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CFD-aided optimization of a laboratory-scale centrifugation for a shear-sensitive insect cell line

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ABSTRACT

A study has been conducted to assess cell damage during centrifugation and to optimize this operation for the insect cell line Se301. Experiments were carried out in a discontinuous centrifuge using Falcon tubes of different sizes as containers. The cells were easily recovered from the cell suspension and the time required for complete sedimentation was found to be independent of tube size or design. Cell damage was observed once the cells were sedimented, therefore this process depends on both the residence time of the cells in the pellet and on the acceleration applied. The sensitivity of the cells to this operation was higher than for other naked microalgae or for very sensitive red blood cells. Indeed, excessive treatment produced cell damage that reduced the productivity of subsequent cultures. CFD simulations were carried out in 2D and a good agreement was found between the CFD and experimental values in terms of the time required for full sedimentation. In addition, this technique allowed the calculation of shear stress, a key variable in the study of cell sensitivity to flow.

1. Introduction

Insect cells have been extensively used to express biologically active recombinant proteins (Kost et al., 2005) and are currently being investigated for the production of biopesticides (Beas-Catena et al., 2014). Such cells present several advantages compared to mammalian cells and are easier to handle as they often do not require the presence of CO_2. Insect cell expression systems produce proteins similar to those produced in mammalian cells. Moreover, insect cells can be adapted to suspension culture, although some cell lines are very shear sensitive, thus meaning that protective additives are needed (Beas-Catena et al., 2011).

In addition to insect cells, many other cell lines are particularly sensitive to agitation in suspension cultures (Lara et al., 2006). Indeed, although animal cells have traditionally been shown to be especially shear-sensitive, various insect cell lines (Tramper et al., 1986; Chalmers, 1996; Beas-Catena et al., 2011) or microalgae (Gallardo-Rodríguez et al., 2016) are difficult to grow in traditional culture systems. However, not all respond in the same way when subjected to high levels of shear or energy dissipation in a short period of time (Gallardo-Rodríguez et al., 2016). In addition to the culture stage, there are many steps in laboratory procedures and productive bioprocesses in which cells may experience energy dissipation rates, shear stresses or pressures high enough to cause cell damage (Mollet et al., 2004; Xu et al., 2015; García-Briones and Chalmers, 1994). For example, pipetting (Mollet et al., 2004) or centrifugation to recover cells (Xu et al., 2015; Westoby et al., 2011) can often damage them. Indeed, the more sensitive the cell line the more evident and marked the effects will be (Urbina et al., 2016).

Centrifugation is a common cell-harvesting technique in large- (Axelsson, 2002; Hutchinson et al., 2006) and small-scale bioprocesses (Hutchinson et al., 2006; Peterson et al., 2012). However, the prediction of the performance of the centrifugation process at large-scale is very difficult due to the fragile nature of the biological materials (Boychyn et al., 2001). On a laboratory scale, it is an essential operation for the maintenance, preservation, routine subcultivation and setting up of varied protocols (Peterson et al., 2012). Depending on the shear resistance of the cells, the same centrifugation treatment can be harmless or provoke cell damage or cell disruption (Hutchinson et al., 2006; Xu et al., 2015). Excessively intense centrifugation can also affect

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the protein of interest structure (Hutchinson et al., 2006; Neal et al., 2002). A linear relationship between cell disruption by centrifugation and energy dissipation in a pilot-scale disc-stack centrifuge has also been found (Westoby et al., 2011). There is currently a wide variety of centrifugation protocols (i.e. intensity and duration of treatment, equipment, etc.) in the literature, with very few criteria for choosing between them (Peterson et al., 2012). For example, Agathos (2007) recommends 100–200 × *g* for 5 min for subculturing animal cells, whereas Lynn (2016) suggests 1000 × *g* for 10 min for maintenance and storage. We also observed variability in the cell yields obtained with the insect cell line Se301 when subculture was performed after centrifugation applying standard literature methods. Thus, it seems imperative to optimize the centrifugation process specifically for the cells of interest (data not published).

Centrifugal stress is present in different bioprocess steps and, depending on its intensity, distribution into cell suspension and the exposure time of the cells, it may damage cells (Xu et al., 2015) or cause cell surface damage that is undetectable using standard viability tests (Peterson et al., 2012). The source of this variability may also depend on other factors, such as the height of suspensions or the geometry of centrifugation containers, which are usually not reported but which could nevertheless modify the shear stress field experienced by the cells for a given *g* force. Computational Fluid Dynamics (CFD) may be useful for unifying centrifugation protocols since it has been shown to be useful for the characterization of centrifuges (Boychyn et al., 2004; Urbina et al., 2016). CFD allows shear rate fields or energy dissipation rates to be determined in flowing systems with living cells (Jain et al., 2005; Mollet et al., 2004; Gallardo-Rodríguez et al., 2016), which is essential for interpreting centrifugation-associated cell damage. CFD can also be used to reduce the experimental effort in selecting, designing or optimizing harvesting systems.

