Optimization of the medium perfusion rate in a packed-bed bioreactor charged with CHO cells

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Abstract

In the present study, the optimal medium perfusion rate to be used for the continuous culture of a recombinant CHO cell line in a packed-bed bioreactor made of Fibra-Cel® disk carriers was determined. A first-generation process had originally been designed with a high perfusion rate, in order to rapidly produce material for pre-clinical and early clinical trials. It was originally operated with a perfusion of 2.6 vvd during production phase in order to supply the high cell density ($\sim 2.5 \times 10^7$ cell ml^{-1} of packed-bed) with sufficient fresh medium. In order to improve the economics of this process, a reduction of the medium perfusion rate by -25% and -50% was investigated at small-scale. The best option was then implemented at pilot scale in order to further produce material for clinical trials with an improved second-generation process. With a -25% reduction of the perfusion rate, the volumetric productivity was maintained compared to the first-generation process, but a -30% loss of productivity was obtained when the medium perfusion rate was further reduced to -50% of its original level. The protein quality under reduced perfusion rate conditions was analyzed for purity, *N*-glycan sialylation level, abundance of dimers or aggregates, and showed that the quality of the final drug substance was comparable to that obtained in reference conditions. Finally, a reduction of -25% medium perfusion was implemented at pilot scale in the second-generation process, which enabled to maintain the same productivity and the same quality of the molecule, while reducing costs of media, material and manpower of the production process. For industrial applications, it is recommended to test whether and how far the perfusion rate can be decreased during the production phase – provided that the product is not sensitive to residence time – with the benefits of reduced cost of goods and to simplify manufacturing operations.

Introduction

The biotech industry makes an extensive use of mammalian cells for the manufacturing of recombinant glycoproteins for human therapy.

Today, fed-batch and perfusion cultures are the two dominant modes of industrial operation for the mammalian cell culture processes that require large amount of proteins (Hu and Aunins 1997). Whatever the production technology of choice is,

development efforts are continuously invested in order to obtain production processes that warrant: high volumetric productivity, batch-to-batch consistency, homogenous product quality at low costs.

The decision between fed-batch or perfusion production mode is mainly dictated by the biology of the clone and the property of the product, and has to be done case-by-case during the course of the development of a new drug product (Kadouri and Spier 1997).

When the selection is a perfusion process, one of the culture systems of choice is stationary packed-bed bioreactor in which cells are immobilized onto solid carriers. This system is easy to operate and with appropriate carriers and culture conditions very high cell density (of $\sim 10^7$–10^8 cell ml^{-1}) can be achieved.

A consequence of this high cell density is the need for an intensive medium perfusion rate (feed and harvest) that should be used in order to keep the cells viable and productive. It appears that the perfusion rate is one of the central parameters of such a process: it drives the volumetric protein productivity, the protein product quality and has a very strong impact on the overall economics of the process.

Therefore, at industrial scale the optimal stationary packed-bed bioreactor process should operate with a perfusion rate as low as possible without compromising on quantity and quality of the product.

In the course of reducing the perfusion rate, several studies were conducted where the concentration of glucose (Dowd et al. 2001; Wang et al. 2002) is used as an indicator of other nutrients level in the feed medium in order to operate the bioreactor at a low perfusion rate without accumulating in the culture high levels of toxic by-products such as lactate and ammonia (Racher et al. 1993; Sugiura and Kakuzaki 1998). Modification of culture parameters such as pH or temperature (Chuppa et al. 1997) is also a common strategy to optimize culture conditions and reduce medium perfusion needs.

In order to achieve optimal medium perfusion rate, three approaches can be considered:

(a) **To fix perfusion rate** at a constant value during the entire production run. This approach is usually preferred in industrial production processes, since it is simpler to operate in a robust and consistent way. It also has the advantage to define medium costs of the process as there is no variation in perfusion rate from run to run.

(b) **To adjust perfusion rate** in response to cell number and/or nutrient consumption such as glucose (Oh et al. 1994; Dowd et al. 2001; Gorenflo et al. 2003), glutamine (Gorenflo et al. 2002), or oxygen (Kyung et al. 1994). Although this approach provides a more scientific rationale for adjusting the perfusion rate, it may lead to an overgrown culture and an 'out-of-control' increase of the perfusion rate. When cells are cultured in suspension mode, a 'culture bleed' is done to avoid overgrowing the culture, but this is not possible when cells are immobilized on a carrier. So in general, this approach is not preferred for manufacturing operations as it is difficult to operate in a robust and consistent manner and the medium perfusion rate needs to be readjusted on a daily basis.

