ACCELERATING PROCESS DEVELOPMENT OF RECOMBINANT PROTEIN PRODUCTION IN PERFUSION CULTURES OF CHINESE HAMSTER OVARY CELLS

by

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Abstract

Perfusion processes provide high productivity, low product residence times and consistent culture conditions, but require long process development times. It can require a week or more to reach each steady state result for process optimization, slowing process development and causing increased production cost. The objective of this work was to accelerate process development in perfusion cultures. Recombinant CHO cells producing t-PA were used, and the effects of temperature, pH and dissolved oxygen were investigated in pseudo steady state perfusion cultures at $\sim 10^7$ cells/mL. It was found that the t-PA concentration increased over 50% at temperatures lower than 37 ^{0}C, with concentrations over 200 mg/L produced at 33 ^{0}C. Cell specific t-PA productivity also increased at low temperatures, reaching a maximum at 33 ^{0}C. A low DO concentration of 16% air saturation had a detrimental effect on t-PA productivity and concentration compared to 70%, while 100% air saturation had no significant effect. Cell specific t-PA production and titre were also negatively affected by pH values ≤ 6.85. To decrease the time needed to achieve steady state, a method to use non-steady state transient responses was developed to qualitatively predict steady state production performance. The method was tested using 3 day temperature shifts in perfusion cultures scanned down by 2 ^{0}C from 37 ^{0}C to 31 ^{0}C, then scanned up to 37 ^{0}C. Transient results showed that higher t-PA concentrations could be predicted at lower temperatures, as pseudo steady state results confirmed. In most cases, transient values on the 3rd day were also in close concordance with pseudo steady state values. To further accelerate process development, transient scanning was applied to small scale, non-instrumented cultures. Similar results were produced, although quantitative t-PA values were 15-30 times lower than in perfusion

culture. The method was further refined by investigating 1 day transient shifts in temperature. More variability was observed in the 1 day transient response, suggesting that cells are still adapting to the new environment, but the overall response still qualitatively predicted the pseudo steady state results. By using a 1 day transient scanning method in small scale culture, process development time could be reduced by as much as 90% when compared to steady state evaluation.

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List of Abbreviations

APR	ammonium production rate [mmol/h]
A-stat	accelerostat
AT III	antithrombin III
BHK	baby hamster kidney
BUN	blood urea nitrogen
CHO	Chinese hamster ovary
CNBr	cyanogen bromide
CSAPR	cell specific ammonium production rate [pmol/cell·d]
CSGUR	cell specific glucose uptake rate [pmol/cell·d]
CSLPR	cell specific lactate production rate [pmol/cell·d]
CStPAPR	cell specific t-PA production rate [pg/cell·d]
DMSO	dimethyl sulphoxide
DO	dissolved oxygen
EPO	erythropoietin
GFP	green fluorescent protein
GUR	glucose uptake rate [mmol/h]
HEL	human embryo lung
hFSH	human follicle stimulating hormone
IFNγ	interferon-γ
LDH	lactate dehydrogenase
LPR	lactate production rate [mmol/h]

MAb	monoclonal antibody
MEM	Minimum Essential Medium
SD	scale-down
SEAP	secreted alkaline phosphatase
SEM	standard error of mean
SFM	serum-free medium
t-PA	tissue plasminogen activator
VtPAPR	volumetric t-PA production rate [mg/L·d]

List of Symbols and Units

^0C	degrees Celsius
d	day
D	dilution rate [h^{-1}]
Da	Dalton [g/mol]
D_{bleed}	dilution bleed rate [h^{-1}]
F	flow rate [mL/d or mL/h]
g	gravitation acceleration [9.81m/s^2]
M	molarity [mol/L]
q_p	cell specific production or consumption rate of *p* [pmol/cell·d or pg/cell·d]
Q_{O2}	volumetric oxygen supply rate [mol/m^2·h]
T	temperature [^0C]
t	time [d or h]

U	International Unit, the amount of enzyme required to catalyze 1 μmol of substrate in 1 min
V_r	working reactor volume [L or mL]
v/v	volume/volume
X_v	viable cell concentration [cells/mL]
$X_{v,LM}$	log mean viable cell concentration [cells/mL]
$Y_{lac/gluc}$	yield of lactate on glucose [-]
μ	cell specific growth rate [h^{-1}]
μ_{app}	apparent cell specific growth rate [h^{-1}]
μ_{calc}	calculated cell specific growth rate [h^{-1}]
↑	increasing
↓	decreasing

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1 Introduction

1.1 Recombinant Protein Production

The production of therapeutic and diagnostic proteins is a major area of interest, as related to bioprocessing. Some proteins of interest currently being produced include Factor VIII, which helps blood coagulation in haemophilics, erythropoietin (EPO), which induces red blood cell production, and tissue plasminogen activator (t-PA), which helps the dissolution of blood clots. The production of these therapeutics is made complex by the fact that many require post-translational modifications in order to function *in-vivo*. These modifications can include disulfide bond formation, glycosylation, and γ-carboxylation (Hu and Peshwa, 1991). Since most bacterial systems cannot perform these post-translational modifications, mammalian cell culture is increasingly used for the production of these proteins. The drawback of mammalian cell culture lies in low cell and protein productivities (Hu and Piret, 1992). Accordingly, research is focused on further optimization of mammalian cell processes.

In this work, t-PA was used as a model recombinant protein. t-PA converts plasminogen to plasmin in the fibrinolytic system. Increasing the concentration of activators such as t-PA can increase the fibrinolytic activity in the blood and in turn, quicken blood clot dissolution (Builder et al., 1988). t-PA was the first approved human therapeutic protein derived from a recombinant continuous cell line (Lubiniecki et al., 1988), and is currently produced by mammalian cells at the 10 000 L scale (Wiebe and Builder, 1994). t-PA is a 66-68 kDa serine protease containing 527 amino acids, 35 cysteine residues,

1 O-glycosylated site and 4 potential N-glycosylated sites (Vehar et al., 1984). t-PA normally exists in two forms: a type I glycoform with three N-glycosylated sites, and a type II glycoform with two N-glycosylated sites (Mori et al., 1995). This variable glycosylation has an effect on both *in vitro* activity and protein clearance rate (Kimura and Miller, 1997).

1.2 Optimization of Bioprocesses

The goal of bioprocess optimization is generally to increase product titres and/or volumetric productivities to minimize cost of production and facilitate downstream processing. Product quality and efficacy is also a consideration in optimization. Protein production is affected by the cell line, culture mode and process parameters, such as temperature, dissolved oxygen, and pH. Other considerations include nutrient requirements, shear sensitivity of cells, waste product accumulation, and process control complexities (Chu and Robinson, 2001).

Stirred suspension is the technology primarily used in large-scale cell culture (Chu and Robinson, 2001). There are a number of reactor and process types used in recombinant protein production. Batch cultures are simple to operate and have short run times, decreasing the risk of contamination. However, the culture environment is not constant and may cause heterogeneity in the product (Curling et al., 1990). As well, toxic byproducts may accumulate over the course of the culture.

Another culture mode commonly used is the fed-batch system, where nutrients are added to extend production and culture viability. Feeding strategies have been designed to avoid nutrient depletion and minimize lactate and ammonium formation by controlling glucose and glutamine concentrations (Ljunggren and Haggstrom, 1992; Xie and Wang, 1994). Fed-batch cultures can substantially increase final product concentrations and volumetric productivities, and so many research groups have focused on these systems (Bibila and Robinson, 1995). Fed-batch systems have the disadvantages of a dynamic culture environment and toxic byproduct buildup. It has also been shown that product quality can be adversely affected (Andersen and Goochee, 1994).

Continuous cultures allow for a steady state culture environment to be maintained. Using the dilution rate to set the growth rate, continuous cultures are a popular system for kinetic studies on cell growth and metabolism. They are not typically used for production purposes due to the low cell and product concentrations produced.

In semicontinuous cultures, a large fraction of the culture is harvested and replaced with fresh medium at regular intervals. This method, also referred to as repeated-batch, can reduce reactor down time between cleaning and sterilization (Henry, 2000). However, changing culture conditions and accumulation of byproducts can be a problem, as with batch cultures.

In perfusion culture, a cell retention device is used to maintain a high cell concentration in the reactor, while fresh medium is added and spent medium removed. These high-

density cultures can increase cell concentration to over 10^7 cells/mL, compared to 1-5 x 10^6 cells/ml for comparable batch and fed-batch cultures (Bibila and Robinson, 1995). Volumetric productivities can be 2-fold to 70-fold higher than in batch (Trampler et al., 1994; Ryll et al., 2000), and product concentrations up to 5-fold higher (Woodside et al., 1998). Due to the continuous nature of perfusion cultures, time-varying conditions are diminished, and product residence time is minimized. However, the design and operation of these systems can be complicated. Perfusion system design must include physical retention of the cells in the bioreactor, which adds to the complexity.

1.3 Thesis Objectives and Organization

Perfusion systems have high volumetric productivities and product concentrations, but require relatively longer process development times, compared to fed-batch. Therefore, the majority of this work has focused on optimization in perfusion culture. The first objective was to determine the effect of the process parameters temperature, pH and dissolved oxygen on protein concentration and cell specific productivity in steady state glucose perfusion culture. To verify the advantages of perfusion culture, a comparison of batch and perfusion culture was performed. From the results of the first objective, a notion developed that time spent in process development could be reduced through a method of transiently scanning through process parameters. This lead to the second objective of this work, which was to investigate transient responses in perfusion culture as a result of step changes in temperature, used as a model process parameter. The third objective was to use small-scale semicontinuous cultures to further evaluate the transient scanning method using a simpler culture mode.

To fulfill these objectives, the research is presented in the following order. A literature review on factors affecting protein production and perfusion process development is presented in Chapter 2. Chapter 3 details the materials and methods used for the thesis work. Chapter 4 presents results of batch and pseudo steady state perfusion cultures, while Chapter 5 presents transient response results, including scale-down work. Finally, conclusions and suggestions for further research are presented in Chapter 6.

2 Literature Review

2.1 Factors Affecting Protein Production

Protein production is affected by a number of cell culture parameters. Environmental factors such as temperature, pH and oxygen limitations play an important part. Production and accumulation of inhibitory byproducts such as lactate and ammonium also contribute to culture productivity.

2.1.1 Temperature

Temperature is an important factor in cell culture, affecting cell growth, viability and protein synthesis. Typically, mammalian cell culture is operated at 37 ^{0}C, to simulate the normal body environment. However, there have been a number of studies investigating the effect of temperature, mainly below 37 ^{0}C. Table 2.1 is a summary of some of these studies. A number of similar trends can be seen across many cell lines, most notably in terms of decreased cell growth and increased viability at lower temperatures. Reuveny et al. (1986) found hybridoma cells had higher viability at temperatures below 34 ^{0}C, with 28 ^{0}C having a detrimental effect on cell growth. Ludwig et al. (1992) grew BHK cells at temperatures between 39 and 28 ^{0}C in batch culture and monitored growth rate and viability. Cell viability was highest at 28 ^{0}C. The growth rate decreased linearly with decreasing temperature until 28 ^{0}C, where the growth rate was 83% lower than the growth rate at 37 ^{0}C. A study with recombinant CHO cells in batch culture showed similar results with maximum cell growth at 36 ^{0}C and no growth below 32 ^{0}C, but increased cell death at the higher temperatures (Furukawa and Ohsuye, 1998).

Table 2.1 Temperature Studies.

Cell Line Product Reactor Type	Range (^{0}C)	Effect (with ↓ T)	Reference
Hybridoma MAb Batch	28 – 37	↑ viability ↓ CSGUR ↓ q_{MAb}	(Reuveny et al., 1986)
Hybridoma MAb Batch	29 – 42	↓ CSGUR ↓ CSLPR ↓ q_{MAb}	(Sureshkumar and Mutharasan, 1991)
Hybridoma MAb Batch	34 – 39	↓ μ no effect q_{MAb}	(Bloemkolk et al., 1992)
BHK Batch	28 – 39	↑ viability ↓ μ ↑ shear stress resistance	(Ludwig et al., 1992)
BHK AT III Batch	30 – 37	↓ μ ↓ CSGUR ↓ CSLPR $q_{ATIII} \neq f(T)$	(Weidemann et al., 1994)
HEL cells t-PA Repeated batch microcarrier	33 – 39	↓ μ (slight) ↑ $q_{t\text{-}PA}$ to max at 33^{0}C	(Takagi and Ueda, 1994)
CHO AT III Fed-batch	33 – 37	↓ CSGUR ↓ CSLPR no change q_{ATIII}	(Rossler et al., 1996)
Recombinant hamster cells Therapeutic protein Perfusion	34 – 37	↓ μ (slight) ↓ CSGUR, ↓ CSLPR no effect [P] no effect q_p	(Chuppa et al., 1997)
CHO 799BgIIIα-AE Batch	30 – 37	↑ viability, ↓ μ ↓ CSGUR ↑ q_p	(Furukawa and Ohsuye, 1998)
CHO 799BgIIIα-AE Perfusion	34 – 37	↓ CSGUR ↑ q_p	(Furukawa and Ohsuye, 1999)
CHO SEAP Batch	30 – 37	↑ [SEAP] ↑ q_{SEAP}	(Kaufmann et al., 1999)
CHO t-PA Batch	32, 37	↓ Xv, ↓ μ ↑ [t-PA] no effect on t-PA activity	(Hendrick et al., 2001)
CHO Protein Continuous packed bed	32 – 37	no effect on μ until 32^{0}C (no growth) ↓ CSGUR ↓ CSLPR	(Ducommun et al., 2002)
CHO EPO Batch	30 – 37	↓ Xv ↑ CSGUR, ↑ CSLPR at 30^{0}C ↑ [EPO] at 33^{0}C, ↓ [EPO] at 30^{0}C ↑ q_{EPO}	(Yoon et al., 2003)

Flow cytometry has revealed that a decrease in temperature can cause cells to remain in the G1 phase of the cell cycle, related to a decrease in the specific growth rate (Bloemkolk et al., 1992). The extended culture viability at lower temperatures can be due to the delayed onset of apoptosis (Moore et al., 1997).

