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Effects of shear stress on the microalgae *Chaetoceros muelleri*

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Abstract The effect of shear stress on the viability of
Chaetoceros muelleri was studied using a combination of a
rheometer and dedicated shearing devices. Different levels
of shear stress were applied by varying the shear rates and
the medium viscosities. It was possible to quantify the
effect of shear stress over a wide range, whilst preserving
laminar flow conditions through the use of a thickening
agent. The threshold value at which the viability of algae
was negatively influenced was between 1 and 1.3 Pa.
Beyond the threshold value the viability decreased sud-
denly to values between 52 and 66%. The effect of shear
stress was almost time independent compared to normal
microalgae cultivation times. The main shear stress effect
was obtained within 1 min, with a secondary effect of up to
8 min.

Keywords Microalgae · *Chaetoceros muelleri* ·
Shear stress · Viability · Aquaculture feed ·
Photobioreactor design

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Introduction

Microalgae are cultivated to serve as feed for aquaculture
(shellfish, shrimps and fish), animal feed (pets and farm-
ing), human nutrition, cosmetics and the production of
high-value ingredients, such as polyunsaturated fatty acids
and pigments [1]. The current market size of microalgal
biomass is about 5,000 t dry matter/year of which at least
1,000 t dry matter/year is produced for aquaculture [2, 3].

We are interested in the production of microalgae as a
feed for bivalves. In general, cultivation of microalgae for
these applications takes place in transparent plastic bags.
Because the capacity of these systems is relatively small,
labor cost per amount of feed for bivalve production is
high. This explains why alternative large scale production
systems for microalgae are being investigated. Such sys-
tems can consist of open ponds, bubble columns, flat-plate
photobioreactors (PBRs) and tubular PBRs [4–6]. PBRs
seem to be most promising in cultivating these microalgae
on a large scale, because of low contamination risk, low
space requirement, almost no CO_2 loss, high adaptability to
many species and high biomass concentration [7, 8].
However, it is suspected that the large hydrodynamic for-
ces present in the PBR could cause shear stress levels that
might be too severe for the sensitive microalgae, which
would lead to reduced growth or even cell death [9].
Nevertheless, a high mixing intensity is necessary to keep
microalgae in suspension, to achieve a sufficient light
distribution and to enhance mass transfer. For the optimal
design of PBRs, it is therefore important to know the
maximum level of shear stress that can be tolerated by the
microalgae.

Damage because of shear stress has been demonstrated
both in bubble columns due to gas sparging [10–14] and in
tubular PBRs due to pumping action [12, 15, 16]. Although

cases of shear damage were described, shear stress was only quantified indirectly by using the liquid flow rate or the number and frequency of pump passages. A first step towards quantification of the shear rate or shear stress was done by Contreras et al. [17] and García Camacho et al. [18]. They used the energy dissipation to calculate an average shear rate or shear stress. For *Phaeodactylum tricornutum*, a shear rate value of 7,000 s^{-1} led to the highest growth rate caused by a balance between mass transfer limitations and shear damage [17]. Sensitivity to shear stress is dependent on the microalgae species used. This has been shown in a study with the dinoflagellate *Protoceratium reticulatum*, where a damage threshold was observed already at an average shear stress of 0.16 mPa, which was equivalent to a shear rate of 0.12 s^{-1} [18].

In order to apply a well-defined shear stress, Couette devices have been used in several studies. Couette devices consist of coaxial cylinders, where either the inner cylinder rotates and the outer remains stationary or vice versa. The effect of shear stress on photosynthesis of *Spirulina platensis* has been studied using a Couette device [19]. It was found that the oxygen production rate and the chain length of *Spirulina platensis* started to decrease when the shear stress exceeded 0.3 Pa. Experiments on cyanobacteria [20] indicated that a shear rate of as little as 2.2 s^{-1} reduced nitrogenase activity and CO_2 fixation. However, the applied shear stress levels were not given, because the shear viscosity was not measured. Couette shearing devices have also been used to study the effect of shear stress on bioluminescence of different dinoflagellate species [21–24]. Bioluminescence threshold values were to the order of 0.1–1 Pa, where the shear stress was calculated as a function of the shear rate and the viscosity of the medium. Shear stress thus appears to trigger bioluminescence in these cases, but the relationship to cell damage or viability is unclear.