(c) **To combine both strategies** (a) and (b) with an initial cell propagation phase (or 'growth phase') where the perfusion rate is progressively increased according to cell growth requirements during the growth phase followed by a shift of culture conditions such as temperature and/or pH in order to stabilize and keep cell metabolism at a relatively low and constant level. At this stage, the perfusion rate can be reduced to a fixed value, matching the reduced need of the cells throughout the production phase.

This article describes such a process based on the high cell density culture of recombinant CHO cells in a packed-bed bioreactor, in which perfusion rate was adjusted according to cell growth requirements during the growth phase and then pronouncedly reduced during the production phase without compromising on process productivity or protein quality.

A first-generation process had originally been designed with the aim to rapidly produce material for pre-clinical and early clinical trials.

This process was designed with a high perfusion rate of 2.6 vvd in order to supply the high cell density ($\sim 2.5 \times 10^7$ cell ml^{-1} of packed-bed) with fresh medium during production phase. This saved development time as we avoided spending time to develop a balanced medium formulation that is

well adapted to the cell culture needs, and the product degradation was not a concern since the high dilution rate imposed to the culture maintained a low residence time of our product in the bioreactor environment.

At a later stage of the development, we investigated a reduction of the medium perfusion rate by -25% and -50% in order to improve the economics of this process. A small-scale system was used to run the tests, and the selected conditions were then implemented at pilot scale in order to further produce material for clinical trials with an improved second-generation process.

It is well known that modification of the perfusion rate during a perfusion process, as well as modification of other bioprocess factors, can influence the recombinant protein quality and in particular its glycosylation pattern (Jenkins et al. 1996; Andersen et al. 2000). Glycosylation is usually recognized as an important function in the solubility, immunogenicity, and pharmacokinetic properties of human glycoproteins and those are the key parameters in the safety and clinical efficacy of a product (Goochee et al. 1991).

In this study, we used as a model a recombinant CHO cell line expressing a heavily glycosylated therapeutical protein for which the purity, N-glycan sialylation level, abundance of dimers or aggregates, were closely monitored in order to ensure consistent product quality. This model served as a basis for the study of the impact of a reduction of the medium perfusion rate on the process performance and product quality.

Materials and methods

Cell culture – experimental system

A packed-bed bioreactor (Ducommun et al. 2002a, b) with Fibra-Cel® carrier (Bibby Sterilin, UK) was used to cultivate CHO cells (Laboratoires Serono, Corsier-sur-Vevey, Switzerland) in a serum free medium (Sigma C-9486). In the small-scale system that was used to investigate a reduction of the perfusion rate, the bioreactor and packed-bed had a working volume of 15 and 5 l, respectively.

The bioreactor was perfused with 2.6 vvd (as defined in Table 1) during growth and production phase. This basic perfusion rate is chosen as the reference 100%, stated as run-100.

These conditions were applied for all sets of bioreactor experiments: medium was perfused at 100% during growth phase at 37 °C. The temperature was regulated at 37.0 °C during growth phase, and then reduced in two steps down to 32.5 °C. The pH was regulated at 7.00 and dissolved oxygen concentration (DO) was maintained at 70% of air saturation throughout the culture.

Due to the fact that counting of cells and cell number determination in a packed-bed bioreactor is a complex and inaccurate analysis, we used glucose consumption rate (GCR) as an indirect method to estimate cell growth and density in the packed-bed bioreactor. In our system, we have determined by direct cell counts on a number of packed-beds cultures that a GCR of 300 g of glucose per kilogram of Fibra-Cel® disks per day corresponds to approximately 2.5×10^7 cells per ml of packed-bed bioreactor volume (data not shown).

This stage has been defined as the end of the cell propagation phase at 37 °C, and when GCR reached a level of 300 g of glucose per kilogram of Fibra-Cel® disks per day, the cultures were switched from 37.0 °C to the production mode by lowering the temperature to 33.5 °C. At this stage, in one set of bioreactors the perfusion was kept at 100% (run-100) and the two other sets were performed with a medium perfusion rate of 75% (run-75) and 50% (run-50) of the maximal level, as summarized in Table 1. The temperature was

Table 1. Perfusion rates tested during the production phase for run-100, run-75 and run-50.

	Perfusion rate (1 $kg_{Fibra-Cel}^{-1}$ day^{-1})	Dilution rate (vvd*)	Replicates (n)
run-100	100 ± 3.5	$2.6 . \pm 0.1$	8
run-75	75	2.0	3
run-50	50	1.3	2

Average of n replicates (± 2 standard deviations for run-100).
*The dilution rate D expressed in vvd is calculated as litre of medium per litre of total system working volume per day (total volume = packed-bed + conditioning tank volume).

further decreased to 32.5 °C at a later stage of the production phase to prevent further cell growth and to promote production.

