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Simultaneous environmental manipulations in semi-perfusion cultures of CHO cells producing rh-tPA

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Abstract We evaluated the combined effect of decreasing the temperature to a mild hypothermia range (34 and 31ºC) and switching to a slowly metabolizable carbon source (glucose substituted by galactose) on the growth and production of a recombinant human tissue plasminogen activator (rh-tPA) by Chinese hamster ovary cells in batch and semi-perfusion cultures. In batch cultures using glucose as a carbon source, decreasing the temperature caused a reduction in cell growth and an increase in specific productivity of rh-tPA of 32% at 34ºC and 55% at 31ºC, compared to cultures at 37ºC. Similar behaviour was observed in cultures at 34ºC using galactose as a carbon source. Nonetheless, at 31ºC, the specific productivity of rh-tPA strongly decreased (about 58%) compared to the culture at 37ºC. In semi-perfusion culture, the highest rh-tPA specific productivity was obtained at 34ºC. Similarly, whether a decrease in the temperature is accompanied of the replacement of glucose by galactose, the rh-tPA specific productivity improved about 112% over that obtained in semi-perfusion culture carried out at 37ºC with glucose as the carbon source. A semi-perfusion culture strategy was implemented based on the combined effect of the chosen carbon source and low temperatures, which was a useful approach for enhance the specific productivity of the recombinant protein.

Keywords: CHO cells, galactose, glutamate, low temperature, perfusion culture, rh-tPA.

INTRODUCTION

Mammalian cell cultures have become the main system for the production of recombinant proteins for clinical applications because of their capacity for proper protein folding, assembly and post-translational modifications (Sunley and Butler, 2010). Chinese hamster ovary (CHO) cells are preferred for large-scale production because they are stable hosts for the expression of heterologous genes (Barnes and Dickson, 2006) and because they easily adapt to non-adherent growth in serum and protein-free media. However, the cultivation of these cells in bioreactors still has several areas to be optimized: cell growth, culture longevity, protein productivity, prevention of apoptosis induction and improve quality production (Mohan et al. 2008). In an effort to increase the productivity of recombinant proteins, the effect of various environmental parameters, such as pH (Yoon et al. 2005; Trummer et al. 2006a), media formulation (Altamirano et al. 2000; Berrios et al. 2009) and temperature (Yoon et al. 2003a; Yoon et al. 2003b; Fox et al. 2004; Yoon et al. 2007), have been extensively investigated. CHO cell cultures that are utilized for the production and investigation of recombinant proteins are typically cultured *in vitro* at 37ºC. However, lower temperatures have shown to increase the specific productivity of the protein of interest (q_P) (Yoon et al. 2003a; Yoon et al. 2003b; Fox et al. 2004; Yoon et al. 2007). Similarly, low temperature cultivation has shown to decrease the specific growth rate (µ) and arrest the cell growth. This effect enables the maintenance of a high viability state for a longer period, reducing the glucose and glutamine or glutamate consumption rates, as well as the lactate and ammonia production rates (Yoon et al. 2003b; Trummer et al. 2006b). The reason why the decrease in the culture temperatures increases the specific productivity of a wide range of recombinant proteins still has not been clearly determined yet (Becerra et al. 2012). Among the possible causes that could be involved in this phenomenon, it could be mentioned the following: cell cycle arrest in G1 phase, considered as the most metabolically active (Yoon et al. 2003a, Yoon et al. 2003b; Bi et al. 2004;

Trummer et al. 2006b), increase in the levels of transcript of the protein and an increase in the stability of the mRNA of the recombinant protein (Yoon et al. 2003a; Fox et al. 2004; Al-Fageeh et al. 2006), increase in the capacity of folding by chaperone proteins of the endoplasmic reticulum (Baik et al. 2006; Masterton et al. 2009), among others.

Biphasic cultures have been developed based on the increased specific productivity and decreased specific growth rate that result from a decrease in the culture temperature. Fox et al. (2004) have studied the effect of low temperatures (32ºC) on batch cultures, achieving a 2-fold increase in specific productivity compared to a control at 37ºC. These authors have also proposed a biphasic cultivation strategy based on a down-shift in temperature for batch cultures. This strategy was optimized, yielding to the volumetric productivity increased by 40% when compared with a monophasic culture at 32ºC and by 90% when compared to a monophasic culture at 37ºC. Yoon et al. (2006) have developed a similar strategy, showing that the increase in volumetric productivity depends on the time it takes to make changes to the operating conditions (when the culture temperature is reduced from 37 to 32ºC). Following this work, Yoon et al. (2007) have developed a biphasic perfusion culture using a temperature reduction from 37 to 28ºC, achieving a 20-fold increase in follicle stimulating hormone (FSH) concentration at 28ºC compared to that obtained at 37ºC. However, this drastic reduction in temperature promotes the induction of apoptosis in cells. In this context, Bollati-Fogolín et al. (2005) developed a biphasic culture of CHO cells which a decrease from 37ºC to 33ºC resulted in an increase in the production of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) without affecting its quality in terms of glycosylation and terminal sialic acid units.

