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## Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*

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**Abstract** The effect of mechanical agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum* was investigated in aerated continuous cultures with and without the added shear protectant Pluronic F68. Damage to cells was quantified through a decrease in the steady state concentration of the biomass in the photobioreactor. For a given aeration rate, the steady state biomass concentration rose with increasing rate of mechanical agitation until an upper limit on agitation speed was reached. This maximum tolerable agitation speed depended on the microalgal species. Further increase in agitation speed caused a decline in the steady state concentration of the biomass. An impeller tip speed of  $>1.56 \text{ m s}^{-1}$  damaged *P. tricornutum* in aerated culture. In contrast, the damage threshold tip speed for *P. cruentum* was between 2.45 and  $2.89 \text{ m s}^{-1}$ . Mechanical agitation was not the direct cause of cell damage. Damage occurred because of the rupture of small gas bubbles at the surface of the culture, but mechanical agitation was instrumental in generating the bubbles that ultimately damaged the cells. Pluronic F68 protected the cells against damage and increased the steady state concentration of the biomass relative to operation without the additive. The protective effect of Pluronic was concentration-dependent over the concentration range of 0.01–0.10% w/v.

**Keywords** Microalgae · *Phaeodactylum tricornutum* · *Porphyridium cruentum* · Shear damage · Photobioreactors · Pluronic

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### Introduction

Microalgae and photosynthetic bacteria are potentially important as sources of novel high-value compounds [1–3], producers of biohydrogen [4] and other fuels [5], and agents for absorbing carbon dioxide from effluent gases and confined atmospheres such as that of a spacecraft. Making use of these photosynthetic cell factories typically requires their controlled culture in photobioreactors.

Tubular photobioreactors with shallow light paths have been used commonly for photosynthetic cultures [6, 7], but they demand large placement areas. More compact gas-agitated bubble columns and airlift bioreactors have been assessed for culturing large quantities of microalgae [8–10], but these devices have a relatively deep light path and, therefore, a photosynthetically inefficient dark zone. This limits their productivity. In a deep culture vessel, productivity can be improved significantly by improving the radial mixing of fluid from the darker core of the culture vessel to its periphery [8, 11]. Mechanical agitation is known to enhance radial mixing. Unfortunately, mechanical agitation can be detrimental to algal cells [12–16]. Consequently, shear-sensitivity of cultures can severely restrict the attainable productivity in bioreactors. This work identifies the beneficial and detrimental regimens of mechanical agitation for the diatom *Phaeodactylum tricornutum* and the red alga *Porphyridium cruentum* in aerated continuous cultures. The use of a shear-protective additive, Pluronic F68, is shown to protect *P. cruentum* against damage caused by hydrodynamic forces. *P. tricornutum* and *P. cruentum* are potentially important sources of high-value polyunsaturated fatty acids such as eicosa-pentaenoic acid [17].

Damage to microalgae and cyanobacteria under processing conditions can be a real problem [6, 13–15, 18–20], but susceptibility to damage is dependent on the species. Algae such as *Dunaliella* are extremely fragile [18, 19], but many other species will tolerate high levels of turbulence and sparging with gas. For example, the

microalga *Ochromonas malhamensis* (8–20  $\mu\text{m}$  average diameter cells) has withstood an impeller tip speed of up to  $1.4\text{ m s}^{-1}$  with low-level sparging. In airlift bioreactors, the microalga *P. tricornutum* readily withstands a specific power input of about  $270\text{ W m}^{-3}$ . In contrast, the microalga *Dunaliella tertiolecta* has a damage threshold of  $98\text{ W m}^{-3}$  in bubble columns although more robust strains of this organism appear to exist [21].

*Dunaliella* lacks a rigid wall. Only a thin cytoplasmic membrane maintains the integrity of the cell. Silva et al. [18] showed that *Dunaliella* was susceptible to damage by gas bubbles as well as by turbulence in the liquid. Supplementing the culture with carboxymethyl cellulose or agar improved cell survival. In one study with *D. tertiolecta*, culture in a bubble column was quite successful, but when the bubble column was converted to an airlift device by inserting a vertical baffle, the productivity declined [19]. Under conditions that were earlier identified as optimal, no growth was observed in the airlift reactor, whereas good growth occurred in the bubble column. Microscopic examination showed significant disruption of the cells in the airlift device [19]. This was associated with the hydrodynamic stresses generated as the culture flowed over the upper edge of the baffle into the downcomer [19]. In the bubble column, the growth was sensitive to aeration rate: growth rate increased with increasing superficial gas velocity until a velocity of about  $0.6\text{ m min}^{-1}$ . Further increase in aeration rate reduced growth, apparently because of hydrodynamic stresses in the fluid [19]. Under non-growth conditions (no light), the specific death rate in the bubble column was shown to increase with superficial gas velocity for velocities exceeding  $0.6\text{ m min}^{-1}$  [19]. At a fixed aeration velocity, the specific death rate decreased with increasing height of the culture fluid in the column [19], probably because the specific power input and, hence, the turbulence intensity declined. Similar behavior has been reported with animal cells in bubble columns [22–26].