In the results section, the bars displayed on the figures represent an interval of four standard deviations (±2 standard deviation) measured for the eight replicates ran under the conditions of run-100.

Assays

Samples were removed daily during the culture. Glucose and lactate concentrations were quantified with an EML 105 analyzer (Radiometer Medical, Brønshøj, Denmark).

The recombinant protein was quantified with an ELISA test.

The quality of the recombinant protein was assessed with an RP-HPLC method in combination with SDS-PAGE methods (ExcelGel SDS Homogeneous 12.5% (Cat# 80-1261-01, Pharmacia). The gels were stained specifically by Western blot in order to detect high molecular weight (i.e. dimers, aggregates) or low molecular weight variants of the r-protein. Non-specific staining by Silver Stain was also used to assess the relative intensity of the r-protein compared to impurities after scanning (Scanner ARCUS 2, Afga) of individual bands in order to determine their relative intensity.

Protein sialylation, and in particular the abundance of neutral, mono-, di-, tri- and tetra-sialylated *N*-glycans was analyzed by separation of the *N*-glycans according to their charge, as described by Gervais et al. (2003). The *N*-glycans were specifically cleaved from the r-protein by hydrazinolysis (*N*-glycanase E-5006B or E-5006C, Glyko Inc.), labelled with the 2-aminobenzamide fluorescent dye (Signal 2-AB labelling kit K404, Glyko), and separated by a weak anion exchanger chromatography column (DEAE GlycoSep C, Glyko) before passing through a fluorescent detector. The proportions of *N*-glycans species could then be determined after integration of the HPLC peaks corresponding to neutral, mono-, di-, tri-, and tetra-sialylated forms.

Results

The first attempt was to reduce the perfusion rate from 2.6 to 2.0 vvd and 1.3 vvd during the production phase (Figure 1) and to follow its effect on cell
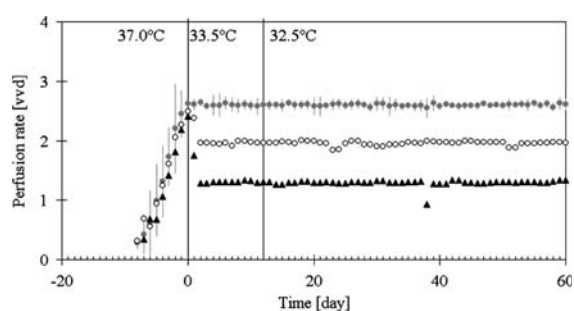

Figure 1. Medium perfusion rate during continuous cultures of CHO cells in a packed-bed bioreactor, with perfusion levels of 2.6 vvd (●), 2.0 vvd (○) and 1.3 vvd (▲). The bioreactor runs have been labelled as run–100 for 100% perfusion (●), run–75 for 75% perfusion (○) and run–50 for 50% perfusion (▲). The maximum perfusion rate of 100% corresponds to 2.6 vvd that were used as the reference conditions.

metabolism, volumetric productivity and product quality.

The concentration of glucose and lactate were measured for all three perfusion rates (Figure 2), and the residual glucose level remained above 0.5 g/l.

The results in Figure 3 show the GCR levels for the three sets of medium perfusion rate tested. These results indicate that a reduced perfusion rate
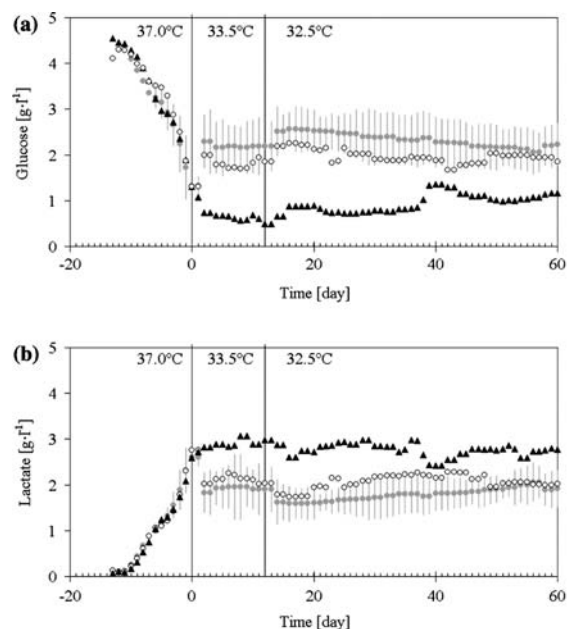