On the other hand, media formulation has been implemented as a strategy for the regulation of cell proliferation and improvement in protein production yields. In this context, using slowly metabolizable substrates leads to a reduction in cell growth, usually without significantly affecting the specific productivity of the protein of interest (Ryll et al. 1994; Altamirano et al. 2000; Hills et al. 2001). Altamirano et al. (2000) have reported that substitutions of glucose by galactose and glutamine by glutamate reduce the cell growth rate in batch CHO cell cultures, improving the cell longevity without affecting the specific production rate of the recombinant human tissue plasminogen activator (rh-tPA). In a similar way, Altamirano et al. (2001) have developed a biphasic perfusion strategy for CHO cells cultures, in which an initial proliferative phase is followed by a second phase that is productive, but non-proliferative. It can be point out from this work that the change of carbon source (galactose instead of glucose) permits the cell growth arrest without altering the specific production rate of rh-tPA (q_{tPA}).

Simultaneous manipulation of the temperature (decrease) with other operational conditions has shown synergistic positive results for the system productivity. However, there are few antecedents to this in the literature. Yoon et al. (2005) and Yoon et al. (2006) have manipulated the temperature and pH in batch cultures, achieving a volumetric productivity that was increased by 1.4 times and maximum specific productivity of erythropoietin (EPO) that was increased by 1.5 times in comparison to the control at 37ºC and pH 7.2. Similar results have been obtained using simultaneous changes in temperature and pH to increase the volumetric productivity of the fusion protein Epo-Fc in batch cultivation. In this case, the volumetric productivity was increased 1.4 times by switching to 33ºC and pH 6.9, yielding an increase in specific productivity of 30% (Trummer et al. 2006a). Han et al. (2009) have utilized simultaneous reductions in temperature and hyperosmolarity in biphasic batch cultures, achieving an increase of 5.3 times for the maximum concentration of interferon-β as compared to a control. This result was achieved by reducing the temperature to 32ºC and increasing the osmolarity to 470 mOsm/kg.

As shown in this background, the simultaneous handling of temperature and other operational variables can have synergistic positive effects on the specific productivity. Thus, the objective of this work was to evaluate the effect of low culture temperatures (31 and 34ºC) and simultaneous substitution of glucose by galactose on rh-tPA production in semi-perfusion cultures of CHO cells.

MATERIALS AND METHODS

Cell line and culture medium

The cell line CHO TF 70R, kindly provided by Pharmacia & Upjohn S.A. (Sweden), was manipulated genetically to produce rh-tPA. A proprietary, serum-free and low protein basal medium, denominated

BIOPRO1 (Lonza Group Ltd., Belgium), was utilized for this work. This medium was supplemented with 1,8 (mg/ml) of vitamin B12 (V6629, Sigma, USA), 15 (ml/L media) of lipids and cholesterol (11905, GibcoBRL, USA), 0,47 mM of proline (P5607, Sigma, USA), 0,78 mM of serine (S4311, Sigma, USA) and 0,45 mM of aspartic acid (A9256, Sigma, USA) (Altamirano et al. 2006). The basal medium was also supplemented with 20 mM of glucose (G7021, Sigma, USA) or galactose (G5388, Sigma, USA) and 6 mM of glutamate (G8415, Sigma, USA) in the different experiments discussed here.

Batch cultures

Batch cultures were started in spinner flasks (Techne, UK) with a working volume of 100 ml and were agitated at 50 rpm in an incubator (Forma Scientific CO_2 incubator, Thermo Fisher Scientific Inc., USA) at 95% relative humidity in an atmosphere of 5% CO_2. The cultures were inoculated with cells from the mid-exponential phase of growth at a cell concentration of 0.20 x 10^6 to 0.25 x 10^6 cells ml^{-1}. The culture temperature was controlled at temperatures of 31ºC, 34ºC and 37ºC ± 0.1ºC.

