Project: Strategies for Continuous Culture

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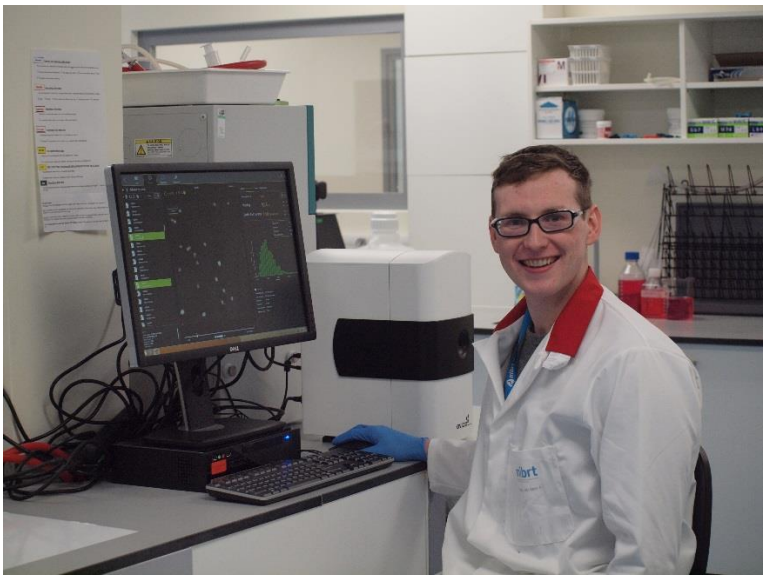

Background:

(a) Chemostat cultures are established without cell separation and allow steady state where growth rate= dilution rate. Chemostat cultures allow analysis of steady state with each parameter change (*Jan et al 1997; Kunkel et al, 2000*)

(b) Perfusion cultures have an associated cell separation eg: ATF or acoustic filter This allows high cell densities (>10^7/ml with associated process intensification (*Rodriguez et al, 2010*)

Proposal

(a) To establish control points in metabolism of producer cells.

Identifying critical process parameters that affect productivity through analysis of steady states

(b) Design a scale down process for biomanufacturing using a cell separator (eg: ATF)

Establish methods of control for enhanced productivity

Jan,D.C.H., Petch, D., Huzel, N. and Butler, M. 1997. The effect of dissolved oxygen on the metabolic profile of a murine hybridoma grown in serum-free medium in continuous culture. Biotech. Bioeng. 54 (2), 153-164.

Kunkel, J.P., Jan, D.C.H., Butler,M. and Jamieson, J.C. 2000. Comparisons of the glycosylation of a monoclonal antibody produced under nominally-identical cell culture conditions in two different bioreactors. Biotechnol. Progress 16 (3), 462-470.

Rodriguez J, Spearman M, Tharmalingam T, Sunley K, Lodewyks C, Huzel N and Butler M. High productivity of human recombinant beta-interferon from a low-temperature perfusion culture. J.Biotechnol.. 150: 509–518 2010.

Adam Bergin and Michael Butler. Bioproduction Congress, KNect365, Dublin in October 2018. "*On-line and Off-line monitoring of CHO cell health in a bioreactor to allow for early identification of cell death*."

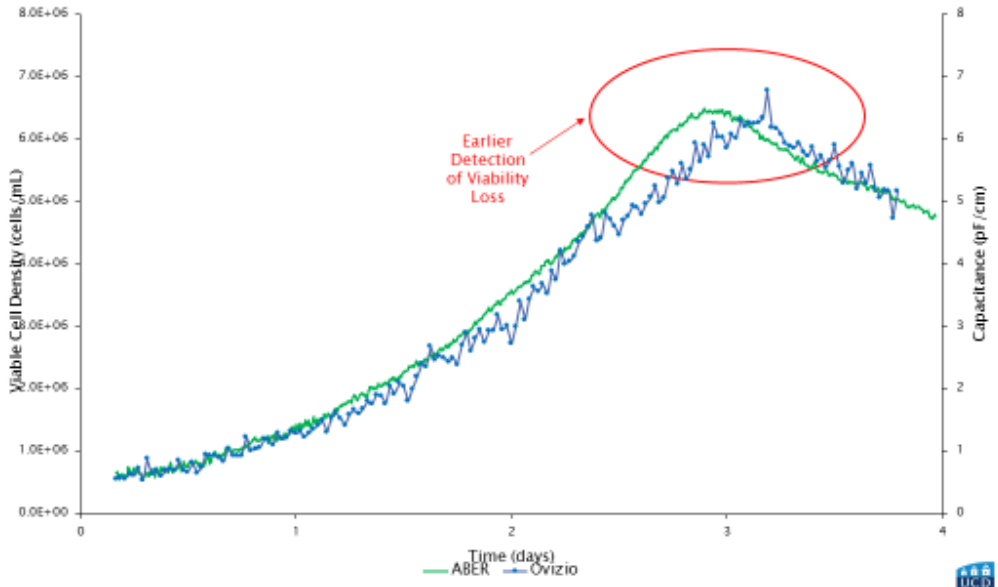

Figure 5: Ovizio VCD curve plotted against ABER capacitance curve

The figure above shows continuous on-line monitoring of the growth of CHO cells in an Applikon bioreactor as measured by biocapacitance (Aber) and by light holography (Ovizio).

Strategies for continuous culture of mammalian cells.

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Background

Batch/fed-batch processing is the most common method of monoclonal antibody (mAb) production at commercial scale. **Continuous bioprocessing** is advantageous over batch/fed-batch for reasons including higher achievable cell densities, less residence time of the product in culture and greater process flexibility. Optimisation of inline process analytical technologies (PAT) will **enhance process performance** and product critical quality attributes (CQA). Cell capacitance readings via an inline biomass probe can be converted to estimate the cell density of a culture. In conjunction with an alternating tangential flow (ATF) harvest column, mammalian cultures can be run at higher VCD ranges with improved product CQA's.