In this study, CFD-aided optimization of a discontinuous benchtop centrifugation process for the *Spodoptera exigua* Se301 insect cell line was addressed in terms of a novel variable named excess of treatment (*ET*), related to the height of suspension in tubes, *g* force and centrifugation time. Evaluation of the impact of centrifugation on subcultivation of cells was carried out using cells previously subjected to different *ET* values.

2. Materials and methods

2.1. Cell line, culture medium, additives, and maintenance

The insect cell line Se301, originally isolated from *Spodoptera exigua* (Hara et al., 1995), was used throughout this work and was kindly donated by the Department of Virology at Wageningen University (the Netherlands). Cells were grown in suspension culture in Ex-Cell 420 serum-free media (Sigma–Aldrich, Ref. 14420C) supplemented with 100 U L^{-1} penicillin-streptomycin (Sigma–Aldrich, Ref. P4333) and 0.125 μg mL^{-1} amphotericin B (Sigma–Aldrich, Ref. A2942). The protective additives against shear stress Polyvinyl Alcohol (PVA, Sigma–Aldrich, Ref. P8136) and Polyvinylpyrrolidone (PVP, Sigma–Aldrich, Ref. P2307), both at a concentration of 0.2% (w/v), and the disaggregant Dextran sulfate (DS, Sigma–Aldrich, Ref. 31404), at a concentration of 25 μg mL^{-1}, were used (Beas-Catena et al., 2013). The density and viscosity of the culture medium with additives was 1010 g mL^{-1} and 1.26 Pa s, respectively. Inoculum was grown in Erlenmeyer flasks of 250 mL (50 mL of culture) agitated at 75 rpm in an orbital shaker with an orbital diameter of 1.9 cm. Flasks were placed in an incubator at 27 °C and humidification was achieved using a water tray on the bottom of the incubator. Cells were passaged every 4 days at a cell density of 5–6 × 10^5 cell mL^{-1}. Cells in the exponential phase were used in all assays.

The cell diameter was determined using the AnalySIS® software from images taken under the light microscope with a Pixelink camera (model no. PL-A662). The mean cell diameter was 18 μm.

Cell concentration and viability in all experiments were measured using a hemocytometer under a light microscope and the Trypan Blue dye-exclusion method.

2.2. Centrifugation assays

Suspensions at a cell concentration of 1.5 × 10^6 cells mL^{-1} and a viability above 97%, obtained from the inoculum as described in Section 2.1, were deposited in Falcon tubes and clarified in a benchtop centrifuge (model SIGMA 4-15C) using a rotor with a maximum radius of 18.2 cm. After every centrifugation, the supernatant was withdrawn and the cell pellet carefully resuspended in 5 mL of fresh medium. Cell concentration and viability were measured for the resulting suspension and the efficiency of the clarification process (η_c) was defined as follows,

$$\eta_c = \frac{N_p}{N_i} \quad (1)$$

where N_p and N_i are the number of total cells in the pellet and in suspension prior to treatment, respectively.

Two types of Falcon tubes (F15 and F50, with a maximum capacity of 15 and 50 mL respectively) were used. The characteristic dimensions of the tubes based on length (l), inner diameter (ϕ), cone height (h_c) and cone base (b_c) were as follows: (i) F15 (l = 118.5, ϕ = 14.6, h_c = 24.0, b_c = 2.0 mm); F50 (l = 114.4, ϕ = 27.0, h_c = 14.0, b_c = 4.6 mm). Experiments were designed by selectively combining the following factors: type of tube (F15 and F50), height of suspension in the tubes (h, 22 and 90 mm), *g* force (g_c, 15 levels ranging from 0 to 4000 × *g*) and centrifugation time (t_c, 7 levels ranging from 0 to 45 min). A total of 68 trials were completed in duplicate. Experimental sedimentation times were determined from η_c versus time at η_c near 100% curves. Theoretical sedimentation times were estimated from Stoke's settling velocity equation given by,

$$v_{stoke} = \frac{2}{9} g_c \frac{r_c}{\mu} (\rho_s - \rho_l) \quad (2)$$

where g_c is the acceleration applied, r_c the radius of the cell, μ the viscosity of the culture media, ρ_s and ρ_l the densities of the cell (1.025 g mL^{-1}) and the culture media, respectively.

2.3. Sub-cultivation of previously centrifuged cells

To evaluate whether a given centrifugation treatment might have caused cell damage that was not detectable using viability measures, or if the damage observed by reduction of cell viability was reversible, previously centrifuged cells were sub-cultured. Briefly, cell pellets were resuspended in 4.5 mL of fresh medium and cultured for 7 days under the same environmental conditions as those for the inocula described above. Initial cell concentrations were fixed near 5 × 10^5 cells mL^{-1}. A control culture was carried out with non-centrifuged cells. All experiments were conducted in duplicate. Cell concentration and cellular viability were monitored at the beginning and end of the culture period (7 days).

Sub-cultured cells were obtained from different centrifugation treatments performed in F15 tubes, at an h of 22 mm, g_c varying from 0 to 4000 *g*, and t_c ranging from 0 to 16 min.