The aim of this study is to determine the shear sensitivity of *Chaetoceros muelleri*. In addition, the time scale in which the effects of shear stress take place will be examined. The purpose was to understand whether the adverse effects were caused by a prolonged exposure to excessive shear stress or if the algal cells were damaged instantaneously. The combination of shear cylinders as Couette devices and rheological measurements was used in order to study the effect of a uniform and well-defined shear stress on the viability of *Chaetoceros muelleri*.

Materials and methods

Culture preparation

The diatom *Chaetoceros muelleri* (CCMP 1316) was obtained from NIOO (Netherlands Institute of Ecology,

Yerseke, The Netherlands). *Chaetoceros muelleri* was cultivated in natural seawater from the Easterscheldt (location Yerseke) enriched with Walne medium modified from Laing [25]. The salinity of the seawater used was 32 g L^{-1}.

Cultures of *Chaetoceros muelleri* were maintained in 10 mL test tubes, 250 mL Erlenmeyer flasks containing 100 mL medium and 3 L Erlenmeyer flasks containing 2 L medium at 20 °C. Light was supplied continuously by white fluorescent light. Every week 10% of the culture was transferred to new medium.

All shear stress experiments were carried out with batch cultures from the 3 L Erlenmeyer flasks after 1 week of growth, at which point the algae were in the early stationary phase. Locust bean gum (LBG) was applied as a thickener to increase the medium viscosity. In the rest of this article, the term medium refers to the enriched seawater including the thickener. The thickener was used in the following concentrations: 0, 0.3 and 0.5%. Cell concentrations in the experiments varied between 5 and 10 million per mL. The cell concentrations in the LBG solutions were 50% of the original culture, because 50 mL of the original culture was mixed with 50 mL of 0.6 or 1.0% LBG solution.

Shearing the algae

The effect of shear stress on the viability of *Chaetoceros muelleri* was studied, using four shear cylinders developed at the Laboratory of Food Process Engineering. The shear cylinders have the following dimensions: an inner cylinder with a length of 145.5 mm and a radius of 20 mm and an outer cylinder with a radius of 21 mm. Between the inner and the outer cylinder is a gap of 1 mm. The total volume between the cylinders is 20 mL. The inner cylinder rotates and the outer cylinder is stationary. The shear rate applied in the shear cylinders is proportional to the rotational speed; the conversion factor of rotational speed (rpm) to shear rate (s^{-1}) is 2.157. The maximum shear rate of the shear cylinders is 1,079 s^{-1} obtained at a rotational speed of 500 rpm. The shear rate is given as:

$$\dot{\gamma} = \frac{2R_o R_i \omega}{R_o^2 - R_i^2} \tag{1}$$

where $\dot{\gamma}$ is the shear rate (s^{-1}), ω is the angular velocity of the outer cylinder (s^{-1}), R_o is the outer cylinder inner radius (m) and R_i is the inner cylinder outside radius (m) with:

$$\omega = \frac{2\pi n}{60} \tag{2}$$

where n is the rotational speed (rpm) of the shear cylinder.

In the first experiment *Chaetoceros muelleri* without LBG was exposed to different levels of rotational speed,

which were 0, 4, 20, 100 and 500 rpm, respectively. The levels of rotational speed applied to the algae in 0.3% LBG were 0, 4, 10, 15, 20, 100 and 500 rpm, respectively. The algae in 0.5% LBG were exposed to the following levels of rotational speed: 0, 2, 4, 10, 20 and 100 rpm. The exposure time to the different levels of shear stress was 1 h and the temperature 4 °C. All exposures in the shear cylinders were done in triplicate.