Damage to the red alga *P. cruentum* in high-shear centrifugal pumps has been documented to greatly reduce culture productivity [16]. Similarly, damage to cells of the marine microalgae *Skeletonema costatum* and *Haslea ostrearia* has been reported during passage through pumps and valves [15]. *H. ostrearia* is a single-celled diatom whereas *S. costatum* grows as chains of connected cells. Both centrifugal and positive displacement rotary vane pumps reduced the chain length of *S. costatum*. The centrifugal pump was more damaging than the positive displacement device. Larger algal filaments were the most susceptible to damage. The rate of decrease in chain length was greater for chains with a longer initial length. In studies with the highly motile microalga *Tetraselmis suecica*, Jaouen et al. [14] observed a loss of motility on the passage of culture suspension through centrifugal and rotary vane positive displacement pumps but not through a peristaltic pump.

In cultures of *Haematococcus pluvialis*, a producer of astaxanthin, early onset of the stationary phase has been

associated with turbulence-induced deflagellation of the cells [6]. Fragility of species such as *H. pluvialis* depends also on the phase of growth [6]. The stationary phase red cysts of *H. pluvialis* are significantly more robust than the green vegetative cells [6]. The cyanobacterium *Spirulina* is another species that is susceptible to damage by excessive shear forces. In addition to an organism's morphology, the biochemical composition of the cell wall has been associated at least qualitatively with the cell's susceptibility to hydrodynamic stresses [12]. For a cell-wall-lacking the mutant of *Chlamydomonas reinhardtii* in bubble columns, aeration at a velocity of  $0.076\text{ m s}^{-1}$  produced a specific cell death rate of  $0.46 \pm 0.08\text{ h}^{-1}$  [21].

Several laboratory studies have attempted to characterize shear tolerance of algal cultures in stirred tank photobioreactors. For example, damage to 8–20  $\mu\text{m}$  average diameter cells of the microalgae *Ochromonas malhamensis* was investigated by Yang and Wang [27] for various agitation–aeration combinations. A 14 L fermenter with a six-bladed Rushton turbine was used. Sparging at low levels had no damaging effect in the absence of mechanical agitation. In the absence of sparging, agitation speeds below 350 rpm (tip speed of  $1.4\text{ m s}^{-1}$ ) produced no damage, but damage occurred above 350 rpm apparently because high-intensity agitation was accompanied by gas entrainment due to vortexing. Under damaging conditions of agitation, aeration at rates that alone had caused no cell damage, enhanced the damaging effect [27].

Under given conditions of mechanical shear, e.g., at a constant impeller tip speed, changes in viscosity may enhance or reduce damage to microbial and other cells. Reduced damage has been reported for the mold *M. javanicus* [12], animal cells [28, 29], and the halotolerant microalga *Dunaliella* [18]. An opposite effect is sometimes observed. Increasing shear damage with increasing culture viscosity has been reported for the protozoan *Tetrahymena pyriformis* [30] and for red blood cells [31]. These seemingly contradictory results are easily explained: whether changes in viscosity increase or reduce shear damage depends on the peculiarities of the mechanism causing the damage [13, 32]. For a given impeller tip speed, increasing viscosity will not affect the tip speed-associated shear rate, but the turbulence will be reduced; hence, the shear rate in the fluid should be lower. However, the shear stress around a particle may increase if the fluid eddy length scale remains comparable to the dimensions of the particle [32]. In yet other cases, increased viscosity may reduce both shear rate and shear stress: only eddies that are larger than the particle may survive and there may be little or no relative motion between the fluid and the particle [32]. In some cases, the frequency of passage through high-shear zones may decline with increasing viscosity.