Effects on cell specific metabolic consumption and production rates are also affected by temperature. Studies with hybridoma, BHK and CHO cells have mainly illustrated that the rate of cellular metabolism decreases at lower temperatures (Reuveny et al., 1986; Sureshkumar and Mutharasan, 1991; Weidemann et al., 1994; Rossler et al., 1996; Chuppa et al., 1997; Ducommun et al., 2002). A decrease in temperature from 37 to 34 ^{0}C resulted in a 41% decrease in the cell specific glucose consumption rate (CSGUR) in hybridoma cells (Reuveny et al., 1986). Sureshkumar and Mutharasan (1991) used hybridomas as well, and found a 40% decrease in the CSGUR between 37 and 33 ^{0}C. In the same study they determined the cell specific lactate production rate (CSLPR) decreased by 61%. Chuppa et al. (1997) grew recombinant hamster cells in perfusion at 37 and 34 ^{0}C and found a 48% decrease in the CSGUR, and a 30% decrease in the CSLPR. Another study with BHK cells in batch culture, the CSGUR and CSLPR decreased 22% and 65% respectively, for the temperature range of 37 to 33 ^{0}C (Weidemann et al., 1994). Two studies with CHO cells with different modes of operation both gave similar results. Rossler et al. (1996) conducted a fed-batch study in the temperature range of 37 to 33 ^{0}C, while Ducommun et al. (2002) used a continuous packed bed system at 37 and 32 ^{0}C. In both cases, a 50% decrease was found for both the CSGUR and the CSLPR. One exception to this trend of lowered metabolic rates is a

study involving CHO cells producing EPO in batch culture (Yoon et al., 2003). The group found that at 33 ^{0}C, CSGUR and CSLPR were similar to values at 37 ^{0}C. However, lowering the temperature further to 30 ^{0}C resulted in a 44% and 56% increase in CSGUR and CSLPR, respectively.

Although cell growth and metabolic rates give similar trends across cell lines, this does not hold true for the effect of temperature on productivity. Reuveny et al. (1986) found a 60% decrease in the cell specific monoclonal antibody (MAb) production rate when the temperature was decreased from 37 to 34 ^{0}C. Sureshkumar and Mutharasan's (1991) hybridoma study produced similar results, with a 42% decrease in the cell specific production rate. However, there have been reports of increased productivity. Furukawa and Ohsuye (1999) observed a 2-fold increase in the cell specific protein production rate when the temperature was changed from 37 to 34 ^{0}C in a CHO perfusion culture. The final protein concentration obtained was also higher (1.6-fold). Yoon et al. (2003) showed a 2.5-fold increase in EPO concentration, and a 4-fold increase in the cell specific EPO production rate when the temperature was lowered from 37 ^{0}C to 33 ^{0}C for a CHO culture. Another study investigating 37 and 33 ^{0}C reported a 41% increase in the cell specific t-PA production rate from repeated batch microcarrier cultures of HEL cells (Takagi and Ueda, 1994). Hendrick et al. (2001) reported an increase in t-PA concentration when the temperature was shifted from 37 to 33 ^{0}C. They determined that the G1 phase is the t-PA accumulation phase for CHO Tf70R cells. Still other studies have reported that temperature can have no significant effect on the cell specific productivity (Bloemkolk et al., 1992; Weidemann et al., 1994; Chuppa et al., 1997).

Taken together, it is apparent that each cell line must be evaluated individually to determine its temperature dependent protein productivity characteristics.

2.1.2 Dissolved Oxygen Concentration

Dissolved oxygen (DO) is another factor that has the potential to significantly influence cell metabolism and protein production. Oxygen limitations are especially a problem in high-density cell cultures, such as perfusion systems. The effect of dissolved oxygen has been studied by a number of groups, as presented in Table 2.2. In general, cases of critically limited dissolved oxygen, and large excesses of oxygen seem to have detrimental effects to cell metabolism.

Table 2.2 Oxygen Studies.

Cell Line Product Reactor Type	Range (% air saturation)	Variable	Effect	Reference
Hybridoma MAb Batch	25, 60	[MAb]	↑ 1.4-fold at lower DO	(Reuveny et al., 1986)
		CSGUR	↓ 40% at lower DO	
Hybridoma MAb Continuous	0.1 - 100	Xv	↑ with ↓ DO to max at 0.5%	(Miller et al., 1987)
		Viability	↑ with ↓ DO	
		CSGUR	↓ with ↓ DO to 0.5% , then large ↑ < 0.5%	
		q_{Mab}	max at 50% DO	
		[MAb]	max at 50% DO	
Hybridoma MAb Continuous	0 - 100	Xv	max at 35% DO ↓ < 5% and at 100% DO ~ constant btwn 5-78%	(Ozturk and Palsson, 1990)
		CSGUR	~ constant > 1.2% DO ↓ < 1.2% DO	
		CSLPR	↑ with ↓ DO	
		CSAPR	~ constant btwn 1.2 - 100 ↑ < 1.2 % DO	
		q_{MAb}	no significant change	
		[MAb]	max at 30% DO	

Table 2.2 Oxygen Studies (continued)

Cell Line Product Reactor Type	Range (% air saturation)	Variable	Effect	Reference
Hybridoma MAb Batch	1 - 100	μ	~ constant btwn 20 - 80% DO $\downarrow$ < 10%, > 80%	(Ozturk and Palsson, 1991)
		CSGUR	min at 10% DO	
		CSLPR	min at 10% DO	
		[MAb]	max btwn 20 - 50% DO $\downarrow$ 50% at 1% DO $\downarrow$ 30% at 100% DO	
CHO hFSH Perfusion	10 – 90	Xv	~ constant	(Chotigeat et al., 1994)
		q_{FSH}	$\uparrow$ 3.7-fold at highest DO	
HEL cells t-PA Repeated batch microcarrier	15 – 60	μ	$\uparrow$ with $\uparrow$ DO $\geq$ 30% ~ constant btwn 30 - 60%	(Takagi and Ueda, 1994)
		$q_{t\text{-}PA}$	max at 20% DO $\uparrow$ 1.6-fold at 20% over 60%	
		[t-PA]	max at 30% DO over 2-fold $\uparrow$ btwn 60 and 30	
CHO Interferon-γ Stirred batch	28, 48, 67, 95	Xv	95% DO, cell growth inhibited max btwn 28 - 67%	(Dunster et al., 1997)
		[IFNγ]	70% $\downarrow$ at 95% DO ~ constant < 67% DO	
Hybridoma MAb Continuous	10 - 125	Xv, μ	$\downarrow$ at > 50% DO	(Jan et al., 1997)
		CSGUR	$\uparrow$ with $\uparrow$ DO almost 2-fold $\uparrow$ at 125 from 10%	
		CSLPR	$\uparrow$ with $\uparrow$ DO almost 2-fold $\uparrow$ at 125 from 10%	
		CSAPR	higher at 10%, 125%	
		q_{MAb}	no significant variation with DO	
CHO t-PA Perfusion	5 - 40	μ	no effect	(Heidemann et al., 1998)
		$q_{t\text{-}PA}$	no effect	
		Ylac/gluc	slight $\uparrow \leq$ 5% DO	

Many studies have examined dissolved oxygen effects on cell growth. Miller et al. (1987) conducted an extensive study with hybridoma cells in continuous culture and found a critical DO concentration of 0.5% air saturation. Decreasing from 100% air saturation to this DO_{crit}, cell concentration and viability increased. However, in Ozturk and Palsson's continuous study of hybridomas (1991), the growth rate remained constant between 20 and 80% air saturation. Below 10%, and at concentrations higher than 80% air saturation, the growth rate decreased. This trend of declining growth rate at higher

levels of air saturation has also been shown in other studies (Ozturk and Palsson, 1990; Jan et al., 1997). In non-limiting conditions, CHO cell growth is not greatly influenced by oxygen concentrations (Chotigeat et al., 1994; Heidemann et al., 1998), although growth inhibition does occur at higher DO concentrations (Dunster et al., 1997). Hypoxic and anoxic conditions have a deleterious effect on growth rate. A study by Lin et al. (1993) using CHO cells in perfusion culture investigated hypoxic and anoxic conditions. By manipulating the specific oxygen consumption rate, mild hypoxic (40-60% reduction in q_{O2}), severe hypoxic (80% reduction in q_{O2}) and anoxic conditions (>99% reduction in q_{O2}) were examined. All three levels were at oxygen concentrations less than 3.3% air saturation. Cell concentration decreased by 25% under severe hypoxia, and 50% under anoxic conditions, as compared to a control culture at 33% air saturation. There was no effect on cell concentration under mild hypoxic conditions. HEL cells show yet another result, with growth rate increasing with DO at air saturation values greater than or equal to 30% (Takagi and Ueda, 1994).

It is known that cells can be subjected to oxidative stress under high levels of dissolved oxygen. This is due to the presence of free radicals produced from "leakage" of electrons from the mitochondrial electron transport chain, which reduce oxygen to O_2^- (Lin and Miller, 1992; Dunster et al., 1997). All aerobic cells have the ability to dismutate O_2^- to hydrogen peroxide with the antioxidant enzyme superoxide dismutase (Dunster et al., 1997). Hydrogen peroxide presents its own dangers as an oxidant given its ability to produce the damaging free radical $OH^\cdot$ (Lin and Miller, 1992). Oxidative damage stress can explain why higher DO concentrations tend to yield lower cell growth and viability.

Cell metabolism is also affected in part by dissolved oxygen, but the effect is varied across cell lines. Under non-limiting conditions, the cell specific glucose uptake rate decreases with decreasing DO (Reuveny et al., 1986; Miller et al., 1987; Ozturk and Palsson, 1991; Jan et al., 1997). Reuveny et al. (1986) showed a 40% decrease in the CSGUR of hybridoma cells in batch when the DO was decreased from 60 to 25% air saturation. Ozturk and Palsson (1991) also observed a 40-50% decrease in CSGUR, albeit from 100% to 10% air saturation. This decrease may be due to lower maintenance energy requirements, for at higher DO, more energy may be needed to repair or avoid oxidative stress (Miller et al., 1987; Ozturk and Palsson, 1991). Hyperoxic conditions were investigated in one study of continuously grown hybridomas, and revealed an almost 2-fold increase in the CSGUR at 125% compared to 10% air saturation (Jan et al., 1997). An investigation of the metabolic flux of glucose by Jan et al. (1997) indicates that increased use of glucose at higher DO concentrations is due to a increase in anaerobic metabolism caused by oxygen toxicity, as shown by a decrease flux through the TCA cycle, and significant increases in flux through both the glycolysis and pentose phosphate pathways.

Under hypoxic conditions, the cell metabolism reacts differently. Lin et al. (1993) has shown that the CSGUR increases with the degree of hypoxia in CHO cells producing t-PA. Studies with hybridoma cells have shown similar increases in CSGUR at very low oxygen concentrations (Miller et al., 1987; Ozturk and Palsson, 1990). This is due to the

Pasteur effect, whereby an increased glycolytic flux is incurred due to increased glucose utilization for energy production when oxygen is limiting (Ozturk and Palsson, 1990).

Cell specific lactate production rates increase under hypoxic conditions as well (Ozturk and Palsson, 1990; Ozturk and Palsson, 1991), which can also be attributed to the Pasteur effect. Under non-limiting conditions, there have been varied effects on cell specific lactate production. A continuous study with a hybridoma cell line demonstrated a 58% decrease in the CSLPR from 125% to 10% air saturation (Jan et al., 1997). Using different hybridoma cells in continuous culture, Ozturk and Palsson (1990) demonstrated a linear increase in the CSLPR with decreasing air saturation. In contrast, a batch study by the same group using the same hybridoma cell line showed a 26% decrease in the CSLPR from 100 to 10% air saturation (Ozturk and Palsson, 1991). Possibly, this is due to a change in medium composition. In the batch study, results were analysed during exponential phase, where the medium composition had high nutrient and low waste product concentrations. In the continuous study, the culture composition would have been different, with lower nutrient and higher waste product concentrations. This could have had an effect on the glucose limitations encountered under batch conditions.

Optimal DO concentrations for antibody production tend to differ from optimal conditions for cell growth. Miller et al. (1987) determined maximum antibody concentration and specific MAb production rate for a hybridoma cell line was optimal at 50% air saturation, in contrast to maximum cell growth at 0.5% air saturation. Reuveny et al (1986) showed that due to higher viable cells available at a lower DO of 25% air

saturation, the antibody production was 1.4-fold higher than at 60% air saturation, where optimal cell growth occurred. A hybridoma cell line cultured by both batch and continuous systems showed no significant change in q_{MAb}, but a maximum antibody concentration near 30% air saturation, differing from the optimal DO concentration of 50% air saturation for cell growth (Ozturk and Palsson, 1990; Ozturk and Palsson, 1991). It should be noted, however, that these were broad maxima in both antibody and cell concentrations. A study by Jan et al. (1997) with hybridoma cells demonstrated no effect of DO on the specific antibody production rate.

Studies on CHO cells have differing results on the effect of oxygen. An investigation of CHO cells in perfusion subjected to hypoxic and anoxic effects indicated a significant decrease (up to 80%) in the specific t-PA production rate (Lin et al., 1993). CHO cells producing hFSH in perfusion showed a 3.7-fold increase in q_{FSH} at 90% over 10% air saturation (Chotigeat et al., 1994). In comparison, another CHO cell line producing t-PA in perfusion showed no effect on $q_{t\text{-}PA}$ in the range of 5 - 40% air saturation (Heidemann et al., 1998). Dunster et al. (1997) investigated stirred batch cultures of CHO cells and found no significant change in the protein concentration at air saturation values less than 67%. However, at 95% air saturation, the protein concentration decreased over 70%. This was due to the growth inhibition encountered at high oxygen levels.

The literature on oxygen effects in cell culture suggests that extremes in oxygen concentration tend to have adverse effects. Ozturk (1996) suggests that most fermentations typically operate at ~50% air saturation.

2.1.3 *Effect of pH*

Variations in extracellular pH can have a significant effect on the growth and metabolism of cells. Most mammalian cells grow optimally in the pH range of 7.2 - 7.6, although specific optimal values vary with cell type (Eagle, 1973). Cell growth and cell metabolism are affected similarly by pH across a number of cell lines, although pH influence on protein production varies. Table 2.3 lists a number of studies that investigated the effects of extracellular pH on cells.