A second experiment was carried out to investigate the time dependence of shear stress effect. Algae in 0.3% LBG were exposed to a rotational speed of 100 rpm for different times of exposure ranging from 1 to 120 min. The temperature was 4 °C as in the first experiment. All tests were done in triplicate.

For all treatments the Taylor number was calculated to find out whether flow instabilities occurred. Taylor vortices will be formed when the Taylor number is higher than the limiting value of 41.3 [26, 27]. The Taylor number is defined as:

$$Ta = \left\lfloor \omega \rho R_i^2 (\delta_{cc} - 1)^{3/2} \right\rfloor / \eta \tag{3}$$

where ω is the angular velocity, ρ is 1,024 kg m^{-3} for seawater with algae at 4 °C, η is the apparent viscosity (Pa s) and δ_{cc} is the ratio of the outer to the inner cylinder radius, which is 1.05.

Rheological characterization

The shear stress was measured with a sample of 3.6 mL of the algae (without LBG, in 0.3 and 0.5% LBG solution) after exposure in the shear cylinders. A rheometer (type Physica MCR 301, Anton Paar) was used to measure the exact shear stress applied in the shear cylinders. The measurements were done at 4 °C.

From the shear stress, the apparent viscosity can be calculated using the following equation:

$$\eta = \frac{\tau}{\dot{\gamma}} \tag{4}$$

where τ is the shear stress (Pa), η is the apparent viscosity (Pa s) and $\dot{\gamma}$ is the shear rate (s^{-1}).

For Newtonian fluids, η is independent of $\dot{\gamma}$ and for non-Newtonian fluids, η depends on $\dot{\gamma}$ [26]. The power-law model was used to describe this dependence [28, 29].

$$\eta = m|\dot{\gamma}|^{n-1} \tag{5}$$

The parameters m and n were measured from a log–log plot with apparent viscosity against shear rate.

The same power-law model is used to measure the dependence between shear rate and shear stress [26].

$$\tau = c\eta^n \tag{6}$$

The parameters c and n were also measured from a log–log plot of shear stress versus shear rate.

Assessment of the viability

The viability of the algae was measured by using the fluorescein diacetate (FDA) staining method. Esterases in viable cells cleave FDA with the formation of fluorescein as a result. Fluorescein is a compound that fluoresces green in viable cells and does not give fluorescence in non-viable cells [30, 31]. A FDA stock solution was prepared by dissolving 46 mg FDA (Sigma) in 10 mL acetone (11 mM) and stored in the dark at −4 °C. One milliliter of the sample containing the algae was incubated with 10 μL FDA stock solution for 20 min [32]. Then the total amount of algal cells and the viable cells were counted in a cell chamber (hemocytometer DHC-B02-5 Büker Türk) using a fluorescence microscope (Olympus IX71). Viability of the algae was taken as the percentage of fluorescing algae.

In LBG, the algal cells did not settle at the bottom of the cell chamber, but were suspended over the 0.1 mm depth of the cell chamber. To count the cells, it was therefore necessary to scan over the depth of the cell chamber by adjusting the focus of the microscope.

Results and discussion

Flow regime characterization

Figure 1 shows the relation between the shear rate and shear stress applied to the algae suspension without LBG, in 0.3% LBG and in 0.5% LBG. As expected, higher shear stress levels could be obtained through an increase in either the shear rate or the viscosity, with the help of a thickener. LBG led to a shear-thinning behavior. The slopes of the flow curves with shear stress as a function of shear rate on a logarithmic scale are less steep for the LBG concentrations. This can also be seen in the power-law model functions where $n < 1$ for the algae in LBG, while $n \approx 1$ for the algae without LBG (Eq. 6).

The shear stress levels that were applied in the further experiments were calculated with the power-law model functions in Fig. 1 and are presented in Table 1.