Figure 2. Glucose (a) and lactate (b) concentration profiles during continuous cultures of CHO cells in a packed-bed bioreactor, for medium perfusion rates of 100% (●) 75% (○) and 50% (▲).
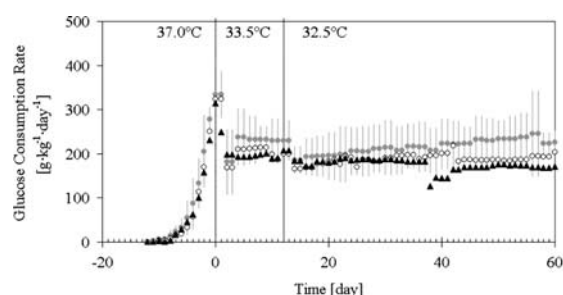
Figure 3. Glucose consumption rate (GCR) profile during continuous cultures of CHO cells in a packed-bed bioreactor, for medium perfusion rates of 100% (●) 75% (○) and 50% (▲). GCR is expressed in grams of glucose consumed per day and per kg of Fibra-Cel carrier.

induces a lower GCR of the culture. However, this effect was barely significant and when the medium perfusion rate was reduced by −25% and −50%, the average GCR measured over the 60-day production phase were reduced by −8% and −15%, respectively (Figure 3).

In parallel, the apparent molar ratio (Figure 4) of glucose conversion to lactate $Y_{lac/glc}$ slightly decreased in response to lower perfusion rate but remained in a range of 1.55–1.65 mole of lactate produced per mole of glucose consumed. The differences observed for the $Y_{lac/glc}$ ratio were not statistically significant since all data points measured for the test runs at reduced perfusion rate are comprised within ±2 standard deviations of values obtained with the reference runs.

Process productivity

The results presented in Figure 5 show a comparison of the recombinant protein produced
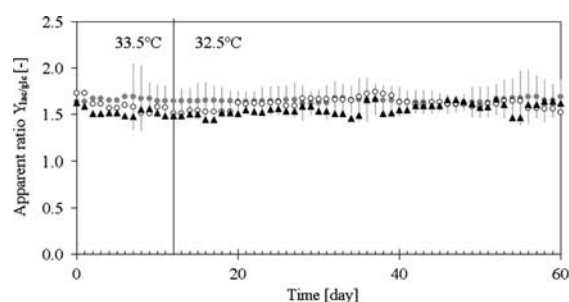

Figure 4. Apparent lactate from glucose molar conversion ratio profile during continuous cultures of CHO cells in a packed-bed bioreactor, for medium perfusion rates of 100% (●) 75% (○) and 50% (▲).
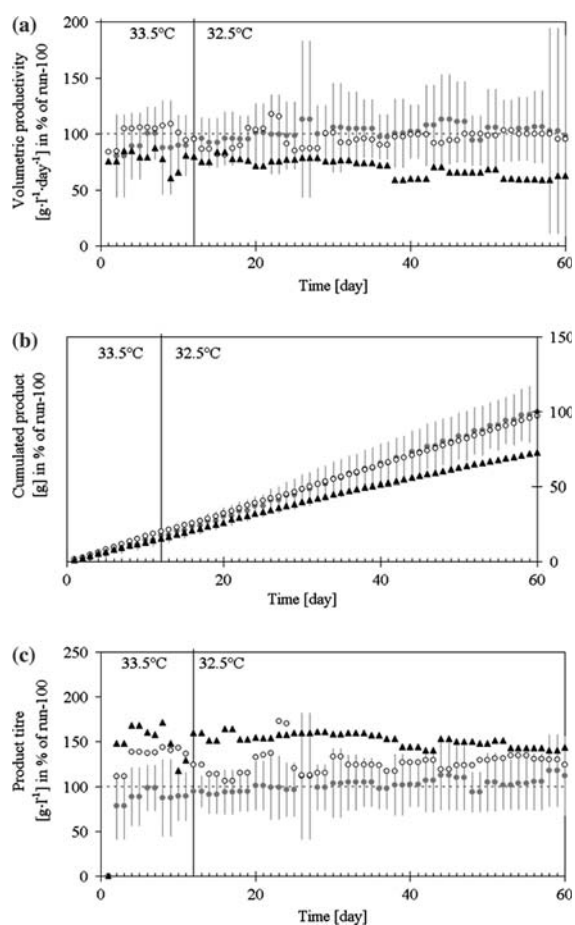

Figure 5. Normalized productivity data (a) volumetric productivity in units of product per total culture volume per day, (b) cumulated product in units of product, (c) titre in units of product per total culture volume, for a r-protein which is produced during continuous cultures of CHO cells in a packed-bed bioreactor, for medium perfusion rates of 100% (●) 75% (○) and 50% (▲). To normalize the data, the average value obtained at 100% perfusion rate over the 60-day production phase was taken as 100% productivity (dotted line).