Cell growth is generally adversely affected by extreme pH values outside the range of 7.0 - 8.0. SP2/0 -derived mouse hybridomas were continuously cultured and subjected to different pH values in a study by Miller et al. (1988a). They found the viability of the culture remained high through pH values between 7.1 - 7.4. At pH 7.7, the viability dropped initially, but recovered to the higher values at 7.1 and 7.4. At pH 6.8, the viability decreased, with no indication of recovery. Ozturk and Palsson (1991) cultivated other murine hybridomas in batch cultures of varying pH values. They determined a maximum cell growth rate at pH 7.2, with a decrease in viable cell concentration of over 80% at pH 6.8. In another batch study of GS-NS0 myeloma cells, the maximum cell growth rate was determined in the pH range of 7.3-7.5, with a decrease of 15% at 7.0 and at 8.0 (Osman et al., 2001). At extreme values of 6.5 and greater than 8.0, there was no cell growth. Similarly, a study with HEL cells showed maximum cell growth at pH 7.4 (Takagi and Ueda, 1994). The effect of pH on hamster cell growth has not been reported extensively, though Eagle (1973) notes that for BHK cells there is a broad pH range between 6.8 - 7.6 that is optimal for cell growth.

Table 2.3 Studies on Effect of Extracellular pH

Cell Line Product Reactor Type	pH Range	Variable	Effect	Reference
Hybridoma MAb Continuous	6.8 - 7.7	Xv, Viability	↓ at pH < 7 ~ constant 7.1 - 7.7	(Miller et al., 1988a)
		CSGUR	↑ as pH ↑	
		$Y_{lac/gluc}$	↑ as pH ↑	
		q_{O2}	no effect	
		[MAb]	no effect	
		q_{MAb}	↑ at pH < 7 ~ constant 7.1-7.4	
Hybridoma MAb Batch	6.9.- 7.65	μ	max at 7.2	(Ozturk and Palsson, 1991)
		CSGUR	↑ as pH ↑	
		CSLPR	↑ as pH ↑	
		$Y_{lac/gluc}$	↑ as pH ↑	
		q_{O2}	no effect	
		[MAb]	max at 7.2	
		q_{MAb}	↑ at pH < 7.2 ~ constant at pH > 7.2	
CHO mPL-I/mPL-II Batch	6.1 - 8.7	CSGUR	↑ as pH ↑ (up to 8.0)	(Borys et al., 1993)
		q_p	↑ with pH to max at pH 7.6-8.0	
HEL cells t-PA Repeated batch microcarrier	6.5 - 8.1	μ	max at 7.4	(Takagi and Ueda, 1994)
		q_{t-PA}	max at 7.3	
		[t-PA]	max at 6.8	
GS-NS0 myeloma MAb Batch	6.5 - 9.0	μ	Max at 7.3-7.5	(Osman et al., 2001)
		Viability	max at 7.3	
		CSGUR	↑ as pH ↑	
		[MAb]	max at 7.0	
		q_{MAb}	constant 6.5-8.0 ↓ pH > 8.0	

Effects on cell metabolism are also comparable across cell lines. The cell specific glucose uptake rate has been shown to increase with increasing pH in a number of studies (Miller et al., 1988a; Ozturk and Palsson, 1991; Borys et al., 1993; Osman et al., 2001). Ozturk and Palsson (1991) observed an 8.5-fold increase in CSGUR from pH 6.8 to 7.65. The CSGUR for GS-NS0 myelomas increased 10-fold from pH 6.5 to 8.5 (Osman et al., 2001). In contrast, Miller et al. (1988a) observed a 50 - 100% higher cell specific glucose

uptake rate when the pH was changed from 7.2 to 7.7. However, the cell viability was low during this period, and after cell recovery, the CSGUR dropped to levels 10-25% higher than at pH 7.2.

Cell specific lactate production rates are also affected by pH. Ozturk and Palsson (1991) showed a 9.5-fold increase in the CSLPR from pH 6.5 to pH 7.65. Osman et al. (2001) found an increase in the CSLPR when the pH was shifted from 7.5 to 8.0 and 8.5. However, when the pH was shifted down from 7.5 to values of 7.3, 7.0 and 6.5, lactate was consumed. Possible pyruvate limitation of cells could be a reason why lactate is consumed (Osman et al., 2001).

Ozturk and Palsson (1991) have proposed some possible justifications for this increase in metabolic rates. It is possible that the glycolytic enzyme activity increases when the pH is increased. Another reason may be that high pH changes the glucose transport rate through the cell membrane by altering the membrane potential. As well, a greater burden on the self-regulation of intercellular pH may explain why lactate production is reduced at lower pH values, while at higher pH values, the elevated amount of lactate produced enables cells to counter alkaline conditions.

Production of antibody or protein is affected by pH, but the effect is not consistent across cell lines as in the case of cell growth. Miller et al. (1988a) found a 3-fold increase in the cell specific antibody production rate at pH 6.8 with respect to pH 7.1-7.4, but no effect of pH on antibody product concentration. This was likely due to the lower growth rate

and cell concentration encountered at low pH. They also reported an increase in the q_{MAb} when the pH was shifted to 7.7 initially, but it then returned to former values obtained at pH 7.1-7.4. Antibody production in the Ozturk and Palsson study (1991) was also higher at low pH, with an almost 2-fold increase in the cell specific antibody production rate at pH 6.9 over 7.2. The q_{MAb} was constant at pH values greater than 7.2 up to 7.65. Antibody concentration was maximum at pH 7.2, corresponding to the value at which growth was also optimal. Osman et al. (2001) found that the cell specific antibody production rate for GS-NS0 myelomas was constant between pH 6.5-8.0, but decreased above pH 8.0. In contrast, a batch study of two CHO cell lines producing mPL-I and mPL-II resulted in a 1.4 to 1.9 -fold increase in the cell specific protein production rate when the pH was increased from 7.2 to 7.6-8.0 (Borys et al., 1993). Miller et al. (1988a) postulate that antibody production is higher during periods of stress. This is supported by the differing optimal pH values for cell growth and antibody production in most cell lines.

There are many other factors that have an influence on cell culture. It is important to note that although the majority of studies have looked at factors one at a time, interactions do occur. For example, Doyle and Butler (1990) have shown that growth inhibition due to ammonia toxicity varied depending on the pH of the culture medium. A combination of hypoxic conditions and low pH can lead to increased cytoxicity in CHO cells, greater than hypoxia or low pH alone (Rotin et al., 1986). It is known that temperature can have an effect on the oxygenation of medium, and influence the oxygen consumption rate (Jorjani and Ozturk, 1999). To evaluate interactive effects, thought should be given to

statistically designed factorial experiments in parallel bioreactors, although this could substantially increase process development time.

2.2 Perfusion Process Development

In terms of investigating the influence of environmental parameters, continuous cultures often have been selected as the mode of operation, primarily due to the straightforward maintenance of a steady state environment (Higareda et al., 1994). Most experimental designs with continuous cultures involve reaching steady state before results are considered reliable. Typically, continuous cultures require three residence times or more before establishment of a steady state. This is likely to take a very long time, and in an industrial environment, time delays translate into major unwelcome expenses. For instance, delays in process transfer from scale to scale can incur costs of $50 000 - $100 000 per week in operational costs, and $500 000 to $1 million per week in lost future revenues (Dutton, 2001). Given this pressure, there is a need to minimize process development time.

2.2.1 Scale-down Systems

One option for reducing process development time is the use of scale-down (SD) systems. There are a number of advantages to using SD systems since more runs can be performed at a lower cost. It has been estimated that SD models allow two orders of magnitude more runs and are two orders of magnitude less expensive (Dutton, 2001). SD methods also offer more flexibility in terms of alternative methods and measurements (Dutton, 2001).

Scale-down systems can be useful for examining the microenvironment of large-scale reactors, where substrate and oxygen gradients may be present due to high mass transfer resistance and concentrated substrates fed to the process. Feeding creates a feed zone, which is an area of high substrate concentration (Bylund et al., 1999). One SD configuration is to combine a stirred tank reactor with a plug flow reactor. The two reactors are set up to mimic the low concentration bulk zone and the high concentration feed zone, respectively. The flow between the two reactors is analogous to the circulation time in the large reactor (Bylund et al., 1999). Other studies have used scale-down systems to investigate gradients of pH (Langheinrich and Nienow, 1999; Osman et al., 2002) and oxygen (Sweere et al., 1988; George et al., 1993).

A number of different vessels can be used as a small scale system. Liu and Hong (2001) described how shake flasks, more typically for microbial and yeast fermentations, can also be used successfully with mammalian cells. They found cylindrical shaped vessels with a height-to-diameter ratio of 3:2 worked best with animal cells. Product concentrations in these shake flasks were comparable to fully instrumented bioreactors, and higher viable cell densities than spinner flasks were obtained. However, it is difficult to monitor pH and oxygen.

A group at the University of Maryland have gone to even smaller capacities and have developed a microbioreactor with a working volume of 2 mL (Kostov et al., 2001). Using optical sensor technology, pH, DO and optical density (OD) can be monitored in a

4 mL cuvette. Comparison of this microbioreactor to a 1 L *E. coli* fermentation yielded similar responses in pH, DO and OD, although the microbioreactor had a slightly higher growth rate and a reduced lag time. This was likely due to a 3 ^{0}C temperature increase from 25 to 28 ^{0}C in the cuvette, a result of heat generation from the optical sensors.

Well plates can also be used for parallel experimentation. Although sampling of well plates can be problematic with such small volumes and may require wells to be sacrificed, the use of green fluorescent protein (GFP) as a reporter gene can make well plates a more feasible approach. Using GFP, cell growth (Girard et al., 2001) and protein production (Cha et al., 1997; Albano et al., 1998) can be measured.

Fully instrumented bioreactors are complex and expensive to set up while shake flasks, spinners, and multiwell plates can be put into operation fairly easily. The challenge becomes whether results in these small scale systems can be as reliable as in monitored, larger scale systems.

2.2.2 Semicontinuous Cultures

Many small scale systems involving shake flasks, spinners or well plates do not have the ability to be operated as fully continuous or perfusion systems. Semicontinuous cultures are a way to overcome this restraint. This method involves removing some of the culture and replacing it with an equal amount of fresh medium at fixed times. If the replacement frequency is high, then culture condition variations can be minimized so they are similar to a continuous system (Westgate and Emery, 1990). The use of semicontinuous cultures

can speed up development, allowing for numerous experiments to be run in parallel with a simplified culture operation.

Hybridoma growth and MAb production have been studied in semicontinuous culture by a number of groups. The ability to use extremely small dilution rates is an advantage available for semicontinuous systems. Leno et al. (1992) studied a range of dilution rates from 0.008 to 0.055 h^{-1} in 1 L roller bottles. They were able to maintain cell density changes of $\pm$ 0.2 x 10^6 cells/mL, with average cell density between 1.5 - 3 x 10^6 cells/mL, depending on the dilution rate. A previous study used a similar method of cultivation in T-flasks, examining the effect of cell cycle and growth phase on hybridoma cultures (Ramirez and Mutharasan, 1990). Both of these studies used a method whereby fresh medium was added to the culture first, then after mixing, an equal amount was removed. Cell concentration was determined both before and after medium addition for accurate growth rate calculations. Henry (2000) also investigated a range of dilution rates in semicontinuous hybridoma cultures, but used a simpler method, sampling only before medium replacement. He showed that a batch model provides a relatively good description of cell growth in semicontinuous cultures.

In a different study (Schneider and Lavoix, 1990), the researchers determined optimal replacement rates for semicontinuous cultivation. They also explored the effect of cell recycling, which would bring the semicontinuous culture closer to simulating a perfusion culture. It was found that a replacement rate of 40%/day was optimal. Above this rate caused cell washout, while below 40% did not provide enough nutrients and did not

remove enough metabolic byproducts. Recycling 33-66% of cells back to the cultivation increased cell concentration and specific productivity. However, total cell recycle caused a further increase in the amount of debris and dead cells and could become detrimental to the system. Schneider and Lavoix (1990) also found that maximum cell density and MAb production could be reached in conditions of maximum growth and also stationary phase, which required replacement rates of ≥ 40%/day with total cell recycling.

2.2.3 *Transient Behaviour*

Investigating the transient response of a system is another possible way to reduce process development time. The non-steady state response of system to an environmental perturbation could give indications of how the culture will react to that perturbation at steady state. In the past, transient behaviour has been used to look at three main areas.

Large scale fermentors can be subject to varied environmental states. Investigation of this microenvironment therefore, has been the focus of many temporal behaviour studies. This is usually carried out with a step, pulse or periodic change of the process variable or substrate concentration under steady state conditions in a continuous reactor (Hsu and Wu, 2002). Buse et al. (1992) have looked at the effect of oscillating DO concentrations on keto acid production in a *Gluconobacter* species. The oscillation durations ranged from 2-5 min. They found that at ~30% DO, which was the minimum level for optimal productivity, oscillations could result in a prolonged lag phase for growth and production. Studies on pH perturbations include Osman et al. (2002), who subjected GS-NS0 myeloma cells to alkali additions. They found a single perturbation up to pH 8.0 had no effect, but a change to pH 9.0 over 10 min caused decreases in viability, with complete

death at 90 min. Another group using *Bacillus thuringiensis* investigated pH oscillations between 7.0 and 8.4 during exponential phase and between 7.0 and 5.8 during stationary phase in order to mimic the typical alkali and acid additions in a culture (Hsu and Wu, 2002). No effect of pH variation was demonstrated. However, such repeated alkali and acid additions would increase culture osmolarity, possibly to a detrimental extent, in animal cell culture. Two-compartment reactors may be used to combat this problem by utilizing a small reactor to represent the surface region where alkali additions are made, and a larger vessel representing the area near the impeller (Osman et al., 2002).

Another focus of transient studies is to explore the regulatory structure of metabolic pathways (Miller et al., 1989a). A number of studies have investigated the transient responses of hybridoma metabolism to changes in oxygen, glucose, glutamine, lactate, ammonia, amino acids and/or vitamins (Miller et al., 1988c; Miller et al., 1988b; Miller et al., 1989a; Miller et al., 1989b; Hiller et al., 1994). In the oxygen study (Miller et al., 1988c), the volumetric oxygen supply rate (Q_{O2}) was stepped down every 5 - 8 days, resulting in DO changes from 10% to 0.1% air saturation, and then back to 10%. The first day after a step change, sharp decreases in viable cell concentration and sharp increases in specific glucose and glutamine consumption rates were observed. However, by the third day, these values were in the range of the steady state values. A lactate and ammonia study (Miller et al., 1988b) showed similar sharp variations during the first day after a step or pulse change, but recovered after 2 or 3 days. Only at very high inhibitory levels was recovery slower, between 3 and 4 days. Newland et al. (1994) also conducted a transient study on ammonia inhibition of hybridomas. They used 2 day step changes of

ammonia concentration in continuous cultures. The temporal response could be divided into three stages: an early transient stage of 5 - 20 h where the culture displays washout kinetics and fluctuations, a late transient stage where a new steady state is approached, and ultimately, the new steady state. They also observed fluctuations during the early transient period, especially pronounced increases in the specific glucose uptake rate, and decreases in cell concentration. By 35 h after the step change, cell concentration and specific glucose consumption had levelled out and approached a constant value.