Semi-perfusion cultures

The semi-perfusion cultures were allowed to grow in batch mode for 3 days before starting the medium replacement operation, following the work of Altamirano et al. (2001). Every 48 hrs, the total content of the reactor was centrifuged (4 min at 1000 g) to retain the cells, the medium supernatant was discarded and new fresh medium replacement was added. In this way, no cells were taken out of the reactor during the medium replacement step, and this experimental procedure could be considered as a semi-continuous perfusion, equivalent to a dilution rate of 0.5 d^{-1} (Altamirano et al. 2001). The semi-perfusion culture that utilized glucose as the main carbon source was cultivated only at 37ºC (named SPC1). Other semi-perfusion culture was conducted only with glucose and maintained at 37ºC for the first 14 days. Subsequently, the temperature was decreased to 34ºC and finally to 31ºC (SPC2). In other semi-perfusion culture, the glucose was replaced by galactose in the feed at the same time that the temperature was reduced from 37 to 34ºC (SPC3). The latter condition was also maintained at 31ºC.

Analytical methods

The cells were counted using a hemacytometer (Neubauer, Germany). Cell viability was determined by the method of exclusion using trypan blue (T8154, Sigma, USA) (1:1 mixture of 0.2% trypan blue in saline and cell sample). After counting, the remainder of each sample was centrifuged (1 min at 5000 g) to remove cells, and the supernatant was frozen for further analysis. The concentrations of glucose and galactose were determined with an automatic biochemistry analyzer (YSI 2700, Yellow Springs Inc., USA). The concentration of rh-tPA was quantified by an enzyme immunoassay (Biopool Imulyse t-PA kit, Diagnostic International, Germany).

Estimation of specific rates

The specific growth rate (μ), specific glucose (and galactose) consumption rate and specific rh-tPA production rate (q_{tPA}) were calculated by plotting total cell concentration, cumulative glucose consumption, galactose consumption and cumulative rh-tPA production versus the integral of viable cells (IVCs). The IVCs was evaluated as a function of culture time at discrete intervals using Equation 1.

$$\int X_V dt = \sum \bar{X}_V (t_2 - t_1)$$

[Equation 1]

Where,

$$\bar{X}_V = \frac{(X_{V1}+X_{V2})}{2}$$

X_{V1} and X_{V2} are the concentration of viable cells at time t_1 and t_2, respectively. $\bar{X}_V$ is the average viable cells concentration within the time interval (t_1-t_2).

The plots were fit with a straight line using a regression coefficient of close to one, as described by Renard et al. (1988). The slope of this line was used as an average specific rate. For the semi-perfusion culture, this linear regression was performed for the two main culture stages (the first stage at 34ºC and the second stage at 31ºC), following the procedure reported by Altamirano et al. (2001).

RESULTS AND DISCUSSION

Cell growth and rh-tPA production in batch cultures

Table 1 shows the influence of the cultivation temperature (31, 34 and 37ºC) on the specific growth rate, culture longevity and specific rh-tPA production rate in batch cultures of CHO cells using glucose or galactose. It is important to bear in mind that these cultures where performed using glutamate instead of glutamine, which has been reported as suitable for increasing cell concentration and rh-tPA production (Altamirano et al. 2000).

Table 1. Effect of the temperature of cultivation on μ, culture longevity and q_{tPA} in batch cultures of CHO cells growing in glucose or galactose. Culture longevity is defined as the time to reach 85% of viability. μ and q_{tPA} were calculated using the integral of viable cells and accumulate variation of the concentrations. Data are reported as means of duplicates with differences of less than 10%.

	Glucose			Galactose		
	37ºC	**34ºC**	**31ºC**	**37ºC**	**34ºC**	**31ºC**
μ (h^{-1})	0.012	0.0094	0.0078	0.0090	0.0089	0.0024
Culture longevity (d)	7	8	13	7	7	11
q_{tPA} (ng 10^{-6} cell h^{-1})	36.2	47.8	56.1	34.4	42.4	14.5