(volumetric productivity, total production, and titre) in the reference run-100 and in the runs with reduced medium perfusion.

When the medium perfusion rate was reduced by −25% and −50%, the average volumetric productivity was reduced by −3% and −30%, respectively (Figure 5a). The lower productivity result obtained for run-50 are statistically different from the reference conditions.

Another difference observed in Figure 5a is the decline of the volumetric productivity over time along the production phase. A stable volumetric

productivity was observed for run-100 and run-75, but in run-50 productivity declined over the duration of the 60-day production phase. More specifically, the productivity level of run-50 was only 60% of the reference value at the end of the 60-day production phase.

The lower productivity of run-50 is also shown on Figure 5b, which represents the cumulated amount of product made over the 60-days production phase.

The corresponding titres measured for the different perfusion rates are presented in Figure 5c, which shows that recombinant protein titres were increased by +25% and +50%, respectively as opposed to the control when the perfusion was decreased by −25% and −50%.

Product quality

With the reduction of the perfusion rate from 2.6 to 2.0 vvd and 1.3 vvd that was tested for run-100 run-75 and run-50, the residence time (τ) of the recombinant protein was increased from 0.4 to 0.5 and 0.8 day respectively ($\tau = 1/D$).

As longer exposure to the environment in the bioreactor could potentially lead to a degradation of the recombinant protein, a preliminary stability study was done before initiating the tests in bioreactors. A purified sample of the r-protein was spiked into cell culture medium, and the quality attributes of the r-protein were monitored after 1, 2 and 5 days of incubation at 37 °C. Since no sign of product degradation was detected by the stability indicating method (data not shown), it was decided to proceed with the bioreactor experiments.

During the tests in bioreactors, the recombinant protein was purified to homogeneity at three points of the bioreactor runs (day 20, 40 and 60) in order to verify that the product quality was maintained for each perfusion rate investigated. The conditions of run-50 were considered as a worst case for protein degradation since this run had the lowest perfusion rate and the longest product residence time in the bioreactor.

To evaluate if product quality was affected by the reduced perfusion rate, final bulks of run-50 were analyzed by SDS-PAGE Silver Stain and SDS-PAGE Western blot, and compared to the profile obtained in the reference conditions of run-

100. The corresponding data obtained for one bulk produced with r-protein harvested at days 47 and 48 of the production phase of run-50 are presented in Figures 6 and 7. No modification of the r-protein quality attributes was detected for run-50 compared to run-100. The electrophoretic purity measured by SDS-PAGE Silver Stain in Figure 6 was higher than 99% purity for all lanes, and no presence of aggregates or truncated forms could be detected as shown on the SDS-PAGE Western blot in Figure 7.

The high level of purity ∼100% of the r-protein produced was confirmed by RP-HPLC for run-100, run-75 and run-50. Finally, the amount of impurities derived from the host CHO cell (Host Cell Proteins) in the purified protein was quantified by HCP-ELISA (data not shown) and was found consistent among run-50, run-75 and run-100.

Samples of the recombinant protein of interest were also submitted to *N*-glycan mapping in order to quantify the proportion of the different sialylated forms of the protein of interest. The *N*-glycan mapping results summarized in Table 2 show that comparable proportions of *N*-glycans are obtained for all perfusion rates tested. This is also illustrated
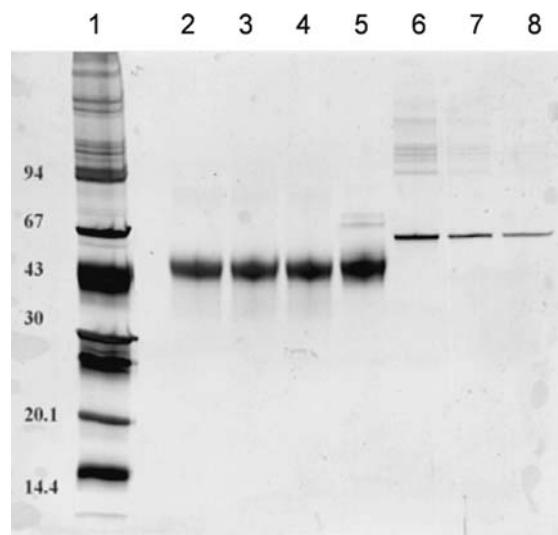