Work reported here confirms that mechanical agitation in an aerated photobioreactor can enhance or reduce biomass productivity of microalgae, depending on the

intensity of agitation. Furthermore, these changes in productivity are not related with possible effects of agitation on gas–liquid mass transfer of carbon dioxide that is necessary for photosynthesis. In addition, excessive mechanical agitation is shown to affect the productivity mainly by affecting the size of the gas bubbles in dispersions and the frequency of rupture of these bubbles. Bubble rupture at the culture surface appears to be the major cause of damage to cells. Surfactants that suppress attachment of cells to bubbles can fully protect the algal cells against damage in agitated-aerated photobioreactors, as proved here.

## Materials and methods

### Organisms and culture conditions

The microalgae *P. cruentum* UTEX 161 and *P. tricor- nutum* UTEX 640 were obtained from the culture collection of the University of Texas, Austin, USA. The algae were grown as separate monocultures. The culture medium was Mediterranean sea water enriched with the following additives (per liter): NaNO<sub>3</sub> (0.85 g), NaH<sub>2</sub>- PO<sub>4</sub>·2H<sub>2</sub>O (0.156 g), MnCl<sub>2</sub>·4H<sub>2</sub>O (0.9 mg), Na<sub>2</sub>- MoO<sub>4</sub>·2H<sub>2</sub>O (242 µg), ferric citrate (4.9 mg), EDTA (9.886 mg), CoCl<sub>2</sub>·6H<sub>2</sub>O (24 µg), CuSO<sub>4</sub>·5H<sub>2</sub>O (25 µg), and ZnCl<sub>2</sub> (13.6 µg). The medium was sterilized (120°C, 20 min) prior to use. When required, the shear protec- tant Pluronic F68 (Sigma, St Louis, MO, USA) was added to the feed medium at the specified concentration prior to sterilization by autoclaving.

The microalgae were grown as continuous cultures at a dilution rate of 0.0139 h<sup>-1</sup> in a 5 liter stirred bioreactor (New Brunswick Scientific, BioFlo III; Edison, NJ, USA). The baffled reactor had the following relevant dimensions: 0.24 m culture depth; 0.17 m vessel diameter; 0.102 m vertical distance between the two four-bladed turbines mounted on the same shaft; 0.094 m clearance of the lower impeller from the bottom of the vessel; and 0.085 m impeller diameter. The length and width of the impeller blades were 0.017 and 0.012 m, respectively. The bioreactor was continuously illuminated with four Osram Dulux EL fluorescent lamps (20 W each) placed around the 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 inci- dent irradiance (photosynthetically active) was 820 µE m<sup>-2</sup>s<sup>-1</sup>. The culture temperature was controlled at 20°C. The pH was maintained at 7.7 ± 0.1 using automatic injection of carbon dioxide, as needed. The vessel was aerated at 1.5 L/min. The aeration gas was filter sterilized by passing through a 0.22 µm Millipore membrane filter cartridge.

Control experiments were carried out in batch cul- tures in unsparged shake flasks to verify that Pluronic F68 did not affect the cellular metabolism, as measured by the growth rate and cell viability. The latter was

measured by the trypan blue dye exclusion method [33]. Low-speed agitation (200 rpm) of the flasks (50 mL) prevented the cells from settling. Different flasks in a given run contained either Pluronic (0.05–0.10%) or no Pluronic. Exponentially growing cells from 500 mL sparged flasks (no Pluronic) were used for the inoculum. Prior to inoculation, the flasks had been autoclaved (120°C, 1 h). The inoculated flasks were placed on an orbital shaker under fluorescent light (Phillips TLD W154) that provided a photosynthetically active photon flux density at the culture surface of 90 µE m<sup>-2</sup>s<sup>-1</sup>. Samples (0.5 mL) taken at various intervals were used to measure the biomass concentration and cell viability. Each experiment was conducted in triplicate and the results were analyzed by a one-way ANOVA.

### Analytical methods

The biomass concentration was estimated by optical density measurements (1 cm light path) in a Hitachi U-1000 spectrophotometer. The wavelength of the measurement was 760 and 625 nm for *P. cruentum* and *P. tricor- nutum*, respectively. The biomass concentration ( $C_b$ , g L<sup>-1</sup>) and the culture absorbance (OD) were line- arly related as follows:

$$C_b = 0.278 \times \text{OD}_{760} \quad (r^2 = 0.977), \quad (1)$$

$$C_b = 0.380 \times \text{OD}_{625} \quad (r^2 = 0.999). \quad (2)$$

The optical density measurements were periodically checked by gravimetry.

