cause of the eventual cell death. Increase in cell size on exposure to inhibitory shear forces has been previously documented for \textit{P. reticulatum} [15]. A declining concentration of dissolved oxygen despite an increasing cell number points to a progressive decline in the photosynthetic activity that too has been attributed to shear-induced oxidative damage [15]. Microscopic examination of the cells showed shrinkage of protoplast (Fig. 8b) as is associated with programmed cell death by oxidative stress [29,30].

In view of the poor results (Fig. 8a), a new batch run was conducted with a reduced impeller speed of 65 rpm. This reduced the damage to cells, but did not completely prevent it (data not shown). Therefore, the next run used an even lower impeller agitation speed of 50 rpm. This run began as a batch culture (Fig. 9) but was later switched to fed-batch operation and subsequently to a semicontinuous culture. In the batch phase, there was an initial increase in cell size, but oxygen continued to be produced (Fig. 9) well past the time at which peak production had occurred in the first batch run (Fig. 8a).

The exact operational strategy used in the fed-batch and semicontinuous phases of the run is summarized in Table 2. The specific instance of addition of the various nutrients to the culture are identified in Fig. 9 and further explained in Table 2.

Photosynthesis also produces reactive oxygen species (ROS). This explains the increasing level of ROS in an otherwise robustly growing population (Fig. 9). On day 16, ROS level reached nearly 90, the highest value observed for cultures of \textit{P. reticulatum}. To prevent possible cell damage as a consequence of an increasing level of ROS, ascorbic acid (AA) was occasionally added to the culture to obtain an AA concentration of 100 μM. Ascorbic acid is an scavenger of ROS and has previously proved useful in countering the harmful effects of ROS produced in response to damaging levels of shear stress [15]. The increased bubbling within the spinfilter up to the full aeration rate from day 35 onwards was also beneficial as it contributed to reducing the DO level. This suggests that an excessive accumulation of DO may be another cause of oxidative stress on cells.

The average specific growth rate (μ = 0.06 days\(^{-1}\)) was similar in both the semicontinuous operation and the fed-batch operation, but the biomass productivity was higher in the semicontinuous culture. This was because the semicontinuous operation attained a higher cell concentration (1.0–1.3 × 10\(^5\) cell mL\(^{-1}\)) (Fig. 9). The average cell productivity (5228 cell mL\(^{-1}\) day\(^{-1}\)) was nearly 3.7 fold greater than observed in static flasks but similar to the value attained in semicontinuous culture in the small bioreactor 2L-STB [7].

Production of yessotoxins (YTXs) by \textit{P. reticulatum} in the various culture modes is shown in Fig. 10. During the first 20 days of operation, the YTXs contents of the cells decreased from 2 pg cell\(^{-1}\) to approximately 1.2 pg cell\(^{-1}\), but remained fairly constant subsequently, i.e. during most of fed-batch and semicontinuous operation. This intracellular concentration of yessotoxins was only about 33% of the average level that has been reported for shake flask cultures [31]. This phenomenon was also observed in earlier studies with the small bioreactor 2L-STB and was attributed to the use of a nutrient-rich medium that does not generally promote the production of secondary metabolites such as yessotoxins.
The strategy used in the third attempt to culture *P. reticulatum* in the large bioreactor (15L-STB) with an impeller of 8.1 cm diameter rotated at 50 rpm. The specific instances of various additions to the culture are pointed out in Fig. 9.

Table 2

<table>
<thead>
<tr>
<th>Day</th>
<th>Culture mode</th>
<th>Incident irradiance (µE m⁻² s⁻¹)</th>
<th>Aeration mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fed batch</td>
<td>241.7</td>
<td>60% surface aeration in headspace; 40% bubbled in spinfilter</td>
</tr>
<tr>
<td>5</td>
<td>Fed-batch with perfusion (1 L/day of L1)</td>
<td>300</td>
<td>20% surface aeration in headspace; 80% bubbled in spinfilter</td>
</tr>
<tr>
<td>6</td>
<td>Fed-batch with perfusion (1 L/day)</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>17</td>
<td>Fed-batch with perfusion (1 L/day of L1 x 3)</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>23</td>
<td>Fed-batch with perfusion (1 L/day of L1 x 3)</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>28</td>
<td>Fed-batch with perfusion (1 L/day of L1 x 3)</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>33</td>
<td>Semicontinuous; removal of 15% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>35</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>36</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>44</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>50</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>55</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
<tr>
<td>63</td>
<td>Semicontinuous; removal of 10% of broth volume</td>
<td>766</td>
<td>100% bubbled in spinfilter</td>
</tr>
</tbody>
</table>

[7]. Nevertheless, the average volumetric productivity of yessotoxins was 9.16 µg L⁻¹ day⁻¹, or comparable to the results reported for the small bioreactor [7].