Viscosity as a function of shear rate is shown in Fig. 2 for the algae without LBG, the algae in 0.3% LBG and 0.5% LBG, respectively. The viscosity of the algae without LBG, which is the original culture of *Chaetoceros muelleri* in the medium, is almost independent of the shear rate. Therefore, the algae without LBG behave nearly Newtonian. This Newtonian behavior suggests that there is little interaction between the algal cells in the suspension and that they behave as separate cells, rather than as clustered cells.

Non-Newtonian behavior is apparent for the algae in LBG, where shear-thinning is evident. Therefore, the
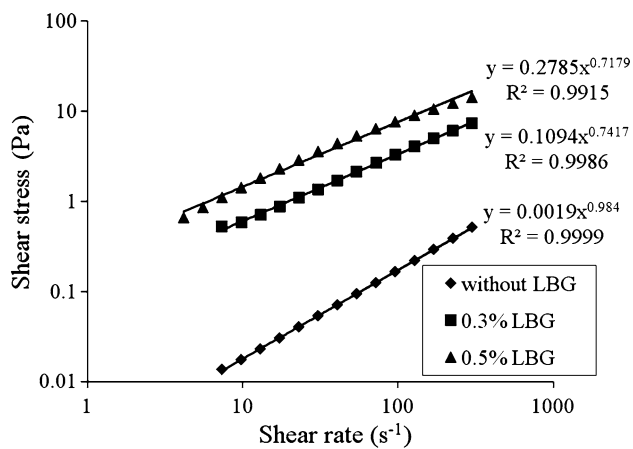
Fig. 1 Shear stress as a function of shear rate: algae without LBG (*diamonds*), algae in 0.3% LBG (*squares*) and algae in 0.5% LBG (*triangles*)

Fig. 2 Viscosity as a function of shear rate: algae without LBG (*diamonds*), algae in 0.3% LBG (*squares*) and algae in 0.5% LBG (*triangles*)

apparent viscosity needs to be determined at the different shear rates for the calculation of the accurate shear stress levels applied. A study of shearing insect cells has also indicated that assuming Newtonian behavior of cell cultures could cause substantial error in shear stress estimates [29].

The Taylor number for every apparent viscosity value was calculated with Eq. 3 (Table 1). In all experiments laminar flow was maintained, except at the highest shear rate of 1,079 s^{-1} in the algae dispersion without LBG. Taylor vortices may have been formed during that experiment and this could have led to secondary flow to a certain extent. The highest shear rate that can be applied for algae without LBG, while keeping the algae in laminar flow, is about 150 s^{-1}. The use of LBG increased the viscosity, leading to a lower Taylor number. For that reason, LBG or other thickeners can be used to reach higher shear stress under stable laminar flow to the extent that they do not affect the viability directly. Maldonado and Latz [24] also used thickeners to increase the viscosity for applying two different shear stress levels at the same shear rate to study the short-term effect of shear stress on a dinoflagellate.
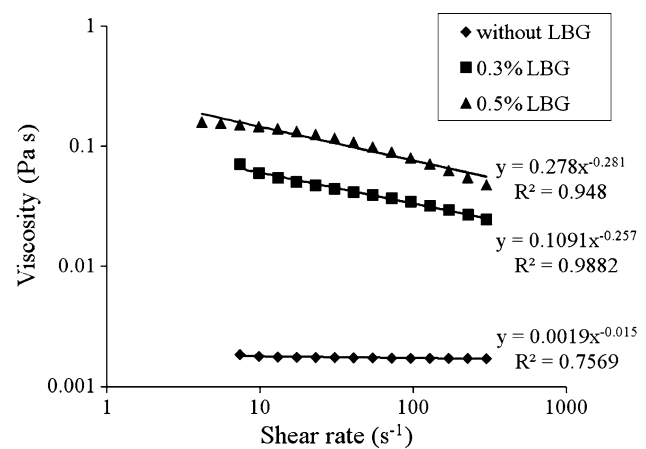
The dimensions of the shear cylinders are also important in creating a stable laminar flow, because the diameter of the inner cylinder and the gap between the inner and outer cylinder are relatively small. A Couette device with a bigger inner cylinder diameter and a relatively bigger gap leads to a smaller critical angular velocity with the formation of Taylor vortices or turbulent flow as a result [33, 34]. It is difficult to determine the applied shear stress if flow instabilities are present.