The viscosity of algal suspensions was measured using a Cannon-Fenske viscometer. Surface tension was measured with a Krüss digital tensiometer K10 ST (Krüss, Hamburg, Germany) using the Wilhelmy plate method. The bulk density of the algal suspensions was measured using a pycnometer.

### Volumetric gas–liquid mass transfer coefficient

The well-known dynamic gassing in method was used to determine the volumetric gas–liquid mass transfer coef- ficient  $k_L a_L$  [34]. Thus, the air flow was stopped and replaced with an equivalent flow of nitrogen to remove dissolved oxygen from the fluid. Once a low value of dissolved oxygen had been attained, the flow of nitrogen was stopped and the gas bubbles were allowed to dis- engage. A preset flow of air then resumed with mea- surements of the dissolved oxygen concentration  $C_L$  as a function of time  $t$ .  $k_L a_L$  was calculated as the slope of the following linear equation:

$$\ln \left( \frac{C^* - C_{Lo}}{C^* - C_L} \right) = k_L a_L t. \quad (3)$$

In the above equation,  $C^*$  is the saturation concentration of oxygen in the liquid phase and  $C_{Lo}$  is the initial

concentration of dissolved oxygen at the instance of commencement of aeration [34].

The  $k_L a_L$  values determined as described applied to the mass transfer of oxygen. These values were corrected for the mass transfer of carbon dioxide [34], as follows:

$$(k_L a_L)_{\text{CO}_2} = (k_L a_L)_{\text{O}_2} \sqrt{\frac{D_{\text{oxygen}}}{D_{\text{carbon dioxide}}}} \quad (4)$$

Here  $D$  is the diffusivity of the specified gas in water.

## Results and discussion

A steady state biomass balance for continuous-flow well-mixed bioreactors shows that the dilution rate  $\hat{D}$ , the specific growth rate  $\mu$  and the specific death rate  $k_d$  are related as follows:

$$\mu = \hat{D} + k_d \quad (5)$$

When no other nutrients are limiting, the specific growth rate depends only on the average irradiance in the culture, the availability of dissolved carbon dioxide and the flashing light effect [11, 35–38].

Biomass concentration versus time data for the continuous culture of *P. tricornutum* are shown in Fig. 1. Initially, at an agitation speed of 150 rpm (i.e. impeller tip speed of  $0.68 \text{ m s}^{-1}$ ), the biomass concentration was constant and at a steady state. The agitation rate was then increased stepwise from 150 to 550 rpm. The biomass concentration changed and attained new steady states for each step change in the agitation rate (Fig. 1). Up to a maximum impeller speed of 350 rpm (impeller tip speed of  $1.56 \text{ m s}^{-1}$ ) the increase in the agitation rate increased the biomass concentration even though the dilution rate was held constant at  $0.0139 \text{ h}^{-1}$  throughout the experiment. Further increase in the agitation speed caused a decline in the biomass concentration, but stable steady states were attained up to the highest impeller speed investigated.

Because the pH was accurately controlled at  $7.7 \pm 0.1$  by injecting carbon dioxide automatically as needed,  $\text{CO}_2$  did not become limiting at agitation speeds greater than the cell damaging threshold speed of 350 rpm. None of the other parameters (aeration rate, incident irradiance, temperature) varied during the experiment. In view of Eq. (5), therefore, the changes in biomass concentration in Fig. 1 were associated with changes in the relative values of the specific growth rate  $\mu$  and the specific death rate  $k_d$  that occurred as a consequence of the changes in impeller agitation speed. Increasing the agitation speed from 150 to 350 rpm increased  $\mu$  relative to the  $k_d$  value because of increased mixing between the light and the dark zones so that no cells resided in continuous darkness for long. Reducing the length of the continuous dark period by increasing the frequency of light–dark cycling (i.e. the flashing light effect) has been proved to enhance biomass productivity of microalgal

cultures [11, 35–38]. Increase in agitation speed above 350 rpm produced an increase in the  $k_d$  value because of hydrodynamics-induced cell damage.

The microalga *P. cruentum* was also sensitive to excessive agitation, as shown in Fig. 2. In Fig. 2 only the steady state biomass concentrations attained at various values of the agitation rates are plotted. The hydrodynamic damage threshold of *P. cruentum* occurred at  $> 550$  rpm (i.e. impeller tip speed of  $> 2.45 \text{ m s}^{-1}$ ) and the culture washed out at 650 rpm. This suggests that compared to *P. tricornutum*, *P. cruentum* is more resistant to hydrodynamic damage. Unfortunately, the data in Figs. 1 and 2 do not permit a definite conclusion to be drawn about relative shear-sensitivity of the two microalgae. This is because the observed changes in biomass concentrations in Figs. 1 and 2 are a net result of the effect of agitation on growth and death processes. There is, therefore, the possibility that the enhancement in the specific growth rate in Fig. 2 as a consequence of increasing agitation speed up to the threshold value of 550 rpm was less substantial than in Fig. 1 for *P. tricornutum*.