Substrate concentration has also been a topic for transient response research. A glucose pulse experiment was performed on a CHO chemostat culture (Hayter et al., 1992). A transient decline in cell concentration was observed after a glucose pulse from 2.75 to 3.8 mM, while cell concentration transiently increased after a pulse from 4.25 to 5 mM. A step change from 2.75 to 4.25 mM resulted in a transient increase in the specific glucose and lactate rates, and a sharp increase in viable cell concentration, which eventually became constant at a higher steady state than before. The Berkeley group also looked at glucose pulse and step changes (Miller et al., 1989a). A much greater glucose pulse from 0.1 to 6.6 mM did not result in any transient changes in hybridoma cell concentration. The specific glucose uptake rate increased to more than twice its previous value immediately after the pulse, but then decreased rapidly over the next five hours. After, the CSGUR continued to decrease and reached its previous level before the pulse after 40 h. The cell specific lactate production rate did not react as quickly as the CSGUR, but did increase transiently over the first 10 h. A step change experiment from 0.1 to 8.4 mM glucose was also performed. Here, approximately steady state values were

obtained after three days, with an immediate increase and decrease in the CSGUR and CSLPR, respectively, over approximately the first 30 h. The increase in cell concentration was initially delayed for about 12 h. Since glucose was initially at a limiting concentration, this delay is likely due to time needed to synthesize additional precursors and enzymes.

In order to establish a steady state, approximately 6-10 days are required, depending on the nature and magnitude of the environmental change (Miller et al., 1988c; Miller et al., 1989a). The swift shift to an approximately constant response within 3-4 days suggests that perhaps obtaining a steady state is unnecessary to evaluate the effect of an environmental parameter. Previous studies, however, have concentrated mainly on substrate and metabolic byproduct concentration effects. Similar studies on environmental parameters such as pH, DO and temperature have not been reported.

The idea of using non-steady state responses of microbial cultures to approximate steady state results has been studied by the development of the accelerostat, or A-stat (Paalme et al., 1995). The A-stat was initially run as a chemostat to obtain a steady state. Then a smooth continuous change in dilution rate was started. This change was defined as the acceleration rate (dD/dt = constant). The A-stat method has been used to estimate the effect of dilution rate on growth in *Escherichia coli* and *Saccharomyces cerevisiae* (Paalme et al., 1995; Paalme et al., 1997a; Paalme et al., 1997b). At lower acceleration rates, growth yields will be nearly the same as in a chemostat, and a close approximation of steady state can be made. However, at higher acceleration rates, these yields are

variable, and steady state conditions cannot be obtained. A study by van der Sluis et al. (2001) examined maximum acceleration rate values where steady state characteristics could be approximated in an A-stat. They found a value of 0.001 h^{-2} to be ideal, with higher acceleration rates resulting in wider deviations. Simulations for *S. cerevisiae* demonstrated the deviations were dependent on the metabolic adaptation rate of the yeast, and also on the rate of change in environmental substrate concentrations during A-stat cultivation. However, it is important to note that if certain dilution rates look promising, for further investigation, one can stop the change in dilution rate to obtain steady state results.

Taken together, transient behaviour shows promise as a method to decrease process development time, since studies indicate that for mammalian cells, only a few days are needed to reach an indication of the steady state response to a shift in a process variable.

3 Materials and Methods

3.1 Cell Line and Medium

Chinese hamster ovary (CHO) cells (CHO 540/24) expressing human tissue plasminogen activator (t-PA) were provided by Cangene (Winnipeg, MB). The cells were grown in a serum-free medium (CNJ SFM 2.1b, Cangene, Winnipeg, MB) containing 25 mM glucose, 4 mM glutamine and a proprietary set of additives.

The inoculum train used for batch and perfusion cultures was slightly modified from the one used by Dowd (2000). A 1 mL frozen vial of between 10^6 and 10^7 CHO cells in cell freezing media (Sigma Chemical, St. Louis, MO) contained 8.7% dimethyl sulphoxide (DMSO) in Minimum Essential Medium (MEM) supplemented with methylcellulose. Cells were resuspended in 20 mL fresh medium in a T-flask (75 cm^2) and grown for 24 h. Cells were then pipetted to further disintegrate methylcellulose strands, and split 1:1 with fresh medium. After 3 days, cells were transferred to a larger T-flask (175 cm^2) in a 1:3 split with fresh medium. Then after 3-4 days, cells were transferred to a 300 mL working volume spinner and grown until cultures could be inoculated at 2-5 $x10^5$ cells/mL, with at least 90% viability. The passage number before inoculation was always kept below 6. The inoculum train for scale-down cultures was identical except the large T-flask was sufficient for inoculation. Cells were maintained in a humidified incubator at 37 ^{0}C, 5% CO_2. The only exception is for the batch culture at 33 ^{0}C, where cells were adapted to 33 ^{0}C on the 2nd passage in a 75 cm^2 T-flask.

3.2 Bioreactor Operation

3.2.1 Perfusion Culture

A 2 L glass Lh bioreactor (Slough, UK) with a working volume of 0.6 L was inoculated at a cell concentration between 2 to 5 x 10^5 cells/mL with approximately 100 mL of inoculum culture. The reactor was run in batch mode for about 7 days until the glucose concentration approached 5 mM. Perfusion was then initiated using 5 mM of glucose as a setpoint for control. Temperature, pH and dissolved oxygen (DO) were monitored and controlled using Anglicon (Brighton Systems, New Haven, UK) digital controllers, and data was logged by personal computer. A submerged silicone tube (length 3.8 m, inner diameter 2.2 mm, wall thickness 0.6 mm, Dow Corning, Midland, MI) allowed bubble free aeration. The pH was maintained through CO_2 addition to the silicone tubing by on/off control of a solenoid valve. DO was maintained by adding pure oxygen via a proportional mass flow controller into the silicone tubing. When high DO concentrations were required (i.e. 100% air saturation), a constant flow of pure oxygen (5-10 mL/min) through the tubing was used in addition to mass flow control. To prevent excess CO_2 and O_2 levels in the headspace, and also to maintain positive pressure within the reactor, a constant air purge of approximately 20 mL/min was added to the reactor headspace. An air flow of 10 mL/min was maintained through the silicone tubing as well. The off-gas exited through a condenser at ambient temperature, returning condensed water to the reactor. A pitched-blade impeller (Lh fermentation, Slough, UK) at 150 rpm was used for agitation. From the time of inoculation until the cell density reached 10^6 cells/mL, agitation was kept at 100 rpm.

Figure 3.1 illustrates the perfusion system used. Cells were retained in the reactor using a BioSep 10L acoustic separator (AppliSens, division of Applikon, Schiedam, The Netherlands). With acoustic separation, cells are subjected to an acoustic field, which causes them to reversibly aggregate in planes and settle as aggregates (Trampler et al., 1994; Woodside et al., 1998).

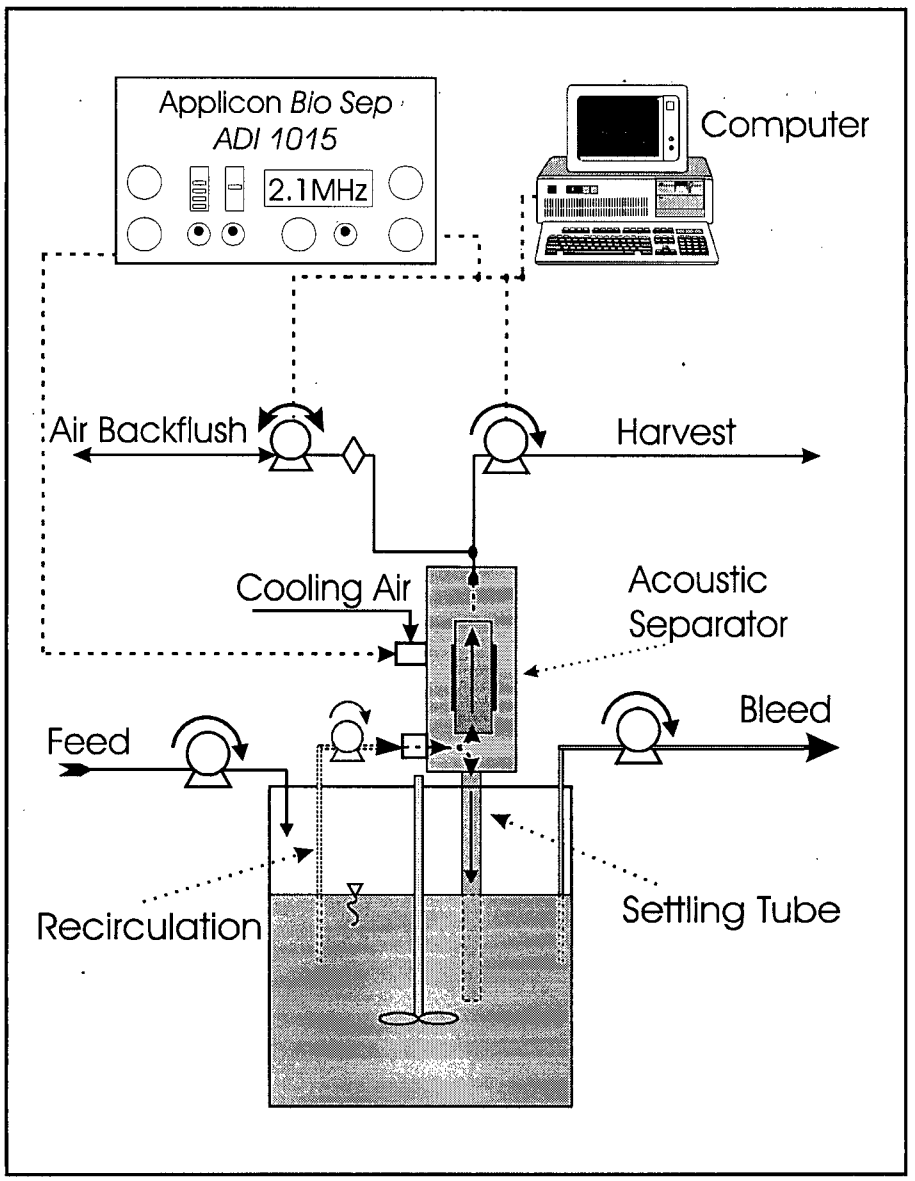

Figure 3.1 Schematic of perfusion system.

Cells were recycled back to the reactor using an air backflush method (Gorenflo et al., 2003), whereby air was periodically pumped down through the acoustic separator into the reactor to clear the separator of accumulated cells. During regular operation, the backflush frequency was set at 12 h^{-1}. During certain times when computer control of the backflush system failed, recirculation was used instead. In this conventional method, culture broth was continuously pumped into the bottom of the separator in order to clear any aggregated cells.

A separate bleed line was used to keep the cell concentration at a constant 10^7 cells/mL, and in the process also remove accumulated cellular debris. The bleed rate was based on the culture growth rate (calculation shown in Section 3.4). The culture volume was maintained using a conductance-based sensor that triggered the harvest pump, preserving a constant liquid level in the reactor. Cole-Parmer (Vernon Hills, IL) Masterflex peristaltic pumps (digital standard drive models 7521-40 and 7550-20) equipped with L/S standard pump heads were used for harvesting, cell bleed, air backflush and recirculation (when needed). Feed medium was added every hour using a P10T peristaltic pump (Dungey Inc., Agincourt, ON). The medium feed, cell bleed and separator operation were controlled with programs created in LabVIEW 6.1 (National Instruments, Austin, TX).

3.2.2 Batch Culture

Batch experiments were performed and monitored using the same Lh bioreactor and Anglicon system as with perfusion cultures. Batch cultures were inoculated at $1.5x10^5$ cells/mL with approximately 100 mL inoculum volume.

3.2.3 Scale-down Culture

Scale-down (SD) cultures were carried out in 100 mL microcarrier spinner flasks (Model 1967-00100, Bellco Glass, Vineland, NJ). The flask were modified and equipped with sample ports. Cultures were maintained in a CO_2 incubator Model 3029 (Forma Scientific, Marietta, OH). The humidification and CO_2 control in the incubator was disabled. Cultures were inoculated at 10^5 cells/mL. Agitation used paddle impellers at 60 rpm and culture volume was kept at 100 mL. The cultures were run in semicontinuous mode, in which a certain amount of culture was removed and replaced by fresh medium to maintain a working volume of 100 mL. Medium exchange was performed manually every 24 h in a biological safety cabinet. The amount of medium exchanged varied from day-to-day in order to return the cell concentration after dilution to 10^5 cells/mL.

A gas mixture of nitrogen, oxygen and carbon dioxide at a total flow rate of 25 mL/min was controlled by gas flowmeters (Cole-Parmer, Vernon Hills, IL). The gas was humidified by bubbling into 300 mL distilled H_20 with 1% v/v gentamycin (GIBCO Invitrogen Canada, Burlington, ON). The gentamycin was used to prevent contamination of the humidifier. The humidified gas then flowed to the headspace of the spinner flask. Each spinner had its own set of flowmeters and humidifiers so oxygen could be controlled independently. Figure 3.2 shows the scale-down setup. Since there were no

pH or DO probes used, a Stat Profile 10 blood/gas analyzer (NOVA Biomedical, Waltham, MA) was used to measure pO_2 and pCO_2 values. A separate spinner was set up with 100 mL fresh medium and sparged directly with 5% CO_2 balance air to obtain pO_2 and pCO_2 values corresponding to 95% DO and 5% CO_2, respectively. A ratio of the sample pO_2 over the control pO_2 from the sparged spinner was used to estimate % air saturation in the SD cultures. A similar ratio was used for % CO_2. A setpoint of 5% CO_2 was used in all SD cultures.

Samples were taken using a 10 mL plastic syringe held upside down in order to minimize any gas mixing in the sample. The samples were also analysed for pO_2 and pCO_2 within 30 s of sampling. Initial tests showed that after 4 min samples had significantly lower pCO_2 and higher pO_2 values due to mixing with ambient air, even when filled plastic microcentrifuge tubes used for sample storage were kept capped.