The CHO cultures with glucose in the medium exhibited a decrease in μ when the temperature was decreased from 37 to 34ºC. This behaviour has been reported in different CHO cell lines (Yoon et al. 2003a; Bollati-Fogolín et al. 2004; Fox et al. 2004) and could be attributed either to a delay in the catabolism of carbon and energy sources by variations at the gene expression level (Baik et al. 2006; Yee et al. 2009) or to a shift in the proportion of cells from the S to the G1 phase of the cell cycle, which results in a state close to growth arrest observed at reduced temperatures (Kumar et al. 2007). By comparing cultures cultivated at 31ºC, a lower μ was obtained in cultures with galactose in comparison with those obtained when glucose was the medium used (Table 1). This result may be due to a lower specific carbon source consumption rate. Indeed, a lower specific galactose consumption rate (4.4-fold decrease) was obtained (data not shown) in comparison to the specific glucose consumption rate. This effect may be because the transport system for the carbon source is sensitive to changes in the culture temperature (Ingermann and Bissonnette, 1983). As shown in Table 1, regardless of the carbon source used, a decrease in the temperature of cultivation from 37 to 31ºC increased the culture longevity (defined as the time to reach 85% viability). Similar results have been previously reported for CHO cell cultures (Berrios et al. 2009). In the CHO cultures with glucose, the q_{tPA} increased when the cultivation temperature was decreased from 37 to 31ºC (Table 1), indicating that 31ºC was the most suitable condition for rh-tPA production. A different result was obtained using galactose. Thus, an increase in q_{tPA} was observed when the temperature was decreased from 37 to 34ºC, whereas the lowest q_{tPA} value was observed at 31ºC. In contrast, Berrios et al. (2009) have reported that the qtPA value can be enhanced in batch cultures of CHO cells conducted at 31ºC, using mannose as the carbon source. Furthermore, Berrios et al. (2011) reported that replacing glucose by mannose in continuous culture, increased specific productivity of t-PA at high concentrations of the

hexose. These findings demonstrate that the result of use of different carbon sources and low temperatures can enhance the qtPA value in CHO cultures, depends on the carbon source used, to the authors knowledge, there is no further publications about the direct effect of different carbon sources and low culture temperature on recombinant protein production.

Cell growth and rh-tPA production in semi-perfusion cultures

Figure 1 shows the profile of cell growth, cell viability and rh-tPA concentration in semi-perfusion cultures of CHO cells cultured at 37ºC with glucose as the main carbon source (SPC1). A maximum cell concentration of 4.6 x 10^6 cell ml^{-1} was obtained after 16 days of cultivation; in this period, the cell viability reached 80%. The maximum rh-tPA concentration reached 10 mg l^{-1} (16 days of cultivation), and an important decrease (also in the viable cells) was observed after that cultivation period (Figure 1). Similar results have been reported by Altamirano et al. (2001) for semi-perfusion cultures of CHO cells utilizing glucose as the main carbon source.

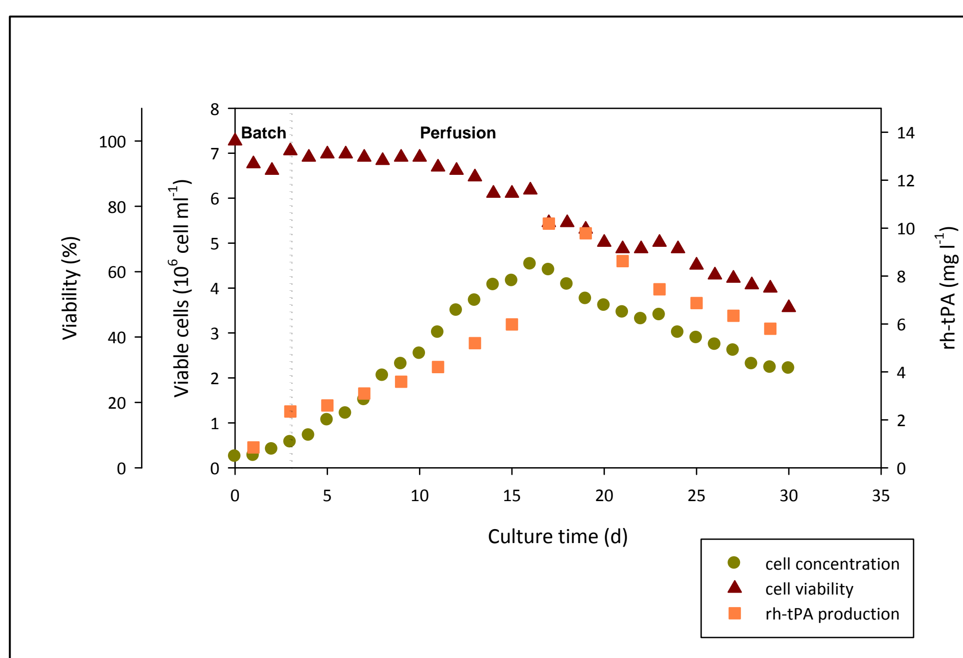

Fig. 1 Viable cell concentration, cell viability and rh-tPA production in semi-perfusion cultures of CHO cells. The semi-perfusion culture was conducted at 37ºC using glucose as the main carbon source. The semi-perfusion cultures were cultivated in culture medium (see **Materials and Methods**) supplemented with 20 mM glucose and 6 mM glutamate. The rh-tPA concentration corresponds to the maximum rh-tPA concentration before replacing the fresh medium (see **Materials and Methods**). Values shown as mean ± standard deviation.