As mentioned earlier, possible limitations in  $\text{CO}_2$  mass transfer did not explain the increase in steady state biomass concentrations seen in Figs. 1 and 2 over certain ranges of agitation speeds. This was because the pH value remained constant at  $7.7 \pm 0.1$  throughout the experiment. To further support this point, the measured values of the overall volumetric mass transfer coefficient  $k_L a_L$  are plotted in Fig. 3 as a function of the impeller agitation speed in a culture of *P. tricornutum*. The aeration rate for all measurements in Fig. 3 was  $1.5 \text{ L min}^{-1}$ , as in all continuous cultures in the present work. The data in Fig. 3 were measured in a batch that contained  $1,031 \text{ mg L}^{-1}$  biomass. The  $k_L a_L$  values measured using oxygen absorption from air were converted to the corresponding values for  $\text{CO}_2$  [34] before being plotted in Fig. 3.

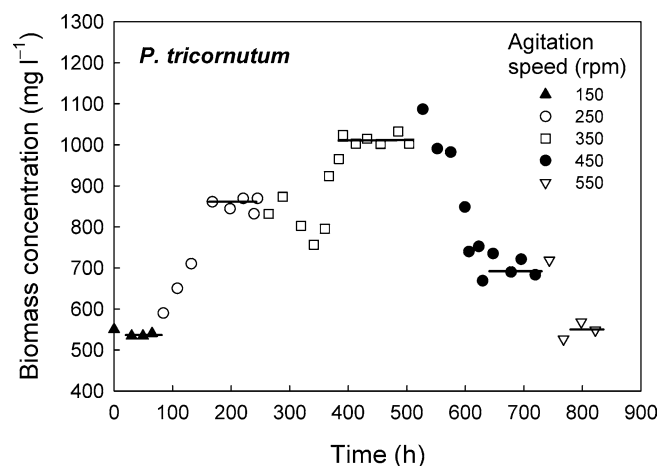
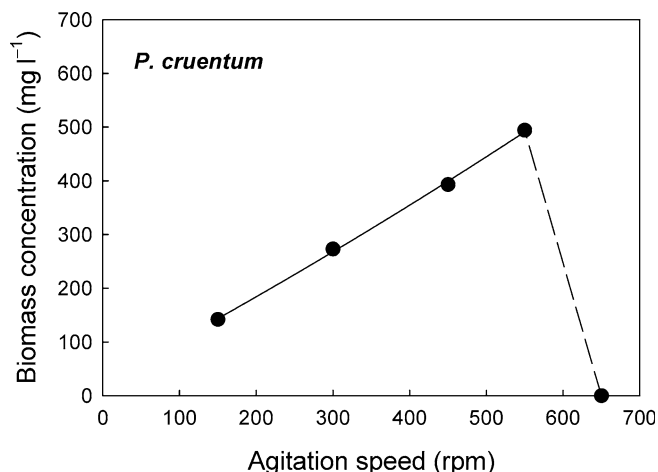


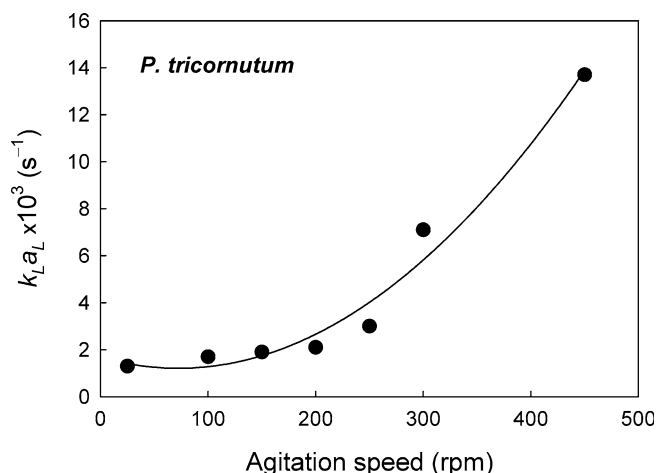
Fig. 1 *P. tricornutum* biomass concentration versus time in the continuous culture at various impeller agitation speeds. Horizontal lines indicate steady states