Figure 6. Electrophoretic purity by SDS-PAGE Silver Stain method. Lane 1: molecular weight markers. Lanes 2, 3, 4: final bulk obtained at 50% perfusion rate (run-50) at production day 47–48 analyzed in triplicate; Lane 5: reference material obtained at 100% perfusion rate (run-100); Lane 6: BSA 5%; 7: BSA 2%; 8: BSA 1%.
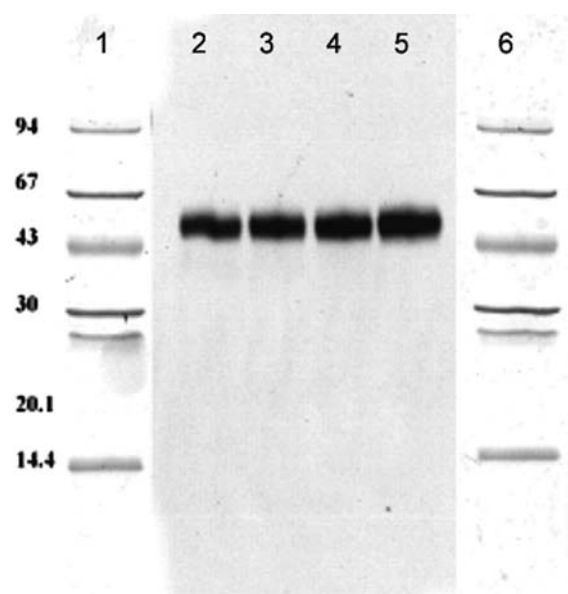
Figure 7. Electrophoretic purity by SDS-PAGE Western blot method. Lane 1: molecular weight markers. Lanes 2, 3, 4: final bulk obtained at 50% perfusion rate (run-50) at production day 47 and 48 analyzed in triplicate; Lane 5: reference material obtained at 100% perfusion rate (run-100). Lane 6: molecular weight markers.

Figure 8. Sialylation by *N*-glycan mapping (RP-HPLC profiles) in intermediate bulk samples at production day 47 and 48 of run-50, run-75 and run-100.

by the corresponding HPLC profiles reported in Figure 8.

A comparison of these data demonstrates that the product sialylation is not altered under conditions of reduced perfusion rate, and the data obtained for run-75 and run-50 are clearly within the range obtained under the standard conditions of run-100.

Discussion

The results show that the strategy to fix the perfusion rate at a defined value allows the system to stabilize and to operate at quasi steady state over
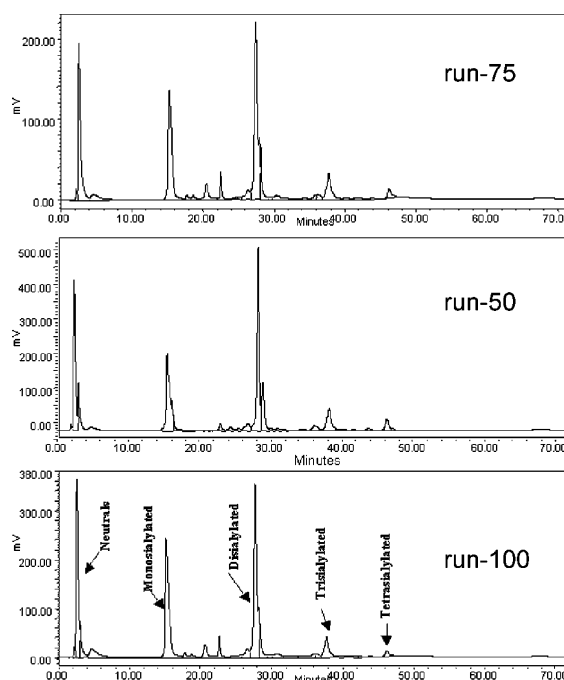
the 60-days production phase at 33.5/32.5 °C, this is confirmed by the glucose and lactate levels (Figure 2) that are maintained at a stable level throughout the production phases in run-100, run-75 and run-50. During the development of a perfusion process producing EPO, it was also observed that a constant perfusion rate allowed the system to approach steady state, in which the concentration of the main components were maintained at a constant level (Wang et al. 2002).