### 4. Concluding remarks

Principles for the successful scale-up of a stirred tank photobioreactor for growing the highly fragile dinoflagellate *P. reticulatum* were assessed. Successful scale-up from a 2 L operation to a 15 L photobioreactor was achieved by increasing the aspect ratio of the reactor to 4.5-fold of the value at the small scale. A relatively large axial flow impeller rotating at a slow speed was used for mixing. Scale up methods for photobioreactors remain largely undeveloped [32]. Although maintaining of geometric similarity is a widely used principle for scale up of stirred tanks, it cannot be directly translated to the case of stirred tank photobioreactors because an unacceptably large increase in culture depth would occur and this will adversely impact the productivity of a photosynthetic microorganism. In this study, the scaled up photobioreactor operated in the semicontinuous mode, had average volumetric productivities of the cells and yessotoxins that were comparable to the best results attained at the small scale.

### Acknowledgements

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### Appendix A. Nomenclature

- **A** \( \text{dimensionless constant in Eq. (2)} \)
- **C<sub>T</sub>** \( \text{dimensionless concentration of tracer} \)
- **D** \( \text{impeller diameter (m)} \)
- **DO** \( \text{dissolved oxygen as percentage of air saturated seawater} \) (%)
- **f<sub>r</sub>** \( \text{dimensionless radius of the spinfilter relative to the radius of the bioreactor vessel} \)
- \[ \text{[H<sup>+</sup>]**}\] \( \text{concentration of tracer} \)
- **h<sub>T</sub>** \( \text{characteristic length of the impeller (m)} \)
- **k<sub>i</sub>** \( \text{slope of linear relation in Fig. 3} \)
- **N** \( \text{cell concentration (cell mL⁻¹)} \)
- **N<sub>i</sub>** \( \text{cell concentration at time } t\text{; (cells mL⁻¹)} \)
- **N<sub>Q</sub>** \( \text{flow number} \)
- **N<sub>R</sub>** \( \text{impeller rotational speed (rpm or rev s⁻¹)} \)
- **Q** \( \text{impeller flow rate (m³ s⁻¹)} \)
- **r<sub>c</sub>** \( \text{radius of the forced vortex zone (m)} \)
- **r<sub>d</sub>** \( \text{radius of impeller (m)} \)
- **r<sub>T</sub>** \( \text{radius of bioreactor (m)} \)
- **L<sub>STB</sub>** \( \text{2L-STB 2 L stirred-tank photobioreactor} \)
- **L<sub>STB</sub>** \( \text{15L-STB 15 L stirred-tank photobioreactor} \)
- **t** \( \text{time of culture (days)} \)
- **T** \( \text{diameter of tank (m)} \)
- **u** \( \text{relative liquid velocity or impeller tip velocity (m s⁻¹)} \)
- **V<sub>L</sub>** \( \text{culture volume (m³)} \)
- **YTXs** \( \text{yessotoxins} \)
- **C<sub>max</sub>** \( \text{local maximum energy dissipation rate (W kg⁻¹)} \)
- **D<sub>p</sub>** \( \text{Averaged energy dissipation rate (W kg⁻¹)} \)
- **E<sub>1</sub>** \( \text{energy dissipation rate (W kg⁻¹)} \)
- **m<sub>mixing</sub>** \( \text{mixing time (s)} \)
- **μ** \( \text{cell-specific growth rate (days⁻¹)} \)
- **μ<sub>L</sub>** \( \text{broth viscosity (Pa s)} \)
- **ρ<sub>L</sub>** \( \text{broth density (kg m⁻³)} \)
- **τ<sub>i</sub>** \( \text{theoretical shear stress on spinfilter surface (mN m⁻²)} \)

### Greek symbols

- **ε<sub>p</sub>** \( \text{Local energy dissipation rate} \)
- **ε<sub>max</sub>** \( \text{Maximum energy dissipation rate} \)
- **θ<sub>m</sub>** \( \text{Particle mixing time} \)
- **λ** \( \text{Kolmogorov’s microscale (m)} \)
- **μ** \( \text{Broth viscosity (Pa s)} \)
- **ρ<sub>L</sub>** \( \text{Broth density (kg m⁻³)} \)
- **τ<sub>i</sub>** \( \text{Stress on spinfilter surface (mN m⁻²)} \)

### References


