EVALUATION OF PSEUDO-PERFUSION FEEDING STRATEGIES FOR mAB PRODUCTION USING A CHO CELL LINE ADAPTED TO CONCENTRATED FEED MEDIA

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Key Words: pseudoperfusion, adaptation, osmolality, basal and concentrated media, feeding strategy

Perfusion has been intensively studied for biopharmaceuticals production due to the high volumetric productivities over long periods in operation. However, high dilution rates (D) used to feed basal media for therapeutic protein production imply in the generation of large harvest volumes, causing a dilution of the product of interest (POI) and increasing purification costs. Previous pseudo-perfusion studies of our group have indicated the possibility of getting a more concentrated POI (a monoclonal antibody produced by a CHO cell line) in the harvest stream using as perfusion feed a concentrated medium (as opposed to basal medium) at lower dilution rates. However, a decrease in cell viability and volumetric productivity (P_v) due to the higher osmolality related to the concentrated media were observed over cultivation time.

In the present work, the same CHO cell line was first adapted to higher osmolality levels by using a concentrated feed that was originally designed for fed-batch processes (TCX2D, Xell AG, Germany), instead of using NaCl or another osmolyte to enhance osmolality. Blends at pre-defined ratios of basal medium (TC-LECC, Xell AG, Germany) and concentrated feed were prepared in order to establish an osmolality range between 280 and 380 mOsm kg^{-1}.Cells were gradually passaged in high osmolality media for approximately one month and considered adapted when the viability recovery was higher than 90%. After adaptation of cells, small-scale pseudo-perfusion runs were carried out, starting the change of medium at the mid-exponencial cell growth phase. The glucose ratio (Glc_{rel}), used as a factor of glucose increment, was calculated assuming the basal media as the control condition, and the range of D for each condition was determined as the inverse proportion related to Glc_{rel}. Hence, basal medium (Glc_{rel} =1.0, 280 mOsm kg^{-1}) was fed in the range of 0.15 – 1 vvd, while media mixtures with Glc_{rel} ~ 2.0 (> 350 mOsm kg^{-1}) were fed at 0.07 – 0.50 vvd.

Cell viability was kept higher than 90% during at least 17 days of experiment, and viable cell densities (VCD) as high as 50E6 cells/mL were reached for all experimental conditions, including for the higher osmolality level (380 mOsm kg^{-1}). Previous studies using cells not adapted to grow in concentrated media indicated a rapid decrease of viability as the Glc_{rel} increased, probably due to high osmolality. A pseudo steady-state at 40-50E6 cells/mL was established for all conditions through bleeding at rates between 0.02-0.32 vvd, to avoid oxygen depletion. For the conditions of higher osmolality (350 and 380 mOsm kg^{-1}), perfusion rate was increased at 1 vvd at late stage of run in order to avoid glucose starvation. The increase of perfusion rate has promoted a residual glucose concentration higher than the control condition (280 mOsm kg^{-1}, fed only with the basal medium) and hence, a cultivation time longer than 20 days was achieved for both conditions.
The profile of accumulated product suggested that a slightly higher osmolality level could enhance product titers over cultivation time. Although high cell density and high viabilities were achieved for all experimental condtions, volumetric productivity (P_v) values were, in average, 50% lower in comparison to the control condition.