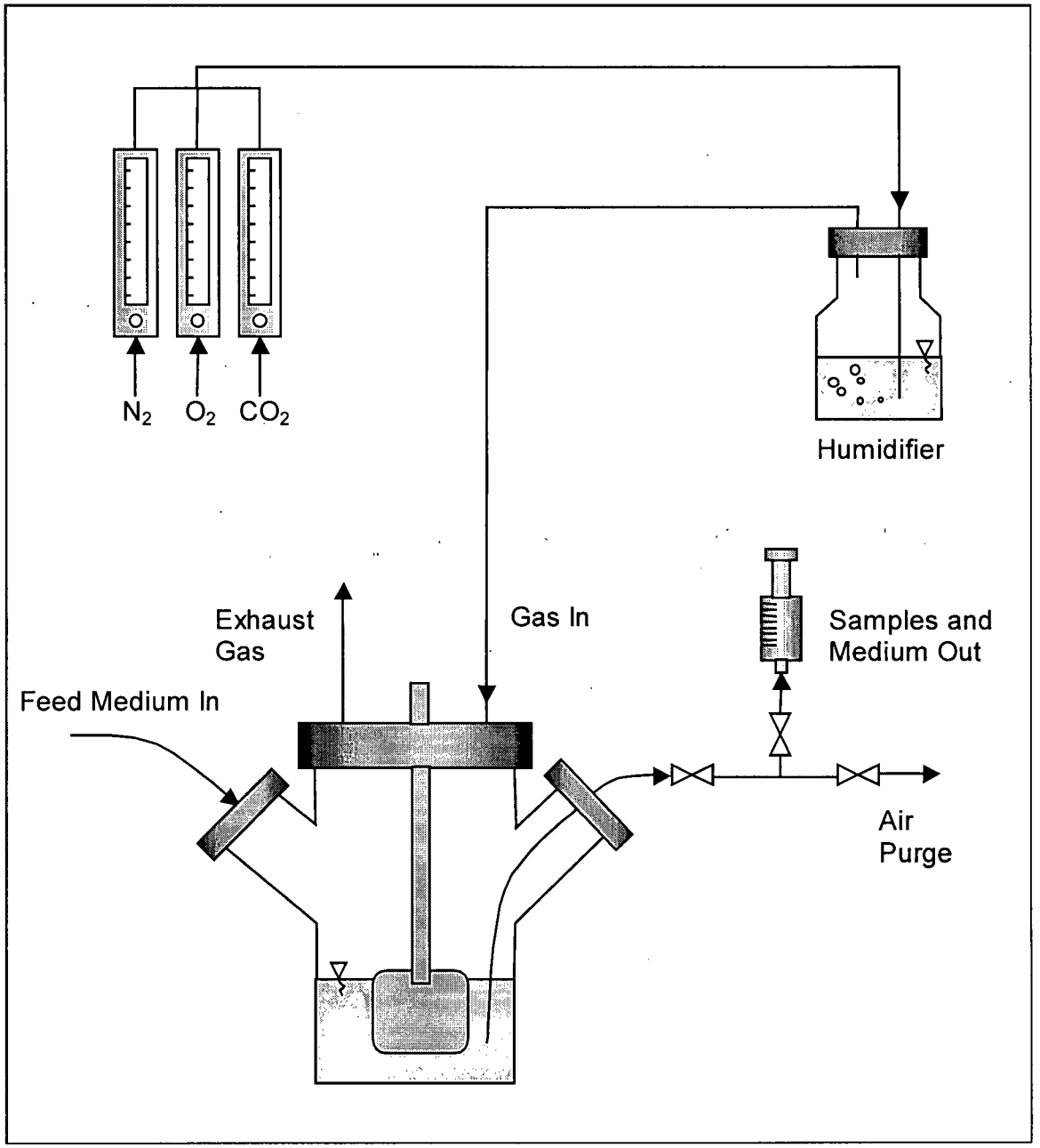

Figure 3.2 Schematic of scale-down culture.

3.3 Analytical Methods

3.3.1 Cell concentration, viability and sample analysis

Total cell numbers and viability were determined by trypan blue dye exclusion and a haemocytometer. To disperse aggregates, 3-5 mL culture samples were mixed 1:1:1 by volume with trypsin-EDTA containing 0.25% trypsin / 1 mM EDTA•4Na (GIBCO Invitrogen Canada, Burlington, ON), and with phosphate-buffered saline (KCl = 0.2 g/L, KH_2PO_4 = 0.2 g/L, NA_2HPO_4 = 1.51 g/L, NaCl = 8 g/L, pH adjusted to 7.4 with NaOH) incubated at 37 ^{0}C for 5 min. Viability was determined using non-trypsinized samples. A Stat Profile 10 blood/gas analyzer (NOVA Biomedical, Waltham, MA) was used to determine glucose, lactate and blood urea nitrogen (BUN) concentrations. The ammonium concentration was calculated using a calibration curve with BUN. Osmolarity was determined using the Advanced Osmometer Model 3D3 (Advanced Instruments, Norwood, MA). Supernatant samples were obtained by centrifugation (300*g*, 10 min) and frozen at –20 ^{0}C for later t-PA analysis.

3.3.2 t-PA Analysis

The enzymatic t-PA activity was determined through a modified colorimetric assay that uses a plasminogen activation system to measure plasmin activity through use of a chromogenic peptide substrate (Ranby and Wallen, 1981; Verheijen et al., 1982). Human t-PA (Calbiochem, La Jolla, CA) was serially diluted with 0.1 M Tris-HCl buffer (adjusted to pH 8.0 with NaOH) and 0.1% v/v Tween 80 to make standards ranging from 0.25 to 10 U/mL. Supernatant culture samples were also diluted with the Tris dilution buffer. 96-well microtiter plates (Nalge Nunc International, Rochester, NY) were loaded

with standards and samples, then a reaction mixture of plasminogen (Boehringer Mannheim Canada, Laval, PQ), CNBr-fragmented fibrinogen (Calbiochem, La Jolla, CA) and the substrate D-Val-Leu-Lys p-nitroanilide (Sigma, St. Louis, MO) was added. The plates were sealed and incubated at 37 ^{0}C for 3 h, during which the p-nitroanilide is cleaved from the substrate by plasmin. The p-nitroanilide was then detected at 405 nm using a microtiter plate reader (Molecular Devices, Sunnyvale, CA) and used to measure t-PA activity. To obtain t-PA concentrations, a conversion factor of 580 000 U/mg (WHO standard specific activity) was applied.

3.4 Equations Used in Analysis

Cell specific rates in batch culture were determined by the time-integral method using the equation:

$$q_N = \frac{dN}{X_v dt} \tag{3.1}$$

where q_N is the cell specific rate of N, X_v is the viable cell concentration. By plotting [N] (μg/mL or μmol/mL) against the time integral of viable cells $\int X_v dt$ (10^6 cell·h/mL), the slope q_N (pg/cell·h or pmol/cell·h) was determined.

For the following equations, time point 1 refers to the previous day, time point 2 refers to the current day, and time point 3 refers to the next day.

The log mean average viable cell concentration ($X_{v,LM}$, cells/mL) between time points 1 and 2 was calculated by:

$$X_{v,LM} = \frac{X_{v2} - X_{v1}}{\ln \frac{X_{v2}}{X_{v1}}} \tag{3.2}$$

Cell specific rates in perfusion culture between time points 1 and 2 were determined by the equation:

$$q_N = \frac{\frac{[N]_2 - [N]_1}{t_2 - t_1} \cdot V_r + F\left([N]_{feed} - \frac{[N]_1 + [N]_2}{2}\right)}{V_r \cdot X_{v,LM}} \tag{3.3}$$

where q_N is the cell specific rate of N (pg/cell·h or pmol/cell·h); t is the sample time (h); V_r is the reactor working volume (L or mL); F is the perfusion feed rate (L/h).

The apparent cell specific growth rate (h^{-1}) in perfusion culture between time points 1 and 2 was calculated by the equation:

$$\mu_{app} = \frac{\ln\left(\frac{X_{v2}}{X_{v1}}\right)}{t_2 - t_1} \tag{3.4}$$

The bleed rate in perfusion culture between time points 1 and 2 was determined by:

$$D_{bleed} = \frac{F_{bleed}}{V_r} \quad \textbf{(3.5)}$$

where D_{bleed} is the bleed rate independent of cell concentration (h^{-1}); F_{bleed} is the amount bled per hour between time points 1 and 2 (mL/h); V_r is the reactor working volume (mL).

The real cell specific growth rate in perfusion culture between time points 1 and 2 was calculated with the equation:

$$\mu_{calc} = D_{bleed} + \mu_{app} \quad \textbf{(3.6)}$$

where μ_{calc} is the calculated cell specific real growth rate (h^{-1}).

Finally, the predicted bleed rate in perfusion culture between time points 2 and 3 was then calculated using the equation:

$$F_{bleed} = \mu_{calc} \cdot V_r \quad \textbf{(3.7)}$$

where F_{bleed} is the amount of culture that will be bled per hour between the current day and next day (time points 2 and 3) (mL/h).

4 Results and Discussion - Batch and Pseudo Steady State Cultures

4.1 Introduction

In this chapter, the effect of temperature, dissolved oxygen and pH on the productivity of a recombinant CHO cell line is presented. The first section details results from batch culture runs, then batch and perfusion productivity is compared. The final section presents pseudo steady state results in perfusion culture, for a range of different environmental conditions.

4.2 Batch Cultures

Batch cultures were run at 37 or 33 ^{0}C. At 37 ^{0}C, cells grew exponentially at a growth rate of 0.019 ± 0.001 h^{-1} for 5 days, then moved into a stationary phase (Figure 4.1). There was no lag phase observed. The maximum viable cell concentration obtained was 2.6×10^6 cells/mL. The 33 ^{0}C culture grew exponentially at a growth rate of 0.005 ± 0.0003 h^{-1} for 12 days, reaching a maximum viable cell concentration of 1.1×10^6 cells/mL. There was no lag phase observed, presumably since the inoculum was adapted for the low temperature culture (i.e. inoculum was grown at 33 ^{0}C for 5 passages before inoculation). The low cell concentration on day 4 in the 33 ^{0}C culture was likely due to a cell counting error. The viability was near 90% during the exponential and stationary phases in both cultures.

At 33 ^{0}C, there was an apparent increase in culture longevity, with cells remaining in stationary phase twice as long as observed at 37 ^{0}C. However, cultivation at 33 ^{0}C resulted in a 74% decrease in the growth rate, and a ~50% lower maximum viable cell concentration when compared to cultivation at 37 ^{0}C. Lowering culture temperature is known to reduce the growth rate. For instance, CHO cell growth rate decreases of 45-50% have been reported (Furukawa and Ohsuye, 1998; Yoon et al., 2003). The difference in DO concentrations between the two batch cultures (70% versus 80% air saturation) was not thought to significantly influence any differences observed. This can be substantiated by later experiments in scale-down cultures, where fluctuations of ±10% air saturation did not influence any results between replicate cultures (Section 5.3).

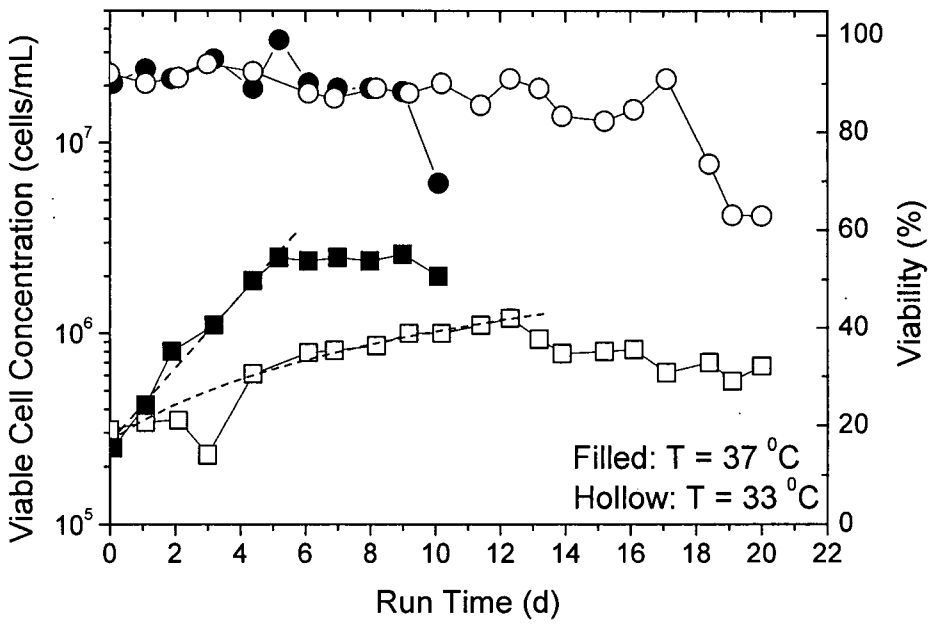

Figure 4.1 Cell concentration (squares) and viability (circles) in CHO cell batch culture at 37 ^{0}C (closed symbols) and 33 ^{0}C (open symbols).

Measurement errors for cell concentration estimated at 10%. Exponential phase denoted by dashed line. For 37 ^{0}C culture: T = 36.8 ± 0.1 ^{0}C, pH = 7.00 ± 0.01, DO = 71 ± 3%. For 33 ^{0}C culture: T = 33.1 ± 0.1 ^{0}C, pH = 7.00 ± 0.08, DO = 82 ± 2%.

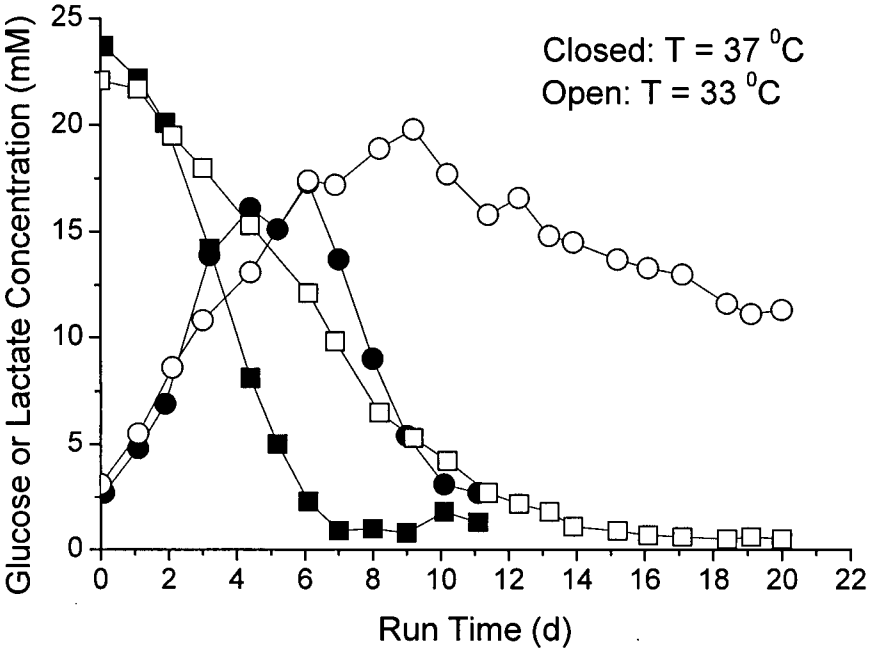

Figure 4.2 Glucose (squares) and lactate (circles) concentrations in CHO cell batch culture at 37 ^{0}C (closed symbols) and 33 ^{0}C (open symbols).