Trypan blue exclusion dye is routinely used for whole cell counting, however offline samples are required to perform these counts. Trypan blue and **biomass probe** counts have shown to give similar readings during exponential cell growth (Braasch *et al.* 2013). This relationship is lost when cells enter the death phase. There is information available in literature as to the reasoning behind this loss of comparability, especially between nuclear and whole cell counting techniques. Crystal violet nuclear staining have shown cell counts increased up to 30% of those seen in whole cell stains such as trypan blue exclusion (Berry *et al.* 1996). Biomass probes measure the **bulk capacitance** of a culture to determine the total biomass.

Project Plan

Compare biomass probe data to trypan blue and crystal violet stains. (Batch Cultures)

→ Media selection for use with CHO cells in perfusion cultures.

→ Establish perfusion cultures with ABER probe installed (5L bioreactor connected to an ATF harvest column)

→ Perform metabolite analysis and examine CQA of product at varying VCD setpoints.

Cell capacitance

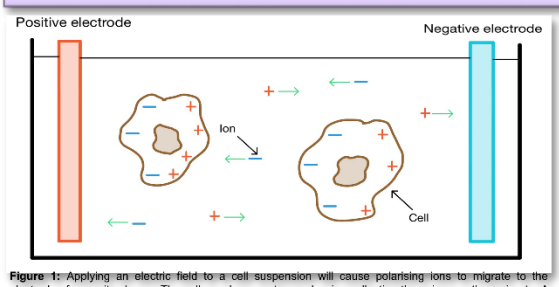

Figure 1: Applying an electric field to a cell suspension will cause polarising ions to migrate to the electrode of opposite charge. The cell membrane acts as a barrier, collecting these ions as they migrate. A charge separation is formed within the cell, which can be measured as capacitance (C). Adapted from (Davey *et al.* 1993)

Higher bulk capacitance values are obtained with an increase in polarized membranes in culture. The change in the direction of the electric field (frequency) can be plotted as a function of the capacitance. Cell capacitance is a function of the dielectric permittivity of cells ($\Delta\varepsilon$), which can be used to determine the biomass of a culture:

$$\Delta\varepsilon \propto NR^4$$

N is defined as the number of cells and R is the cell radius (Braasch *et al.* 2013). The following equation has shown to deviate as cells enter the apoptotic phase of cell growth. We will investigate if this variance is due to any changes in the nuclear structure of cells using a crystal violet stain, which cannot be identified by bulk capacitance measurements.

Continuous perfusion culture setup

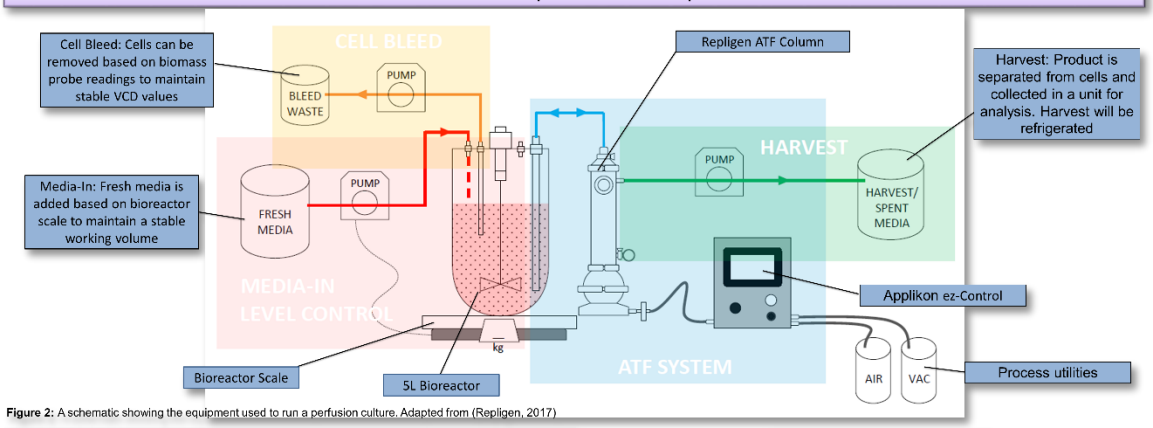

Figure 2: A schematic showing the equipment used to run a perfusion culture. Adapted from (Repligen, 2017)

Repligen ATF Column

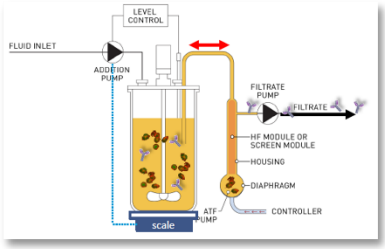

Figure 3: Basic operation of an ATF column. The flow of culture onto the column can be alternated using a diaphragm pump, reducing filter fouling. The screen will retain cells whilst separating the product into the filtrate stream. (Repligen, 2017)

Cited Literature

Berry, J., *et al.* (1996). "The crystal violet nuclei staining technique leads to anomalous results in monitoring mammalian cell cultures." Cytotechnology **21**(1): 73-80.

Braasch, K., *et al.* (2013). "The changing dielectric properties of CHO cells can be used to determine early apoptotic events in a bioprocess." Biotechnology and bioengineering **110**(11): 2902-2914.

Davey, C. L., *et al.* (1993). "Introduction to the dielectric estimation of cellular biomass in real time, with special emphasis on measurements at high volume fractions." Analytica Chimica Acta **279**(1): 155-161.

This work is supported by:

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