The effect of shearing treatment on viability

In all processing conditions tested, the shape of the cells was unaltered. This holds for the viable cells as well as for the non-viable cells. The total cell concentration of the algae that were exposed to the high shear stresses did not decrease either. The viable and non-viable cells could only be discriminated by the FDA staining method, where the viable cells showed fluorescence (Fig. 3). Obviously, the affected cells must be damaged internally without any visible external impact observed in quiescent conditions after the shearing treatment.

Table 1 Shear stresses applied and Taylor numbers in relation with shear rates

Rotational speed (rpm)	Shear rate (s^{-1})	Shear stress without LBG (Pa)	Taylor number 0% LBG	Shear stress 0.3% LBG (Pa)	Taylor number 0.3% LBG	Shear stress 0.5% LBG (Pa)	Taylor number 0.5% LBG
0	0	0	0	0	0	0	0
2	4.31	–	–	–	–	0.795	0.0052
4	8.63	0.016	1.04	0.541	0.031	1.31	0.013
10	21.57	–	–	1.07	0.097	2.53	0.041
15	32.36	–	–	1.44	0.162	–	–
20	43.14	0.077	5.39	1.78	0.232	4.15	0.100
100	215.7	0.376	27.6	5.89	1.76	13.2	0.783
500	1,079	1.83	141	19.4	13.3	–	–

The effect of shear rate on the viability is shown in Fig. 4. Without LBG, no effect of shear rate on the viability of the algae was observed. No effect on the viability of the algae in 0.3% LBG was seen until a shear rate of 22 s^{-1}. Higher shear rates applied to the algae in 0.3% LBG affected the algae negatively, reducing the viability to 56%. At a shear rate of 8.6 s^{-1} applied to the algae in 0.5% LBG, the viability dropped to a value of 59%. Increasing the shear rate reduced the percentage of viable cells slightly to 52%. There seems to be a slight difference between the viability levels of the samples with 0.3 and 0.5% LBG. We believe that these differences might be attributed to the fact that the experiments were done on different days. In addition, LBG did not affect the viability of the algae directly. No negative effect on the viability was seen when the algae in 0.3 and 0.5% LBG were not sheared. Furthermore, 100% viability was seen when low shear rates were applied to the algae in both LBG concentrations.

From Fig. 4, it can be seen that the shear rate is not the determining factor in explaining the negative effect on cell viability. Obviously, a higher viscosity leads to a lower shear rate at which a negative effect can be observed. When analyzing the effect of shear stress on the viability, the effects of shear rate and viscosity can be combined. The effect of all different levels of shear stress on the viability of Chaetoceros muelleri can be seen in Fig. 5.

Shear stress values up to 1 Pa had no effect on the viability. At shear stress levels higher than 1.3 Pa, a sharp drop in viability can be seen with resulting viabilities between 52 and 66%. The threshold value of shear stress for Chaetoceros muelleri is therefore to be found between 1 and 1.3 Pa. A further increase of the shear stress to 19.4 Pa did not reduce the viability significantly. The effect of shear stress on the viability of Chaetoceros muelleri can thus be described as a step response with an almost similar effect beyond the threshold value, which is represented by the dotted line in Fig. 5. This would suggest that only a certain percentage of the cells are sensitive to shear stress
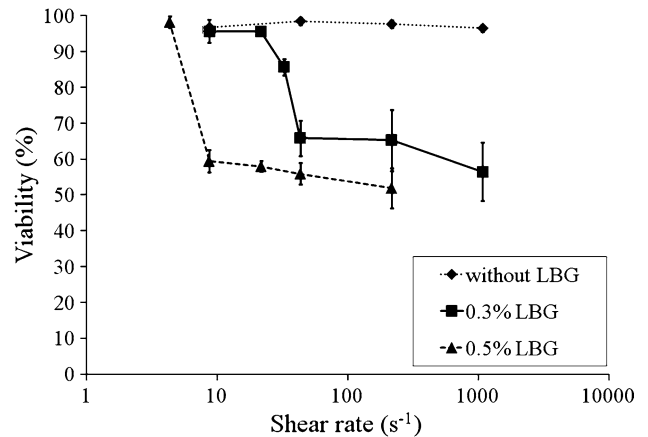