Measurement errors for glucose and lactate are 0.5 mM. For 37 ^{0}C culture: T = 36.8 ± 0.1 ^{0}C, pH = 7.00 ± 0.01, DO = 71 ± 3%. For 33 ^{0}C culture: T = 33.1 ± 0.1 ^{0}C, pH = 7.00 ± 0.08, DO = 82 ± 2%.

Both glucose and lactate profiles for the 37 and 33 ^{0}C batch cultures were similar, with lactate consumption occurring during the stationary phase of both cultures (Figure 4.2). Glucose uptake was rapid during the exponential phase of the 37 ^{0}C culture, reaching a concentration of 5.0 mM on day 5, when exponential growth ceased. Glucose uptake was slower in the 33 ^{0}C culture, reaching 5.3 mM on day 9. Both cultures showed consumption of lactate during stationary phase, although lactate consumption in the 33 ^{0}C culture started during the last 3 days of exponential phase.

Table 4.1 details the results obtained from the two batch cultures. The overall glucose uptake rate at 33 ^{0}C was 0.08 ± 0.003 mmol/h, significantly slower than the GUR at 37 ^{0}C ($p < 0.01$). The average lactate production rate during exponential phase was also lower than at 37 ^{0}C ($p < 0.01$). However, on a cell specific basis, there was no significant effect of temperature on glucose consumption and lactate production. The average CSGUR and CSLPR at 33 ^{0}C were 2.7 ± 0.2 and 3.0 ± 0.4 pmol/cell·d, respectively. The majority of studies have reported decreased cell specific metabolic consumption and production rates at lower temperature, except Yoon et al. (2003) who report similar cell specific glucose and lactate rates at 33 and 37 ^{0}C.

Table 4.1 Summary of Batch Culture Results. All results from exponential phase unless otherwise noted.

Variable	T = 37 ^{0}C	T = 33 ^{0}C	p-value
Maximum Xv (cells/mL)	2.5 x 10^6	1.1 x 10^6	-
Growth rate (h^{-1})	0.019 ± 0.001	0.005 ± 0.0003	$p < 0.01$
GUR (mmol/h)	0.17 ± 0.01	0.08 ± 0.003	$p < 0.01$
LPR (mmol/h)	0.11 ± 0.01	0.08 ± 0.006	$p < 0.01$
LPR* (mmol/h)	-0.15 ± 0.01	-0.03 ± 0.002	$p < 0.01$
CSGUR (pmol/cell·d)	2.8 ± 0.3	2.7 ± 0.2	not significant
CSLPR (pmol/cell·d)	3.5 ± 0.6	3.0 ± 0.4	not significant
CSLPR* (pmol/cell·d)	-1.4 ± 0.1	-0.8 ± 0.05	$p < 0.01$
Maximum [t-PA] (mg/L)	67.4 ± 2.4	81.1 ± 0.8	$p < 0.01$
$q_{t\text{-}PA}$ (pg/cell·d)	6.5 ± 0.8	9.7 ± 0.6	$p < 0.05$

Note: * indicates rate during stationary phase. Cell specific rates calculated using plot of concentration against time integral of viable cells (Yoon et al., 2003).

During stationary phase, lactate in the 37 ^{0}C culture was consumed at an average production rate of -0.15 ± 0.01 mmol/h, or by taking into account the cell concentration, an average cell specific lactate production rate of -1.4 ± 0.1 pmol/cell·d. The lactate

production rate at 33 ^{0}C was significantly higher (i.e. lactate was not being consumed as rapidly) than at 37 ^{0}C, with a value of -0.03 ± 0.002 mmol/h ($p < 0.01$). The cell specific lactate production rate was -0.8 ± 0.05 pmol/cell·d, also significantly higher than at 37 ^{0}C ($p < 0.01$). Since the viable cell concentration during this time does not change very much, the time integral of viable cells does not have a large effect, and therefore, the CSLPR reflects the significant change in LPR. One explanation for lower lactate consumption at 33 ^{0}C is the presence of a higher residual glucose concentration in the culture at the onset of stationary phase. Since lactate consumption is initiated when glucose is exhausted, a lower rate of consumption would be sufficient if glucose was available.

This change in cellular metabolism to lactate consumption has been reported before in fed-batch GS-NS0 cultures, coinciding with glucose depletion and the beginning of cell death (Bibila et al., 1994; Zhou et al., 1997). It has been hypothesized that lactate consumption is initiated for the production of pyruvate. Batch cultures of CHO cells have also shown this change (Zanghi et al., 1999).

The average maximum t-PA concentration obtained at 37 ^{0}C was 67.4 ± 2.4 mg/L (Figure 4.3), while the average maximum concentration at 33 ^{0}C was significantly higher at 81.1 ± 0.8 mg/L ($p < 0.01$). This indicates a lower temperature can be beneficial for production in this CHO cell line, since higher product concentrations facilitate downstream processing. The t-PA concentration started to decrease during the last few

days of the 37 ^{0}C batch culture, most likely due to t-PA degradation from proteolytic enzymes released from dead cells (Dowd, 2000).

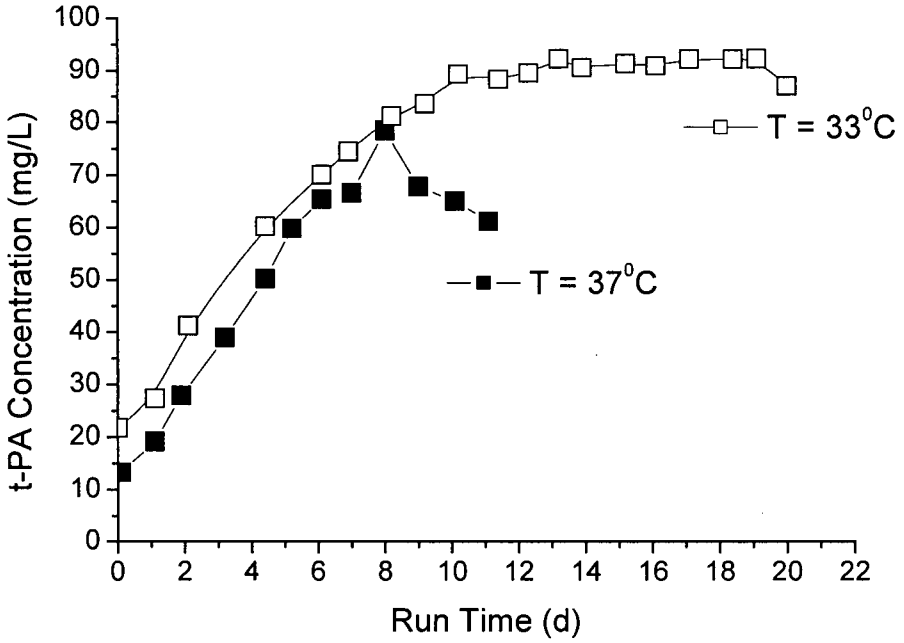

Figure 4.3 t-PA concentrations in CHO cell batch culture at 37 ^{0}C (closed symbols) and 33 ^{0}C (open symbols).

Measurement errors for t-PA concentration are ± 6%. Concentration at start of culture is not zero due to t-PA present in inoculum. For 37 ^{0}C culture: T = 36.8 ± 0.1 ^{0}C, pH = 7.00 ± 0.01, DO = 71 ± 3%. For 33 ^{0}C culture: T = 33.1 ± 0.1 ^{0}C, pH = 7.00 ± 0.08, DO = 82 ± 2%.

Increased cell specific t-PA production rates at 33 ^{0}C is one reason for the higher t-PA concentration obtained at lower temperature (Figure 4.4). At 37 ^{0}C, the cell specific t-PA production rate, or $q_{t\text{-}PA}$, was 6.5 ± 0.8 pg/cell·d, significantly lower than the rate of 9.7 ± 0.6 pg/cell·d observed at 33 ^{0}C by an F-test comparison ($p < 0.05$). However, lower temperature resulted in only a 1.5-fold increase of the cell specific productivity, while

Ducommun et al. (2002) found a 6-fold increase in the specific protein production rate at 33 ^{0}C compared to 37 ^{0}C, although the mode of culture was a packed bed process. The effect of temperature on $q_{t\text{-}PA}$ may be cell line dependent. Yoon et al. (2003) showed a 4-fold increase in specific EPO production in a CHO cell line at 33 ^{0}C over 37 ^{0}C. However, another study with CHO cells showed no effect on productivity (Rossler et al., 1996), confirming temperature effect on productivity is cell line dependent.

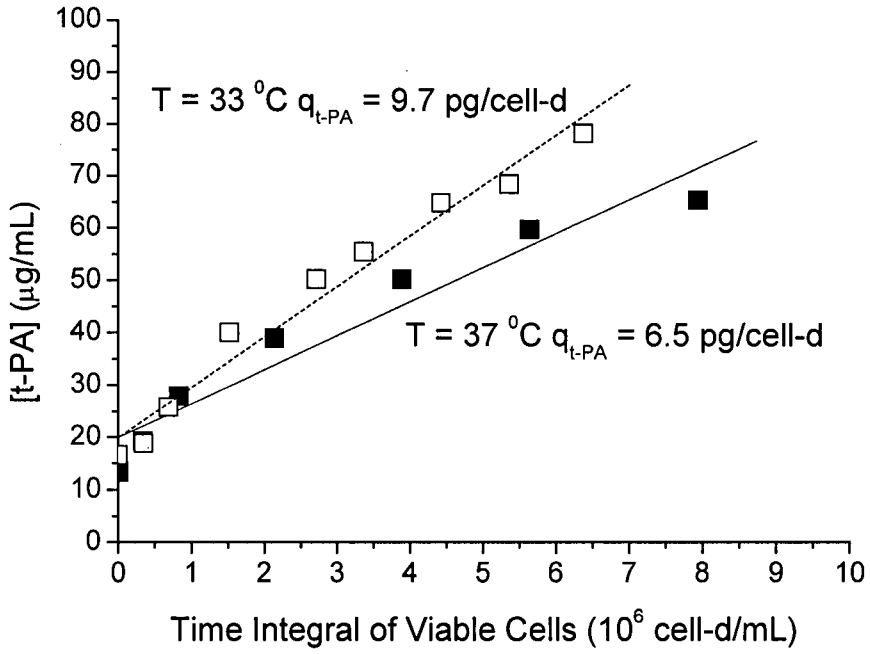

Figure 4.4 Evaluation of cell specific t-PA production rate using integral method for CHO cell batch cultures at 37 ^{0}C (closed symbols) and 33 ^{0}C (open symbols).

Measurement errors for t-PA concentration are ± 6%. Measurement errors for cell concentration estimated at 10%. Concentration at start of culture is not zero due to t-PA present in inoculum. Cell specific rates calculated using plot of concentration against time integral of viable cells (Yoon et al., 2003). For 37 ^{0}C culture: T = 36.8 ± 0.1 ^{0}C, pH = 7.00 ± 0.01, DO = 71 ± 3%. For 33 ^{0}C culture: T = 33.1 ± 0.1 ^{0}C, pH = 7.00 ± 0.08, DO = 82 ± 2%.

Although the cell specific t-PA productivity is higher at 33 ^{0}C, the much lower growth rate and cell concentration at this temperature has a negative effect on volumetric productivity. Overall volumetric t-PA production was ~50% lower at 33 ^{0}C than at 37 ^{0}C. The higher t-PA concentration at the lower temperature was more likely due to prolonged culture longevity, which increased the overall amount of protein accumulation. This has been reported as the main reason for higher product concentrations at low temperature in a number of studies (Furukawa and Ohsuye, 1998; Kaufmann et al., 1999; Yoon et al., 2003). Lower temperatures may also reduce the release of proteolytic enzymes from dead cells. Decreased lactate dehydrogenase (LDH) activity as a measure of cell lysis has been reported at low temperatures (Furukawa and Ohsuye, 1998; Yoon et al., 2003).

To further enhance t-PA production, a biphasic system could be used where cells are grown to high cell density at 37 ^{0}C, then shifted to 33 ^{0}C. However, nutrient supplementation may be needed, since glucose is rapidly depleted at the higher temperature. Rossler et al. (1996) have used a CHO fed-batch system shifted from 37 to 33 ^{0}C. Product concentration was comparable to fed-batch cultivation at 33 ^{0}C alone, but the total culture time required was reduced by 50%. A repeated-batch system has also been used to extend cultivation time, while maintaining a higher viable cell concentration (Weidemann et al., 1994).

4.3 Culture Mode Comparison - Batch versus Perfusion

The cumulative t-PA production versus culture time is plotted for both batch and perfusion culture in Figure 4.5. Perfusion culture production was not at optimal levels for the whole run since various pseudo steady state experiments, where the environmental parameters were changed, were performed during the run. However, even with non-optimized production after 118 days of culture, a total of 8.3 g of t-PA were produced in the perfusion culture.