In order to enhance the production of rh-tPA, two different strategies for semi-perfusion cultures were evaluated. As indicated previously, a semi-perfusion culture was carried out with glucose while changing the culture temperature from 37ºC to 34 and 31ºC (SPC2). In the other semi-perfusion culture, the glucose was replaced by galactose while simultaneously varying the culture temperature (SPC3). Figure 2 shows the profile of viable cells, viability and rh-tPA concentration in the semi-perfusion cultures. In these two semi-perfusion cultures, the viable cells increased during the stage at 34ºC, with a higher maximum cell concentration being found in perfusion cultures that used galactose. The cell viability remained constant (80-90%) during the stage at 34ºC. A different result was observed in the semi-perfusion culture performed at 37ºC using glucose (SPC1), in which the viability strongly decreased after 15 days of cultivation (Figure 1). In the semi-perfusion cultures SPC2 and SPC3, the cell concentration and cell viability decreased with a decrease in the culture temperature to 31ºC (Figures 2a-2b). This behaviour could be linked to a lower specific carbon source consumption rate, as compared to the stage at 34ºC. In this regard, the specific glucose consumption rate was 47 nmol 10^{-6} cell h^{-1} during the 34ºC stage and was 35 nmol 10^{-6} cell h^{-1} during the 31ºC stage in SPC2, whereas in

the semi-perfusion cultures using galactose (SPC3) were 13.9 and 8 nmol 10^{-6} cell h^{-1}, respectively. Furthermore, neither the depletion of carbon source or glutamate, nor the accumulation of toxic byproducts of metabolism in perfusion cultures was observed (data not shown). Considering these results, there is a limitation of how much the culture temperature can be decreased while maintaining culture longevity.

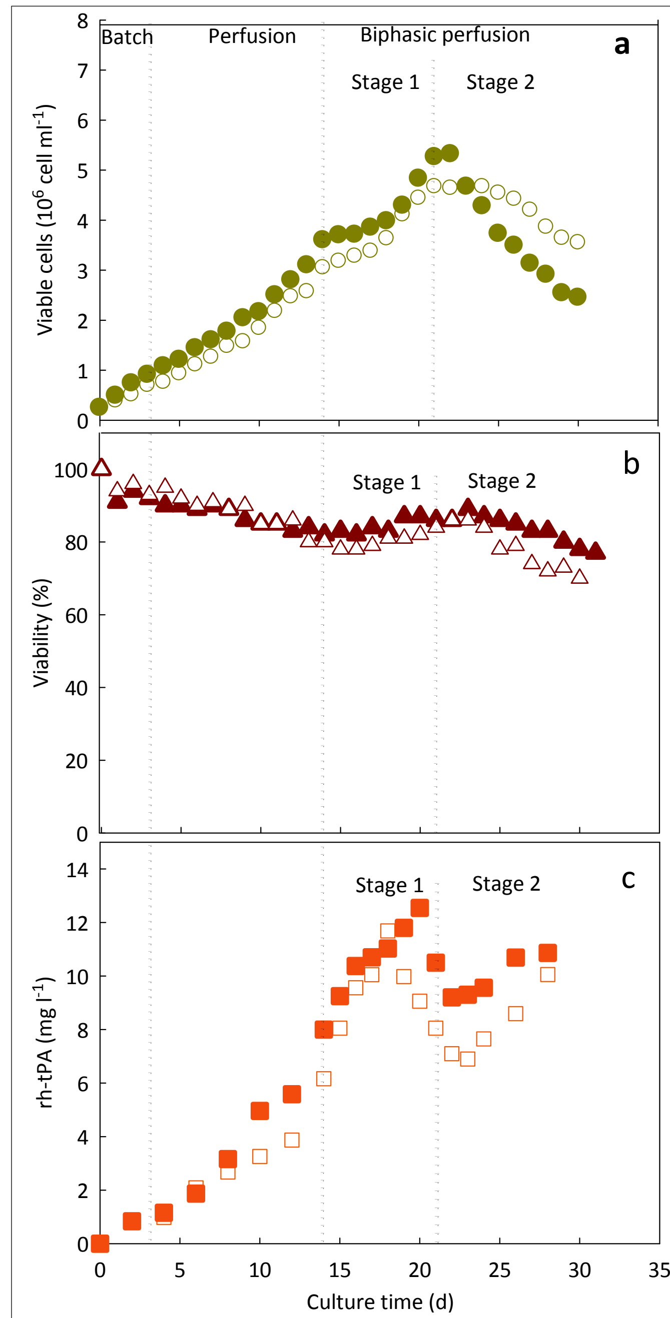

Figure 2. Semi-perfusion cultures of CHO cells to produce rh-tPA. Semi-perfusion cultures were conducted at 34ºC (first stage) and 31ºC (second stage) using glucose or galactose as the main carbon source. Glucose (open symbols); Galactose (closed symbols). The semi-perfusion cultures were carried out in culture medium (see Materials and Methods) supplemented with 20 mM glucose or galactose and 6 mM glutamate. Values shown as mean ± standard deviation.