Fig. 4 Effect of shear rate on the viability of Chaetoceros muelleri; without LBG (diamonds), 0.3% LBG (squares) and 0.5% LBG (triangles). Error bars represent 95% confidence intervals
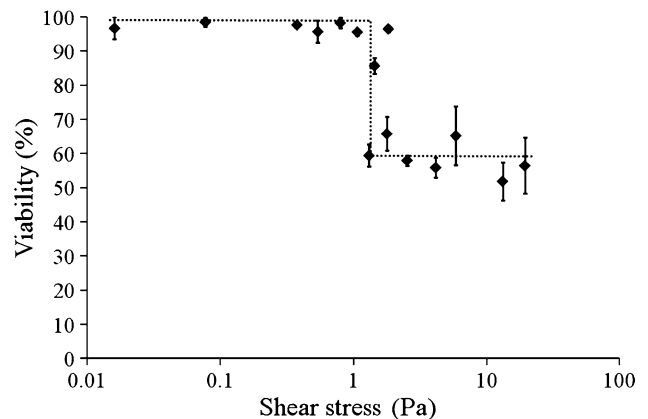

Fig. 5 Effect of shear stress on the viability of Chaetoceros muelleri with all treatments (without LBG, 0.3% LBG and 0.5% LBG) taken together. Error bars represent 95% confidence intervals

and that higher shear stresses than the threshold value, up to the maximum applied value of 19.4 Pa, only affect the sensitive cells. Although it is unclear which cells are more susceptible to shear stress, other studies have speculated that shear stress disrupts cell division [18, 35]. Dividing

Fig. 3 Microscopic pictures of Chaetoceros muelleri; with normal light (**a**) 11 cells can be counted and with fluorescent light (**b**) 8 viable cells show fluorescence
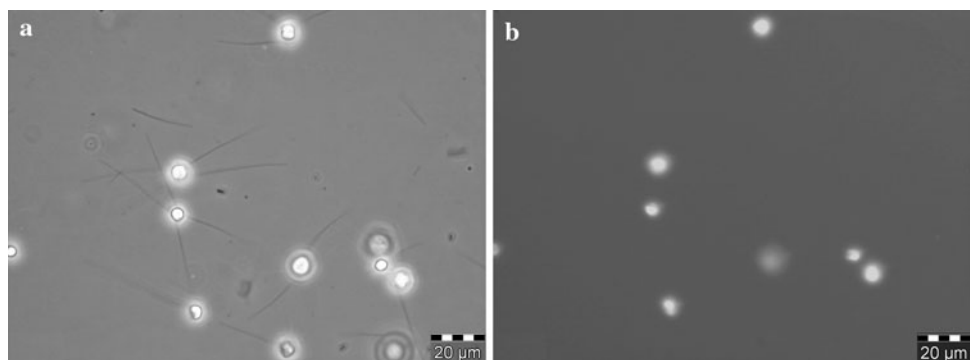
cells are probably more shear sensitive. Step responses to shear stress have previously been reported for enzymes [36] and starch molecules [37, 38], suggesting a more general role of shear stress in deactivation and breakage processes.

The only deviation from the general interpretation of our results is that a shear stress of 1.8 Pa could be applied to the algae without LBG at the highest shear rate without any detrimental effect on the viability. Flow instabilities like Taylor vortices could have occurred in that experiment, so that the results could have been influenced in a way that they are not reliable or comparable.