The reason for a lower GCR observed under lower perfusion rate was investigated. This phenomenon was already characterized in a similar packed-bed culture where a reduction of the cellular specific glucose consumption was measured

Table 2. Fraction of differently sialylated *N*-glycan molecules (given as % of the *N*-glycan groups) in semi-purified samples of drug substance for run-100, run-75 and run-50.

	Fraction of differently sialylated *N*-glycan molecules				
	Neutrals (%)	Mono-sialylated (%)	Di-sialylated (%)	Tri-sialylated (%)	Tetra-sialylated (%)
Run-100	19–21	21–29	39–44	7–11	2–4
Run-75	18–22	20–23	44–50	8–9	3–4
Run-50	19–21	20–25	45–48	8–10	2–4

when the temperature was decreased to 33.5/ 32.5 °C (Ducommun et al. 2002b). The hypothesis that it could be due to cell loss under lower perfusion was ruled out by the fact that the cell density in suspension in the cell culture medium was always below 0.1×10^6 cell ml^{-1} in all runs, which indicates that very few cells detached from the packed-bed over the duration of the runs.

Similar studies were made by Hiller and Miller, where they analyzed the metabolic response of hybridoma cells after changes in dilution rate (Miller et al. 1988; Hiller et al. 1991). When the dilution rate was decreased from 1.0–1.3 to 0.3–0.4 day^{-1} the ratio $Y_{lac/glc}$ decreased from 1.9–2.1 to 1.6 at lower perfusion rate. The response of $Y_{lac/glc}$ to variation in the perfusion rate is not always so marked, and Banik and Heath (1996) observed with another hybridoma cell line that $Y_{lac/glc}$ was constant in a range of 1.39–1.41 and was unaffected by changes in perfusion rates.

In this study, the $Y_{lac/glc}$ ratio was not significantly modified at -25% or -50% perfusion rate since all data points measured for the test runs were comprised within ± 2 standard deviations of values obtained for the reference runs.

As the cellular metabolism remained unchanged under reduced perfusion conditions, one could expect the cellular productivity of the r-protein to be maintained as well. This was in fact observed, and the volumetric productivity was maintained (-3%) compared to the reference process when the perfusion rate was reduced by -25%. But when the perfusion was further reduced by -50% of its original level, the productivity dropped by -30%.

In the conditions of run-50, the lower productivity obtained might either be linked to the depletion of a key substrate present in the cell culture medium (amino acids, vitamins, etc.) or to the inhibition of one of typical by-products (such as lactate or ammonia), two major causes of productivity loss commonly reported under low perfusion conditions (Kurokawa et al. 1993).

To investigate this question, de la Broise et al. submitted a culture of suspension hybridoma cells secreting IgM to variations of perfusion rate, while the cells were fully retained in the bioreactor system by tangential-flow filtration (de la Broise et al. 1991). After equilibrium, the dilution rate was increased from 0.5 to 1.0 vvd, but the available substrates (nutrients and growth factors) were maintained constant by the use of a standard medium half-diluted in an osmotic salt solution. No significant effect on the cell density or viability was observed and the IgM concentration in the filtrate was reduced by nearly half indicating that IgM production rate was also kept nearly constant. Those results showed that in their system, the substrate availability rather than a toxic effect, was the main factor driving the antibody production rate.

A similar study was conducted with hybridoma cells producing an antibody product (Banik and Heath 1996). The cells were totally retained in the cell culture system by a microfiltration module, while the perfusion rate was varied. Different rates of medium supply and waste removal were applied (2.5, 1.5 and 0.75 vvd) to determine whether cell density was limited or inhibited, and whether a substance could be responsible for the correlation between cell density and productivity of an antibody product. The authors found that in that case, nutrients supply was a stronger determinant of cell density than waste removal.

In another study (Racher and Griffiths 1993), the dilution rate had also to be increased from 0.3 to 1.0 vvd to avoid limitations and maximize density of BHK 21 cells expressing a recombinant antibody in order to reach a higher volumetric productivity in a fixed-bed bioreactor system.

This clearly confirms that the optimal perfusion level has to be chosen carefully in order to realize significant medium savings without compromising on productivity (Pinton et al. 1991; Schmid et al. 1992).

In our case, the data available do not allow us to conclude on the reason (inhibition or limitation) for the -30% loss of productivity that was observed when the perfusion rate was reduced by -50%. However, from the literature examples given above, it can be expected that the drop in productivity is most probably due to a limitation of one or more components of the cell culture medium.

Another property that may be influenced by a modification of the perfusion rate is the product quality. A modification of the dilution rate (D) will change the environmental culture conditions as well as the average residence time (τ) of the r-protein of interest in the bioreactor, and for some proteins that are sensitive to degradation, the perfusion rate cannot be reduced without compromising product quality.