During the 34ºC stage, a higher rh-tPA production (12.5 mg l^{-1}) was obtained using galactose (SPC3) as compared to the rh-tPA production (11.7 mg l^{-1}) in SPC2 (Figure 2c). The decrease of rh-tPA production at the end of the 34ºC stage can be partially reversed by decreasing the culture temperature to 31ºC, despite the loss of cellular viability. In cultures with compounds that cause cell damage, such as sodium butyrate, cells have been known to remain productive, increasing the productivity of the recombinant protein (Sung et al. 2004; Goulart et al. 2010).

Table 2 shows the μ and q_{tPA} obtained in the semi-perfusion cultures. A decrease in μ from 0.012 to 0.005 h^{-1} and an improvement in the q_{tPA} were obtained when the culture temperature was decreased from 37 to 34ºC (SPC2 and SPC3). However, a decrease in the cultivation temperature to 31ºC produced a decrease in the q_{tPA} value, as compared to 34ºC. This behaviour can be explained by a loss of cellular viability, as discussed previously. Altamirano et al. (2001) reported that the use of galactose instead of glucose enables cell growth arrest without affecting the q_{tPA} in semi-perfusion cultures at 37ºC (data included in Table 2). However, in our study, an increase of 112% in the q_{tPA} value was obtained using galactose instead of glucose and with the use of a lower culture temperature (34ºC; Table 2). This evidence demonstrates that temperature manipulation (between 31 and 37ºC), together with the use of a carbon source that is slowly metabolized, can have a synergistic effect on the production of rh-tPA. To our knowledge, the present work is the first to report a strategy to cultivate semi-perfusion cultures of CHO cells for producing rh-tPA that takes advantage of the combined effect of changes in the carbon source and culture temperature.

Table 2. Influence of carbon source and temperature of cultivation on μ and q_{tPA} in perfusion cultures and biphasic perfusion cultures of CHO cells.

	Carbon source	Temperature	μ (h^{-1})	q_{tPA} (ng 10^{-6} cell h^{-1})	Percent of change q_{tPA} [a]
SPC1	Glucose	37ºC	0.012	38.5	-
SPC2	Glucose	37ºC 34ºC 31ºC	0.005 0.0	75.9 54.4	+97 +41.3
SPC3	Glucose Galactose	37ºC 34ºC 31ºC	0.005 0.0	82.4 70.2	+112 +82.3
SPC (Altamirano et al. 2001)	Glucose Galactose	37ºC 37ºC	0.010 0.0	21.2 19.9	- -6

[a]The percentage of change was calculated in reference to perfusion at 37ºC using glucose as main carbon source. Positive values mean an increase. Negative value is not significant. q_{tPA} were estimate using the integral of viable cells and accumulate variation of the concentrations. Data are reported as means of duplicates with differences of less than 10%.

CONCLUDING REMARKS

The application of the simultaneous manipulation of operational variables, including a decrease in temperature and replacement of the carbon source, for semi-perfusion cultures of CHO cells led to an increase in the specific productivity of rh-tPA. This increase was found to be higher than that achieved when using reduced temperature or carbon source replacement separately (Altamirano et al. 2001). This suggests a synergistic effect between the two culture conditions, which is further enhanced by the productivity of CHO cells in the culture conditions.

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REFERENCES

AL-FAGEEH, M.; MARCHANT, R.; CARDEN, M. and SMALES, C. (2006). The cold-shock response in cultured mammalian cells: Harnessing the response for the improvement of recombinant protein production. *Biotechnology and Bioengineering*, vol. 93, no. 5, p. 829-835. [CrossRef]

ALTAMIRANO, C.; PAREDES, C.; CAIRÓ, J. and GÒDIA, F. (2000). Improvement of CHO cell culture medium formulation: Simultaneous substitution of glucose and glutamine. *Biotechnology Progress*, vol. 16, no. 1, p. 69-75. [CrossRef]

ALTAMIRANO, C.; CAIRÓ, J. and GÒDIA, F. (2001). Decoupling cell growth and product formation in Chinese hamster ovary cells through metabolic control. *Biotechnology and Bioengineering*, vol. 76, no. 4, p. 351-360. [CrossRef]