**Fig. 2** Steady state biomass concentration of *P. cruentum* at various impeller agitation speeds

Using the known value of the dilution rate and a measured biomass concentration of  $1,031 \text{ mg L}^{-1}$  in the bioreactor, the biomass productivity was shown to be  $14.3 \text{ mg L}^{-1} \text{ h}^{-1}$  at the agitation speed of 350 rpm. Assuming a biomass carbon content of 50% by dry weight, as documented for *P. tricornutum* [33], the calculated  $\text{CO}_2$  demand of the culture was being fully met at the  $k_L a_L$  value of  $8.3 \times 10^{-3} \text{ s}^{-1}$  estimated from the measured data (Fig. 3). Despite this, an agitation rate of greater than 350 rpm reduced biomass concentration (Fig. 1) even though the  $k_L a_L$  value increased with increasing agitation rate (Fig. 3). Thus, a limiting supply of  $\text{CO}_2$  was not an explanation for the observed decline in the biomass concentration (Fig. 1).

Improved light–dark cycling as an explanation for the observed increase in steady state biomass concentration with increasing agitation rate in Figs. 1 and 2 required a demonstration that a dark zone existed in the photobi-



**Fig. 3** Effect of impeller agitation speed on the overall volumetric mass transfer coefficient ( $k_L a_L$ ) of carbon dioxide in the *P. tricornutum* culture at a biomass concentration of  $1,031 \text{ mg L}^{-1}$ . The aeration rate was  $1.5 \text{ L/min}$

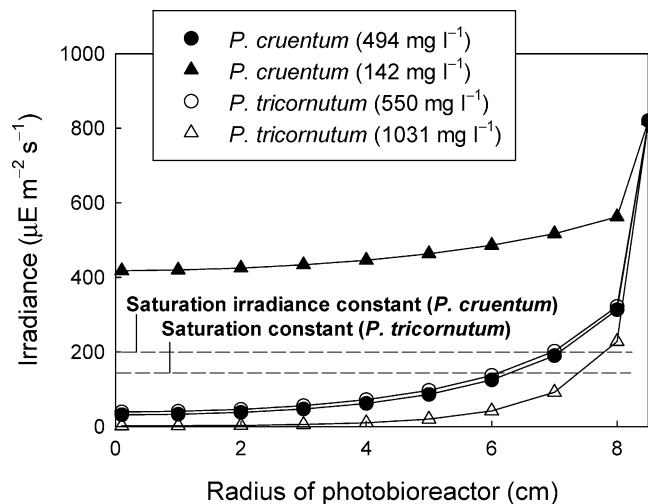
oreactors under the conditions of the experiments. Thus, for low and high biomass concentrations of the two microalgae in the reactor, the radial irradiance profiles were calculated using the Evers's model for diffuse light [40], as follows:

$$I(r) = \frac{I_o}{\pi} \int_0^\pi \exp \left\{ -K_a C_b \left[ r \cos \phi + (R^2 - r^2 \sin^2 \phi)^{0.5} \right] \right\} d\phi, \quad (6)$$

where ( $I_r$ ) was the irradiance at depth  $r$  in the culture,  $R$  was the radius of the bioreactor vessel,  $I_o$  was the incident irradiance,  $K_a$  was the absorption coefficient for the biomass,  $C_b$  was the biomass concentration in the broth, and  $\phi$  was the angle of penetration of the light beam. The light absorption coefficient  $K_a$  was determined spectrophotometrically as described by Molina Grima et al. [39].