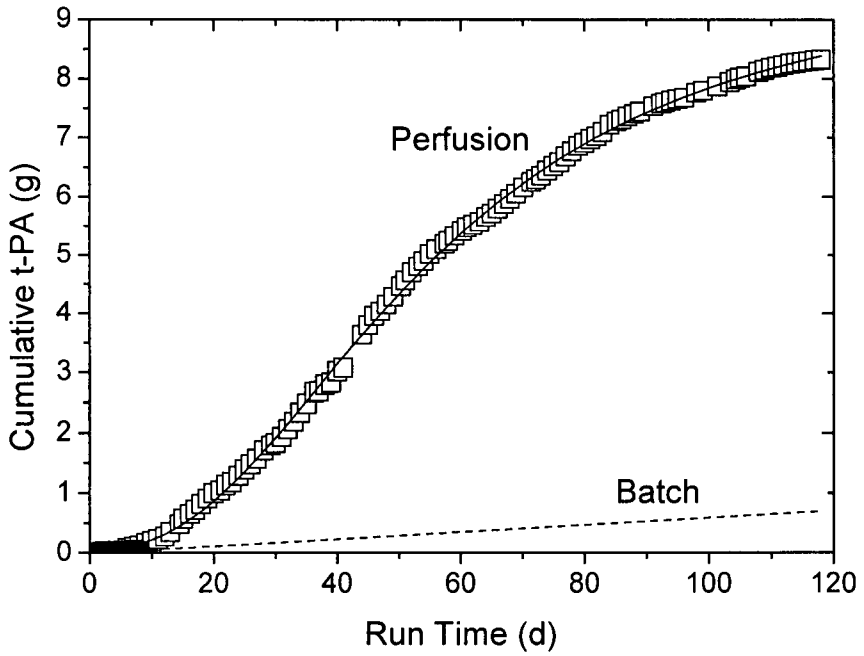

Figure 4.5 Cumulative t-PA produced in an 8-day batch culture (closed symbols) and a 118-day perfusion culture (open symbols).

T, pH and DO varied during course of perfusion culture. Batch culture at T = 36.8 ± 0.1 ^{0}C, pH = 7.00 ± 0.01, DO = 71 ± 3%. Dotted line represents extrapolated potential cumulative t-PA that could be produced by 15 8-day batch cultures during the equivalent time of 118 day perfusion culture.

Extrapolation of an 8 day batch culture would yield only 0.6g of t-PA, indicating perfusion culture allows close to 14-fold higher volumetric productivity than batch culture. The concentration of t-PA in perfusion was 3.4-fold higher than batch (Table 4.2). To produce 8.3g of t-PA in batch culture, 231 batch runs would be needed.

Table 4.2 Comparison of an 8-day Batch Culture and a 118-day Perfusion Culture.

Culture Mode	Run Time (d)	Max [t-PA] (mg/L)	Max VtPAPR (mg/L·d)	Cumulative t-PA (g)
Batch	8	67 ± 2	16.2	0.04
Perfusion	118	228 ± 15	223	8.3

4.4 Pseudo Steady State Perfusion Culture

To determine the influence of environmental parameters on t-PA production, pseudo steady state experiments were performed during the course of three perfusion cultures. The majority of pseudo steady state experiments were performed during run P28 (Figure 4.6), which initially used a control setpoint of 37 ^{0}C, pH 7.0 and 70% air saturation until a steady state in cell specific glucose uptake was achieved. Pseudo steady state values at 37 ^{0}C, pH 7.0 and 100% air saturation were obtained in a different run (P32). Another run (P44) was used to obtain pseudo steady state values at 37 ^{0}C, pH 7.1 and 70% air saturation. Pseudo steady state was assumed when the cell specific glucose uptake rate remained constant (± 0.2 pmol/cell·d) for at least three days, generally 5-8 days after the environmental change. The feed was controlled using a glucose setpoint of 5 mM in the reactor. Cell concentration was kept constant at 10^7 cells/mL through use of an acoustic filter and control of the cell bleed rate. The perfusion culture setup is further described in

Chapter 3, Materials and Methods. To test for significance, the Tukey-Kramer test for multiple comparisons was used ($\alpha = 0.05$).

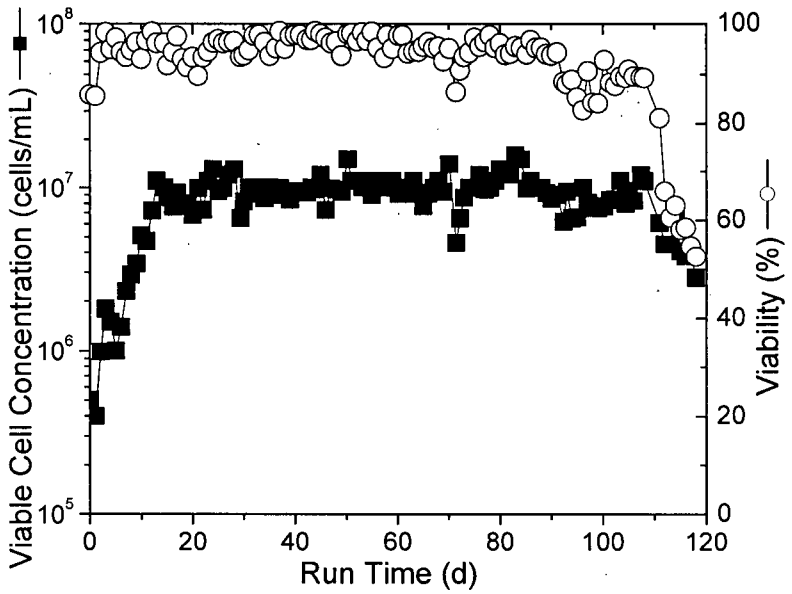

Figure 4.6 Viable cell concentration (closed squares) and viability (open circles) during 118 day perfusion culture (P28).

System was run as a batch culture for the first 7 days, then perfusion was initiated. Cell bleed was started on the 16th day of culture, when the cell concentration reached 10^7 cells/mL. Viability was maintained over 90% for most of the culture. Culture failure was due to experiments at pH 6.67 initiated on day 109.

4.4.1 Effect of Temperature

Three different temperatures were tested: 36.9 ± 0.4, 33.6 ± 0.4 and 31.1 ± 0.0 ^{0}C, with results summarized in Table 4.3. At 37 ^{0}C, the calculated cell specific growth rate was 0.022 ± 0.002 h^{-1}. The growth rate at 33.6 ^{0}C was significantly lower than the growth rate at 37 ^{0}C by 81% ($p < 0.05$). The growth rates obtained in perfusion are comparable to those obtained previously in batch culture (0.019 and 0.005 h^{-1} at 37 and 33 ^{0}C, respectively).

Table 4.3 Pseudo Steady State Data at Various Temperatures.

Variable	T = 36.9 ^{0}C	T = 33.6 ^{0}C	T = 31.1 ^{0}C
μ_{calc} (h^{-1})	0.022 ± 0.002	0.004 ± 0.003	0.003 ± 0.001
CSGUR (pmol/cell·d)	2.15 ± 0.01	1.34 ± 0.08	1.15 ± 0.07
CSLPR (pmol/cell·d)	1.37 ± 0.03	0.67 ± 0.11	0.11 ± 0.04
[t-PA] (mg/L)	147 ± 8	228 ± 15	238 ± 5
CStPAPR (pg/cell·d)	15.1 ± 0.4	17.9 ± 1.0	12.9 ± 0.4
VtPAPR (mg/L·d)	167 ± 16	147 ± 8	132 ± 7

Note: Values are average of last 3 days of pseudo steady state ± SEM, with the exception of values at 31.1 ^{0}C, which used last 2 days average ± SEM. Values for 31.1 ^{0}C taken from run P44, others taken from run P28. For μ_{calc}, values are last 4 days average ± SEM. Calculations for cell specific rates and growth rates are detailed in Chapter 3, Section 3.4.

The cell specific glucose uptake rate decreased with temperature (Table 4.3). The CSGUR was 1.15 ± 0.07 pmol/cell·d at 31.1 ^{0}C, 46% lower than at 37 ^{0}C ($p < 0.05$). At 33.6 ^{0}C, the CSGUR was also significantly lower than at 37 ^{0}C ($p < 0.05$), although not significantly different from the value at 31.1 ^{0}C. Cell specific lactate production also significantly decreased (by ~50%) at 33.6 ^{0}C compared to 37 ^{0}C ($p < 0.05$). The decrease in the CSGUR and CSLPR is in agreement with other reports on low temperature cultivation in perfusion (Chuppa et al., 1997; Furukawa and Ohsuye, 1999), although the extent of decrease is varied. Notably, the decreased rate of cell metabolism stands in contrast to the previous batch culture results, in which no change in the CSGUR or CSLPR was observed.

Temperature did have a significant effect on the cell specific t-PA production rate (Figure 4.7). The CStPAPR at 33.6 ^{0}C was significantly higher than at 31.1 ^{0}C by 1.4-fold ($p < 0.05$). However, there was no significant difference between the cell specific productivity at 37 and 33.6 ^{0}C, and at 37 and 31.1 ^{0}C. Cell specific productivity at 33 ^{0}C was 1.8-fold higher in perfusion culture compared to the previous batch culture. There

are contrasting reports on the effect of temperature on cell specific productivity, indicating cell line dependency. A number of studies have indicated increased productivity at low temperature (Takagi and Ueda, 1994; Furukawa and Ohsuye, 1999).

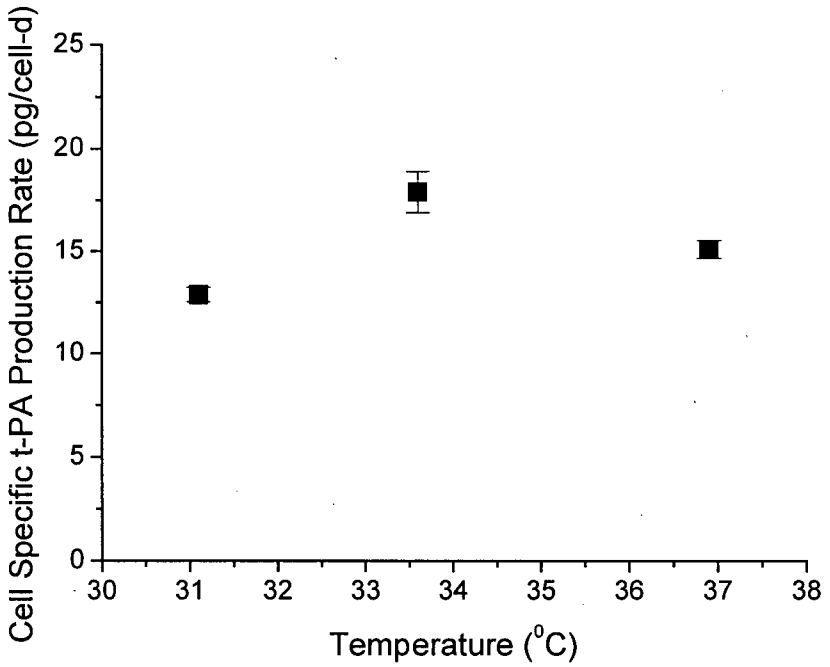

Figure 4.7 Cell specific t-PA production as a function of temperature.

Setpoints used: DO = 70% air saturation, pH = 7.0, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values taken from two perfusion cultures, P28 and P44. Each point represents mean of last 3 days ± SEM at pseudo steady state, except at 31.1 ^{0}C, where last 2 days average ± SEM was used. Values for 31.1 ^{0}C taken from run P44, others taken from run P28.

The tPA concentration was also affected by temperature (Figure 4.8). The concentration at 33.6 ^{0}C was 227.6 ± 14.9 mg/L, 1.5-fold higher than at 37 ^{0}C ($p < 0.05$). The high t-PA concentration at the lower temperatures is likely due to the lower perfusion rate required. The perfusion rate at 31.1 ^{0}C was 0.51 L/L·d, 53% lower than the rate at 37 ^{0}C.

The perfusion rate at 33.6 ^{0}C was 0.63 L/L·d, 43% lower than the rate at 37 ^{0}C. Lower perfusion rates are beneficial for production purposes not only because of the resulting higher product concentrations, but also in terms of decreased media expense. A possible downside is the potential for decreased volumetric productivity. However, although the volumetric productivity decreased at lower temperatures, the decrease was not significant.

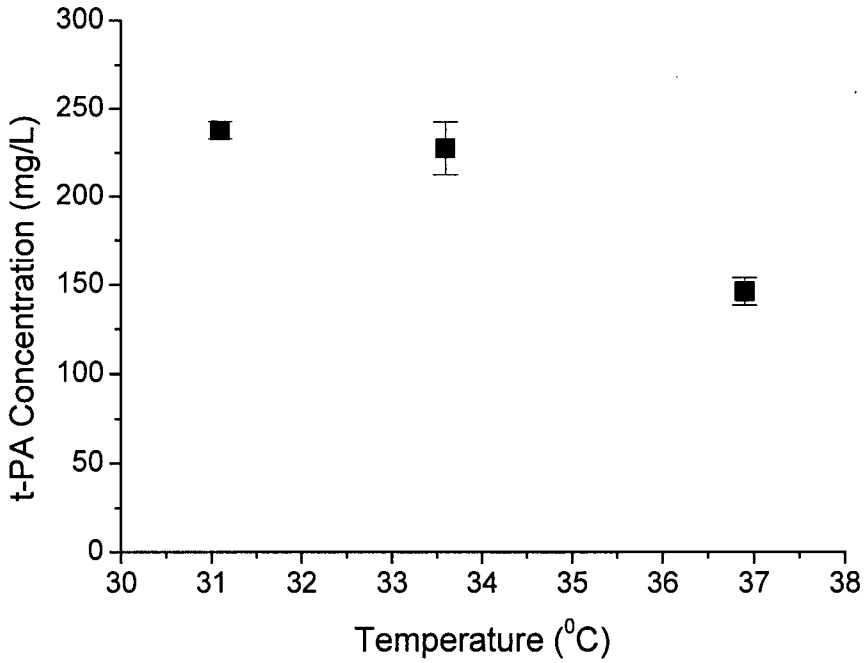

Figure 4.8 Concentration of t-PA as a function of temperature.

Setpoints used: DO = 70% air saturation, pH = 7.0, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values taken from two perfusion cultures, P28 and P44. Each point represents mean of last 3 days ± SEM at pseudo steady state, except at 31.1 ^{0}C, where last 2 days average ± SEM was used. Values for 31.1 ^{0}C taken from run P44, others taken from run P28.

4.4.2 *Effect of Dissolved Oxygen*

Three different dissolved oxygen concentrations were investigated: 16 ± 2, 72 ± 4 and 105 ± 5% air saturation. The setpoint of 100% air saturation was exceeded because pure oxygen was used in order to control DO.

Cell growth was affected by high DO concentrations. At 105% air saturation, cells grew at a rate of 0.016 h^{-1}, 36% lower than the growth rate at 72% air saturation. The growth rate at 16% showed no significant inhibition. Growth inhibition at high O_2 concentrations has been previously observed in other cell lines (Ozturk and Palsson, 1990; Dunster et al., 1997), primarily thought to be due to damage caused by oxidative stress.