For example, recombinant factor VIII is very sensitive to proteolytic degradation and its production process operates with a high medium renewal rate of 6–8 vvd in order to ensure a short residence time ($\tau = 3$–4 h) of the product in the bioreactor (Chuppa et al. 1997). The same authors also demonstrated that the protein activity is maximized when a high dilution rate is combined with a reduction of the temperature from 37 °C to 34–35 °C since the proteolytic activity of key degrading enzymes can be reduced at lower temperature.

Another case of enzymatic degradation was reported for recombinant human IFN-γ (Goldman et al. 1998). The authors showed that the sialylation of glycans of rhIFN-γ was consistent throughout a fluidized-bed perfusion culture, whereas the sialylation declined significantly in the stationary and death phase of a comparable batch culture most likely due to the activity of sialidases released from lysed cells.

A similar observation was made for the glycosylation pattern of a MAb product (Mohan et al. 1993). The MAb was found incompletely glycosylated in batch cultures or in perfusion cultures operated with 0.36 vvd medium renewal rate, and the perfusion rate had to be increased to 0.60 vvd in order to generate fully glycosylated MAb.

Finally, it was also reported for the production of β-secretase from suspension cultures of HEK-293 cells that the highest specific activity was obtained with the highest dilution rate tested (2 vvd), compared to the other conditions applied: 1 vvd, 0.5 vvd and batch culture (Lüllau et al. 2003).

In this study, it was important to evaluate that the product quality was maintained upon reduction of the medium perfusion rate. The run with the lowest perfusion, run–50, has the longest residence time ($\tau = 0.8$ day) and this is the worst case for protein stability since it leaves the product exposed to all degrading activities present in the bioreactor environment for the longest time. Under these conditions we can consider that 99% of the protein will have a residence time in the bioreactor system shorter than 4 day ($5\tau = 4$ day for run-50).

Since the stability study demonstrated that the r-protein could be stored for up to 4 days at 37 °C in crude harvest without significant alterations, it was anticipated that under the range of perfusion rates tested the product would not be degraded. This was confirmed by the results obtained in the bioreactor runs, as all lots of purified protein generated at production day-20, day-40 and day-60 of each perfusion conditions tested met the specifications established with reference material from run-100. So in our study reported here, no sign of product degradation could be detected.

Glucose starvation is also a typical cause of incorrect product sialylation (Goochee and Monica 1990). This effect has been studied for IFN-γ produced from CHO cells, and the product glycosylation was found affected under low glucose residual levels below 0.1 g/l (Hayter et al. 1993; Hooker et al. 1995).

In the conditions reported here, the r-protein sialylation was not affected due to the lower glucose concentrations (Figure 2) reached during the production phase of run-75 and run-50. This can be explained by the fact that the range of residual glucose concentrations (2.6–0.5 g/l) reported here is still much higher than the value of less than 0.1 g/l glucose level (probably inducing some glucose starvation effect) for which incomplete sialylation of IFN-γ was observed.

Based on the results obtained at small-scale, a reduction of −25% medium perfusion was implemented at pilot scale in the second-generation process, which enabled to maintain the same productivity and the same quality of the molecule, while reducing costs of media, material and manpower of the production process.

The −25% reduction on medium translated directly into a −25% saving on: the powder medium and side ingredients, the pre-filters and sterilizing filters, the sterile bags used for media storage after filtration, and the labour costs associated with medium preparation as fewer medium batches were needed.

As the r-protein titre in the crude harvest was increased by +25% in the second-generation process, the downstream processing benefited from similar savings: reduced manpower needs due to smaller volumes to handle, reduced purification cycle time, optimization of the equipment and manpower needs, etc (data not shown).

Conclusion

From the results obtained at small-scale it is clear that reduction of −25% in perfusion rate

combined both benefits of maximizing productivity with a saving of −25% on medium consumption.

Further reduction of the perfusion rate to −50% was detrimental to the process productivity, which declined by −30% under such conditions. Also, with the −50% perfusion rate conditions, volumetric productivity declined over the duration of the 60-day production phase, whereas a stable productivity was maintained with the higher perfusion rates tested.

The implementation of −25% perfusion in our routine pilot scale production runs simplified the operations of both upstream and downstream units, and allowed significant savings on the cost of goods of this production process.

For industrial applications, it is recommended to test whether and how far the perfusion rate can be decreased during the production phase – provided that the product is not sensitive to residence time – with the aim to reduce cost of goods and to simplify manufacturing operations.

Acknowledgments

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