A measured incident irradiance value of  $820 \mu\text{E m}^{-2} \text{ s}^{-1}$  at the surface of the reactor was used in these calculations. Similarly, the measured  $K_a$ -data were used in Eq. (6) to obtain the radial irradiance profiles shown in Fig. 4. The figure proves that for all concentrations of *P. tricornutum* relevant in this study, the bioreactor had an interior region with irradiance levels of less than  $185 \mu\text{E m}^{-2} \text{ s}^{-1}$ , the reported [41] saturation irradiance (i.e. the irradiance value at half the maximum growth rate) for *P. tricornutum*. Thus a dark zone always existed in the culture vessel. Similarly, for the *P. cruentum* cultures, the irradiance values in a large part of the bioreactor were less than the  $\sim 200 \mu\text{E m}^{-2} \text{ s}^{-1}$  saturation irradiance that has been documented [42] for this microalga. In this case, too, a dark zone existed in the reactor for the higher biomass concentration that was relevant to the data in Fig. 2. For the low biomass concentration of *P. cruentum* in Fig. 2, the irradiance value in the culture was everywhere higher than the light-saturation constant for this alga (Fig. 4). Nevertheless, a light-limited zone existed in a large volume of the reactor. This was because the light-saturation irradiance occurs typically at roughly 30% of the nonlimiting irradiance level for microalgae. Indeed the irradiance value above which photosynthesis is not limited by light has been reported for *P. cruentum* as  $400\text{--}550 \mu\text{E m}^{-2} \text{ s}^{-1}$  [43] and  $700 \mu\text{E m}^{-2} \text{ s}^{-1}$  [44]. As expected, this value is roughly 2–3 times the light-saturation constant for *P. cruentum*. Thus, an irradiance value of  $\leq 600 \mu\text{E m}^{-2} \text{ s}^{-1}$  represents a light-limited regimen for *P. cruentum*. Clearly, reducing the length of time of continuous exposure to a light-limited zone (as opposed to a dark zone) by increasing the mixing improves the productivity of the culture, as seen in Fig. 2.

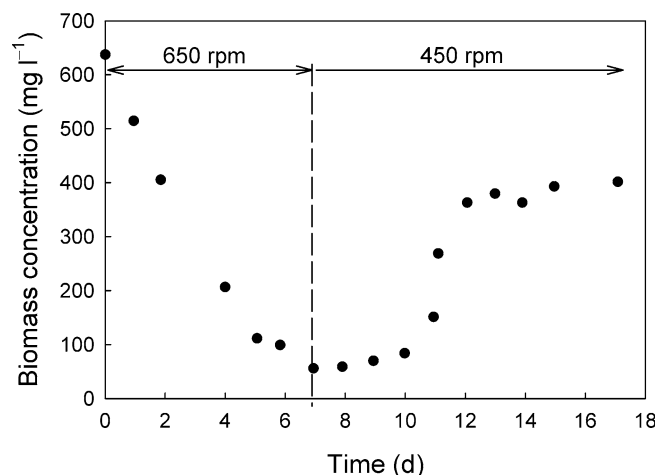
To further demonstrate the cell damaging effect of agitation, a continuous culture of *P. cruentum* was grown to a stable biomass concentration at an agitation rate of less than the damaging threshold of 650 rpm. The agitation rate was then instantaneously increased to 650 rpm. The biomass contents in the reactor declined



**Fig. 4** Radial irradiance profiles in the reactor at low and high biomass concentrations of *P. cruentum* and *P. tricornutum*

continuously over the next 8 days, as shown in Fig. 5. Just before the impending washout of the biomass, the agitation intensity was reduced to 450 rpm and the biomass concentration recovered to a stable steady state value of  $\sim 400 \text{ mg L}^{-1}$  (Fig. 5) as had been attained in a previous run under the same conditions (Fig. 2). This conclusively proved the damaging effect of excessive agitation on the algal cells.

Other studies carried out in bubble columns in the absence of mechanical agitation confirm that *P. tricornutum* is sensitive to the break-up of small bubbles on the surface of the culture [45]. Cell damage could be suppressed by adding a small amount ( $\geq 0.02\%$  by wt) of carboxy methyl cellulose to the culture medium [45]. Methyl cellulose additives have been associated with the reduced attachment of cells to bubbles [46–49].



**Fig. 5** Time course of *P. cruentum* biomass concentration at a constant dilution rate of  $0.0139 \text{ h}^{-1}$ . The biomass was lost continuously from the instance the agitation speed was raised to 650 rpm but recovered on reducing the agitation speed to 450 rpm

Microbial morphology is well-known to influence susceptibility to shear damage [13]. Conceivably, therefore, it may be possible to culture the polymorphic diatom *P. tricornutum* in morphotypes with improved ability to withstand damage causing forces. *P. tricornutum* has three main morphotypes: oval, fusiform and tri-radiate [50,51]. The fusiform morphology generally occurs in photobioreactor cultures such as in the present work, but occasionally the morphology can change suddenly and without an apparent environmental cause [52]. The fusiform morphotype lacks a silica skeleton and is more susceptible to hydrodynamic damage than some of the other forms.

#### The effects of Pluronic F68

The presence of Pluronic F68 in gently agitated unsparged shake flasks had no effect on biomass growth rate or cell viability in comparison with equivalent flasks without the Pluronic additive. In all cases, the viability was close to 100% and no cell damage was observed. In view of these observations, Pluronic F68 had no physiological effect on the cells.