ALTAMIRANO, C.; ILLANES, A.; CANESSA, R. and BECERRA, S. (2006). Specific nutrient supplementation of defined serum-free medium for the improvement of CHO cells growth and t-PA production. *Electronic Journal of Biotechnology*, vol. 9, no. 1, p. 61-67. [CrossRef]

BAIK, J.K.; LEE, M.S.; AN, S.R.; YOON, S.K.; JOO, E.J.; KIM, Y.H.; PARK, H.W. and LEE, G.M. (2006). Initial transcriptome and proteome analyses of low culture temperature-induced expression in CHO cells producing erythropoietin. *Biotechnology and Bioengineering*, vol. 93, no. 2, p. 361-371. [CrossRef]

BARNES, L. and DICKSON, A. (2006). Mammalian cell factories for efficient and stable protein expression. *Current Opinion in Biotechnology*, vol. 17, no. 4, p. 381-386. [CrossRef]

BECERRA, S.; BERRIOS, J.; OSSES, N. and ALTAMIRANO, C. (2012). Exploring the effect of mild hypothermia on CHO cell productivity. *Biochemical Engineering Journal*, vol. 60, p. 1-8. [CrossRef]

BERRIOS, J.; DÍAZ-BARRERA, A.; BAZÁN, C. and ALTAMIRANO, C. (2009). Relationship between tissue plasminogen activator production and specific growth rate in Chinese hamster ovary cells cultured in mannose at low temperature. *Biotechnology Letters*, vol. 31, no. 10, p. 1493-1497. [CrossRef]

BERRIOS, J.; ALTAMIRANO C.; OSSES, N. and GONZALEZ, R. (2011). Continuous CHO cell cultures with improved recombinant protein productivity by using mannose as carbon source: Metabolic analysis and scale-up simulation. *Chemical Engineering Science*, vol. 66, no. 11, p. 2431-2439. [CrossRef]

BI, J.; SHUTTLEWORTH, J. and AL-RUBEAI, M. (2004). Uncoupling of cell growth and proliferation results in enhancement of productivity in p21Cip1-arrested CHO cells. *Biotechnology and Bioengineering*, vol. 85, no. 7, p. 741-749. [CrossRef]

BOLLATI-FOGOLÍN, M.; WAGNER, R.; ETCHEVERRIGARAY, M. and KRATJE, R. (2004). Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF producing CHO cells. *Journal of Biotechnology*, vol. 109, no. 1-2, p. 179-191. [CrossRef]

BOLLATI-FOGOLÍN, M.; FORNO, G.; NIMTZ, M.; CONRADT, H.S.; ETCHEVERRIGARAY, M. and KRATJE, R. (2005). Temperature reduction in cultures of hGM-CSF-expressing CHO cells: Effect on productivity and product quality. *Biotechnology Progress*, vol. 21, no. 1, p. 17-21. [CrossRef]

FOX, S.; PATEL, U.; YAP, M. and WANG, D. (2004). Maximizing interferon-γ production by Chinese hamster ovary cells through temperature shift optimization: Experimental and modeling. *Biotechnology and Bioengineering*, vol. 85, no. 2, p. 177-184. [CrossRef]

GOULART, H.R.; ARTHUSO, F.S.; CAPONE, M.V.N.; OLIVEIRA, T.L.; BARTOLINI, P. and SOARES, C.R.J. (2010). Enhancement of human prolactin synthesis by sodium butyrate addition to serum-free CHO cell culture. *Journal of Biomedicine and Biotechnology*, vol. 2010, p. 1-11. [CrossRef]

HAN, Y.; KOO, T. and LEE, G. (2009). Enhanced interferon-β production by CHO cells through elevated osmolality and reduced culture temperature. *Biotechnology Progress*, vol. 25, no. 5, p. 1440-1447. [CrossRef]

HILLS, A.; PATEL, A.; BOYD, P. and JAMES, D. (2001). Metabolic control of recombinant monoclonal antibody *N*-glycosylation in GS-NS0 cells. *Biotechnology and Bioengineering*, vol. 75, no. 2, p. 239-251. [CrossRef]

INGERMANN, R. and BISSONNETTE, J. (1983). Effect of temperature on kinetics of hexose uptake by human placental plasma membrane vesicles. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, vol. 734, no. 2, p. 329-335. [CrossRef]

KUMAR, N.; GAMMELL, P. and CLYNES, M. (2007). Proliferation control strategies to improve productivity and survival during CHO based production culture. A summary of recent methods employed and the effects of proliferation control in product secreting CHO cell lines. *Cytotechnology*, vol. 53, no. 1-3, p. 33-46. [CrossRef]