These results imply that a PBR for cultivating *Chaetoceros muelleri* should be designed in such a way that the maximum exhibited shear stress is lower than the threshold value. The question that now remains is whether this shear stress can occur in a PBR. Considering the study of Contreras et al. [17], shear rates up to 14,000 s^{-1} can occur, which implies a local shear stress of about 20 Pa. This value clearly exceeds the value reported above. In other words, shear stress is likely to negatively influence the algae in a PBR. However, reducing the fluid velocity too much can lead to other limitations, such as mass transfer, which also negatively influences the growth rate.

Time dependence of shear stress effect

Chaetoceros muelleri in 0.3% LBG was exposed to a constant shear stress of 5.89 Pa at a shear rate of 215.7 s^{-1} in the shear cylinders to elucidate the time dependence of the shear stress effects. The viability already decreased after 1 min to 82% and kept on decreasing at a reduced rate for the following 7 min. Longer exposure times to
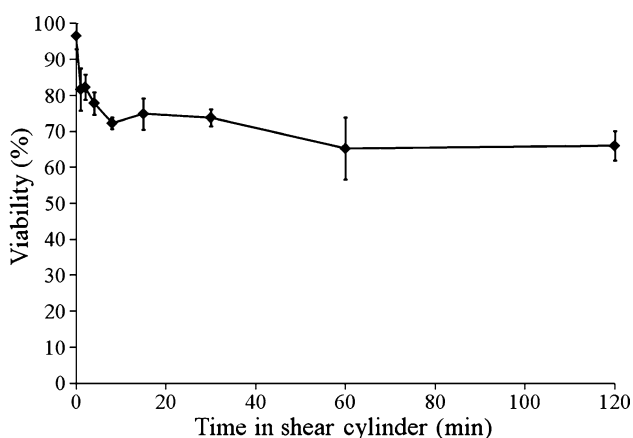

Fig. 6 The effect of time dependency of shear stress on the viability of *Chaetoceros muelleri*. *Error bars* represent 95% confidence intervals

shear stress did not reduce the viability any further. The viability was found to vary between 65 and 75% for all exposure times longer than 8 min (Fig. 6). This would suggest that the effect of shear stress is almost time-independent. Compared to normal cultivation times, the effect can be considered as almost instantaneous. The fact that the viability does not decrease completely indicates that only the more sensitive cells are susceptible to shear stress, while the more resistant cells are not affected by shear stress even over a longer period. This result is in contrast with a study done with hybridoma cells [39]. The viability of these mammalian cells, which were also sheared for time periods of less than 2 h, decreased over time. However, the shear stress levels applied were much higher and varied between 5 and 100 Pa. The time dependence of shear stress was also investigated with plant cells [40]. Although the main conclusion was that the effect of shear stress on plant cells was time dependent, it was clear that the first minutes of exposure to a certain shear stress were the most detrimental. The study of Dunlop et al. [40] also showed that similar shear stress levels and exposure times as in this study gave almost the same viability results. Complete cell death was only reached when significantly higher shear stresses (50–100 Pa) were applied to the cells. These high shear stress values are expected to be unrealistic in PBRs given the low viscosity of the medium used.

Conclusions

Shear stress had an adverse effect on the viability of *Chaetoceros muelleri* with a threshold value between 1 and 1.3 Pa. Shear stress higher than the threshold value caused a sudden decrease in viability to levels between 52 and 66%. It appears that only a certain fraction of the cells are susceptible to shear stress up to 19.4 Pa.

No external damage was observed, meaning that internal damage must have taken place in the affected cells.

The effect of shear stress was almost instantaneous as compared to regular cultivation times. The detrimental effect already took place after 1 min of exposure to shear stress. The viability did not decrease any further with shear stress exposure times longer than 8 min. This would suggest that the sensitive cells were affected by shear stress instantaneously and that high shear stress over a long period did not have a negative effect on the more resistant cells.

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