The steady state biomass concentrations of *P. cruentum* attained in continuous cultures at various agitation intensities with and without added Pluronic F68 are shown in Table 1. The data in the table were obtained at a constant dilution rate of  $0.0139 \text{ h}^{-1}$  in cultures aerated at 0.4 vvm. As shown in the table, Pluronic did not affect the viscosity of the broth and therefore could not directly dampen turbulence in the fluid [28]. However, addition of Pluronic did reduce interfacial tension of the broth relative to that of water. Presence of Pluronic had a clear protective effect on cells. Thus, at an impeller speed of 450 rpm, the presence of 0.01% Pluronic enhanced the steady state biomass concentration by 66% relative to when no Pluronic was added (Table 1). Similarly, at an impeller speed of 650 rpm, the culture washed out in the absence of Pluronic because cell damage reduced the net maximum growth rate to less than or equal to the dilution rate. In the presence of progressively higher levels of Pluronic (0.01–0.10%), no washout occurred at 650 rpm and the attained steady state concentration of the biomass increased with increasing concentration of Pluronic.

The Pluronic-induced enhancement of steady state biomass concentration even at the relatively low agitation speed of 450 rpm (Table 1) indicated that cell damage took place at lower agitation speeds than were necessary to produce culture washout. The data in Table 1 suggest that motion of the impeller blades and turbulence in the fluid were not the direct causes of the observed cell damage. Damage occurred mainly because of the rupture of gas bubbles at the surface of the fluid. Even though the aeration rate was constant in all cases in Table 1, the impeller speed increased and this reduced the average bubble size. As these small bubbles rose to the surface and ruptured, any cells carried with

**Table 1** Steady state biomass concentration attained with and without the Pluronic F68 additive in cultures of *P. cruentum*

Agitation rate (rpm)	Pluronic concentration (w/v %)	Biomass concentration (mg L <sup>-1</sup> )	Viscosity (cP)	Surface tension relative to water
450	0.00	396.3	1	1.00
450	0.00	395.9	1	1.00
450	0.01	655.8	1	0.86
650	0.00	0.0 (washout)	1	1.00
650	0.01	617.2	1	0.89
650	0.05	756.0	1	0.90
650	0.10	1,205.4	1	0.76

Specific air flow rate of 0.4 vvm; dilution rate of 0.0139 h<sup>-1</sup>

the bubbles ruptured also. Rising bubbles, specially small ones, have been shown to carry cells with them in their wakes and adsorbed to the surface [53–55]. Furthermore, the rupture of small bubbles is known to be more damaging to cells than is the rupture of large bubbles [13, 16, 27, 55–58]. Presence of Pluronic, a surfactant, is known to reduce the attachment of cells to bubbles; hence in the presence of Pluronic fewer cells are carried to the surface where the rupture events cause damage. A Pluronic concentration of 0.1% is the recommended upper limit as concentrations greater than this caused excessive foaming and led to operational problems.

## Conclusions

Within limits, increasing agitation rate in continuous cultures of microalgae enhanced biomass productivity by reducing the length of the continuous dark period (or light-limited period) experienced by the cells. Both *P. tricornutum* and *P. cruentum* experienced various levels of cell damage at high agitation rates. Compared to *P. tricornutum*, the microalga *P. cruentum* had a higher tolerance for the otherwise damaging forces. The cause of the cell damage was not mechanical agitation per se, but the rupture of small gas bubbles at the surface of the culture. Increasing agitation rate reduced the bubble size to produce a more damaging environment than that which existed when the bubbles were larger. Addition of Pluronic F68 over a concentration range of 0.01–0.10% protected cells in a concentration-dependent manner. A Pluronic concentration of 0.1% appeared to afford complete protection to *P. cruentum* under conditions that had caused severe damage and culture washout in the absence of Pluronic. These results lead to the following specific conclusions.

1. Enhanced mixing in deep photobioreactors can lead to improved biomass production because of an improved light regimen.
2. Excessive mechanical agitation in the presence of aeration in photobioreactors can damage microalgae because agitation reduces the size of the gas bubbles

rupturing at the surface of the broth and the rupture of small bubbles is more damaging to cells.

3. Different microalgae have different tolerances for the damage causing hydrodynamic forces.
4. Presence of Pluronic F68 in the broth can help protect algal cells against hydrodynamic damage.

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