MASTERTON, R.; ROOBOL, A.; AL-FAGEEH, M.; CARDEN, M. and SMALES, C. (2009). Post-translational events of a model reporter protein proceed with higher fidelity and accuracy upon mild hypothermic culturing of Chinese hamster ovary cells. *Biotechnology and Bioengineering*, vol. 105, no. 1, p. 215-220. [CrossRef]

MOHAN, C.; KIM, Y.; KOO, J. and LEE, G. (2008). Assessment of cell engineering strategies for improved therapeutic protein production in CHO cells. *Biotechnology Journal*, vol. 3, no. 5, p. 624-630. [CrossRef]

RENARD, J.; SPAGNOLI, R.; MAIZER, C.; SALLES, M. and MANDINE, E. (1988). Evidence that monoclonal antibody production kinetics is related to the integral of viable cells curve in batch systems. *Biotechnology Letters*, vol. 10, no. 2, p. 91-96. [CrossRef]

RYLL, T.; VALLEY, U. and WAGNER, R. (1994). Biochemistry of growth inhibition by ammonium ions in mammalian cells. *Biotechnology and Bioengineering*, vol. 44, no. 2, p. 184-193. [CrossRef]

SUNG, Y.; SONG, Y.; LIM, S.; CHUNG, J. and LEE, G. (2004). Effect of sodium butyrate on the production, heterogeneity and biological activity of human thrombopoietin by recombinant Chinese hamster ovary cells. *Journal of Biotechnology*, vol. 112, no. 3, p. 323-335. [CrossRef]

SUNLEY, K. and BUTLER, M. (2010). Strategies for the enhancement of recombinant protein production from mammalian cells by growth arrest. *Biotechnology Advances*, vol. 28, no. 3, p. 385-394. [CrossRef]

TRUMMER, E.; FAULAND, K.; SEIDINGER, S.; SCHRIEBL, K.; LATTENMAYER, C.; KUNERT, R.; VORAUER-UHL, K.; WEIK, R.; BORTH, N.; KATINGER, H. and MÜLLER, D. (2006a). Process parameter shifting: Part I.

Effect of DOT, pH, and temperature on the performance of Epo-Fc expressing CHO cells cultivated in controlled batch bioreactors. *Biotechnology and Bioengineering*, vol. 94, no. 6, p. 1033-1043. **[CrossRef]**

TRUMMER, E.; FAULAND, K.; SEIDINGER, S.; SCHRIEBL, K.; LATTENMAYER, C.; KUNERT, R.; VORAUER-UHL, K.; WEIK, R.; BORTH, N.; KATINGER, H. and MÜLLER, D. (2006b). Process parameter shifting: Part II. Biphasic cultivation-A tool for enhancing the volumetric productivity of batch processes using Epo-Fc expressing CHO cells. *Biotechnology and Bioengineering*, vol. 94, no. 6, p. 1045-1052. **[CrossRef]**

YEE, J.C.; GERDTZEN, Z.P. and HU, W.H. (2009). Comparative transcriptome analysis to unveil genes affecting recombinant protein productivity in mammalian cells. *Biotechnology and Bioengineering*, vol. 102, no. 1, p. 246-263. **[CrossRef]**

YOON, S.; KIM, S. and LEE, G. (2003a). Effect of low culture temperature of specific productivity and transcription level of anti 4-1BB antibody in recombinant Chinese hamster ovary cells. *Biotechnology Progress*, vol. 19, no. 4, p. 1383-1386. **[CrossRef]**

YOON, S.; SONG, J. and LEE, G. (2003b). Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnology and Bioengineering*, vol. 82, no. 3, p. 289-298. **[CrossRef]**

YOON, S.; CHOI, S.; SONG, J. and LEE, G. (2005). Effect of culture pH on erythropoietin production by Chinese hamster ovary cells grown in suspension at 32.5 and 37ºC. *Biotechnology and Bioengineering*, vol. 89, no. 3, p. 345-356. **[CrossRef]**

YOON, S.; KIM, S.; SONG, J. and LEE, G. (2006). Biphasic culture strategy for enhancing volumetric erythropoietin productivity of Chinese hamster ovary cells. *Enzyme and Microbial Technology*, vol. 39, no. 3, p. 362-365. **[CrossRef]**

YOON, S.; AHN, Y. and JEONG, M. (2007). Effect of culture temperature on follicle-stimulating hormone production by Chinese hamster ovary cells in a perfusion bioreactor. *Applied Microbiology and Biotechnology*, vol. 76, no. 1, p. 83-89. **[CrossRef]**

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