Table 4.4 is a summary of the pseudo steady state data obtained at different DO concentrations. The cell specific glucose uptake rate at 72% air saturation was 23% lower than the value observed in batch culture, likely due to the low glucose concentration of 5 mM that was used. When the DO was decreased to 16% air saturation, a significant 1.4-fold increase in the CSGUR was observed ($p < 0.05$). However, increasing the DO to 105% air saturation from 72% did not affect the CSGUR.

The cell specific lactate production followed a similar trend for lower oxygen. At 72% air saturation, the CSLPR was again lower than encountered in batch culture. Decreasing the oxygen concentration resulted in a 2.4-fold increase in the CSLPR to

3.28 ± 0.18 pmol/cell·d, while increasing O_2 to 105% air saturation also increased the CSLPR, although only a 1.5-fold increase.

Table 4.4 Pseudo Steady State Data at Various Oxygen Concentrations.

Variable	DO = 16%	DO = 72%	DO = 105%
CSGUR (pmol/cell·d)	2.96 ± 0.04	2.15 ± 0.01	2.15 ± 0.01
CSLPR (pmol/cell·d)	3.28 ± 0.18	1.37 ± 0.03	2.02 ± 0.08
[t-PA] (mg/L)	63 ± 10	147 ± 8	105 ± 15
CStPAPR (pg/cell·d)	9.3 ± 1.8	15.1 ± 0.4	10.4 ± 2.8
VtPAPR (mg/L·d)	95 ± 19	167 ± 16	96 ± 27

Note: Values are average of last 3 days of pseudo steady state ± SEM. Calculations for cell specific rates and growth rates are detailed in Chapter 3, Section 3.4. Values at 105% air saturation taken from run P32, others from run P28.

The increase in both the CSGUR and CSLPR at 16% air saturation as compared to 72% suggests that the cells are experiencing hypoxic conditions. When O_2 is limited, cells use more glucose for energy production, resulting in a higher glycolytic flux. This is known as the Pasteur effect (Ozturk and Palsson, 1991). However, the air saturation values for hypoxia reported previously have been very low, with a maximum of 10% DO (Miller et al., 1987; Ozturk and Palsson, 1991). As well, in most reported cases, growth inhibition has been observed under hypoxic conditions (Ozturk and Palsson, 1991; Lin et al., 1993), but no growth inhibition was observed with this cell line. At 105% air saturation, the small increase in the CSLPR without corresponding increase in CSGUR may imply an increase in the yield of lactate from glucose ($Y_{lac/gluc}$), indicating a greater dependence on glycolysis. Similar increases in $Y_{lac/gluc}$ have been observed before under hyperoxic conditions, where oxygen toxicity in cells results in a transition to anaerobic metabolism (Jan et al., 1997).

An examination of the cell specific t-PA production rate (Figure 4.9) shows an apparent maximum rate of 15.1 ± 0.4 pg/cell·d at 72% air saturation, but a significant difference ($p < 0.05$) with 16 or 105% air saturation was not obtained. The effect of DO on cell productivity is cell line dependent. A previous study using CHO cells reports no effect of DO on cell specific t-PA production in the range of 5-40% air saturation (Heidemann et al., 1998). However, Chotigeat et al. (1994) report a 3-fold higher protein productivity at 90% compared to 10% air saturation.

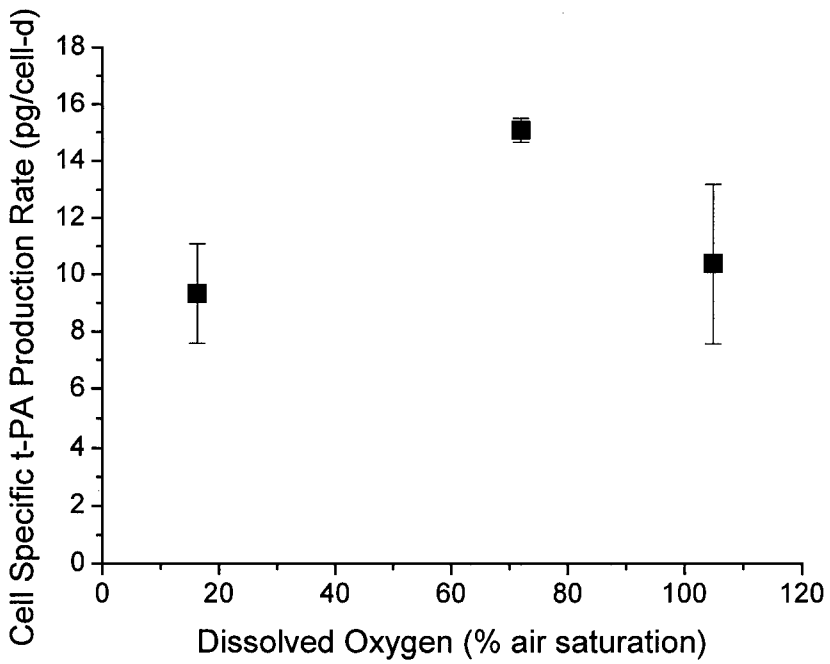

Figure 4.9 Cell specific t-PA production as a function of dissolved oxygen concentration.

Setpoints used: T = 37 ^{0}C, pH = 7.0, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values at 105% air saturation taken from run P32, others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state.

A maximum t-PA concentration of 146.5 ± 7.8 mg/L was produced at 72% air saturation (Figure 4.10). The change in t-PA concentration between 72 and 105% air saturation did not satisfy the significance test ($p < 0.05$), while there was a significant decrease ($p < 0.05$) of 57% in [t-PA] when the oxygen decreased from 72 to 16% air saturation. Increases in the cell specific glucose uptake rate at lower DO resulted in higher feed rates that in turn diluted the t-PA. Decreases in the cell specific t-PA production rate may also have reduced the product concentration. Figure 4.9 and 4.10 show how [t-PA] changes mirror the changes in the CStPAPR. Overall, for this CHO cell line, operation between 20 and 80% air saturation should yield the best results.

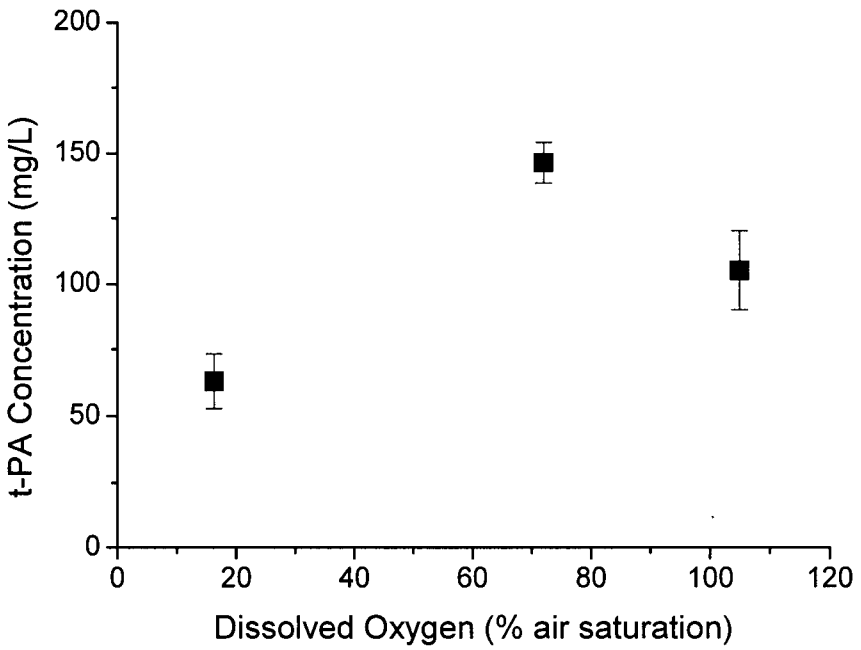

Figure 4.10 Concentration of t-PA as a function of dissolved oxygen concentration. Setpoints used: T = 37 ^{0}C, pH = 7.0, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values at 105% air saturation taken from run P32, others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state.

4.4.3 *Effect of pH*

The effect of pH was also considered for this system. At 37 ^{0}C, pH values of 6.67, 6.85, 7.04 and 7.10 were tested, while at 33 ^{0}C, values of 7.05, 7.19 and 7.25 were studied. pH was controlled by CO_2 addition only, such that more alkaline pH values could not be surveyed.

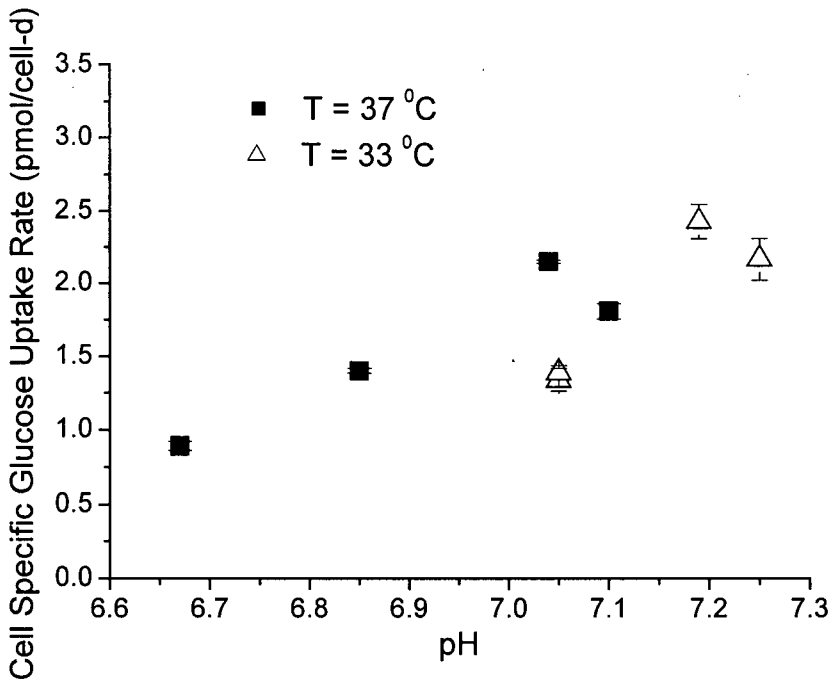

Figure 4.11 Cell specific glucose uptake as a function of pH at 37 ^{0}C (closed squares) and 33 ^{0}C (open triangles).

Setpoints used: DO = 70% air saturation, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values at pH 7.10, 37 ^{0}C taken from run P44, all others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state.

The cell specific glucose uptake rate as a function of pH is shown in Figure 4.11. There was a significant increase ($p < 0.05$) in the CSGUR at 37 ^{0}C as pH increased from 6.67 to 7.05, but a small 15% decrease from 7.05 to 7.10. At pH 7.05, the CSGUR values at 33 ^{0}C were lower than at 37 ^{0}C, which could be attributed to the lower temperature. There was a significant increase (~1.5-fold) in the CSGUR at 33 ^{0}C when pH was increased from 7.05 to 7.25 ($p < 0.05$). Interestingly, an approximately 1.5-fold increase was observed for 0.2 unit increases in pH, regardless of temperature, except at the maximum pH values tested.

It should be noted that the values obtained at pH 6.67 are not pseudo steady state values. At this low pH, cell growth ceased and the viability decreased markedly. The viable cell concentration at this point (8 -10 days after the shift to 6.67) was 3-4 x 10^5 cells/mL, with ~55% viability. The culture did not recover, so the average values presented are the last three days before the culture was terminated. This could impact the reliability of values at 6.67. Other pH values tested at both 37 and 33 ^{0}C did not significantly affect cell specific growth rates.

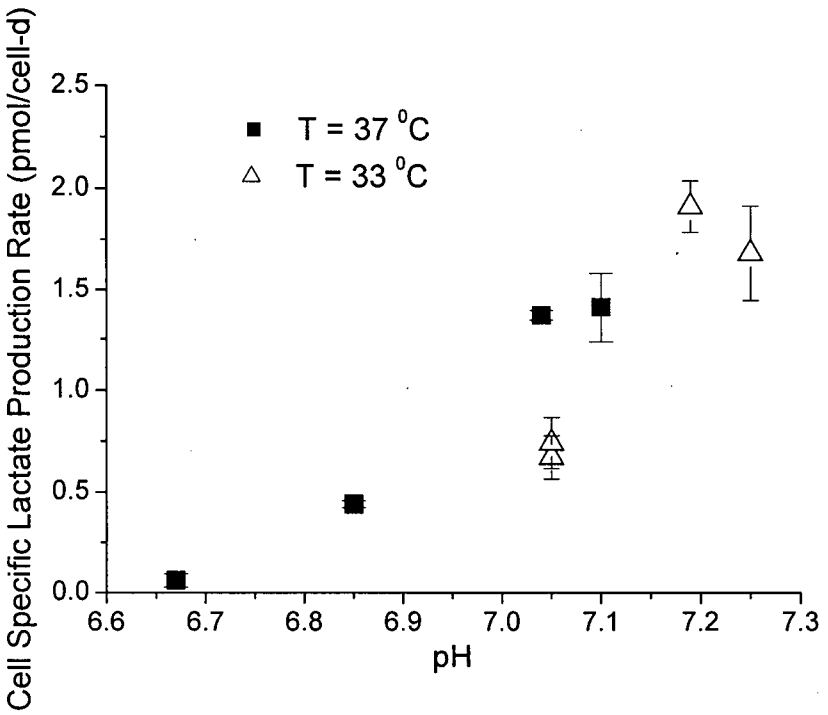

Figure 4.12 Cell specific lactate production as a function of pH at 37 ^{0}C (closed squares) and 33 ^{0}C (open triangles).

Setpoints used: DO = 70% air saturation, [glucose] = 5 mM, $X_v = 10^7$ cells/mL.Values at pH 7.10, 37 ^{0}C taken from run P44, all others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state.

Lactate production showed a similar increase as pH increased (Figure 4.12). At very low pH values, there was a change in cellular metabolism to ammonia production. The cell specific ammonia production rate was tripled from 0.23 ± 0.01 pmol/cell·d at pH 7.05 to a value of 0.64 ± 0.03 pmol/cell·d at pH 6.67. This is likely due to the detrimental effect low pH has on cell growth. At pH 6.67, there was essentially no growth. It has been reported that at low specific growth rates, glucose metabolism may contribute less to the energy supply than at high growth rates (Hayter et al., 1993). Hayter et al. (1993) go on to suggest that the higher ammonia yield may be a result of increased use of ammonia-

producing pathways, since substrates for transamination reactions (derived from glucose) are absent.

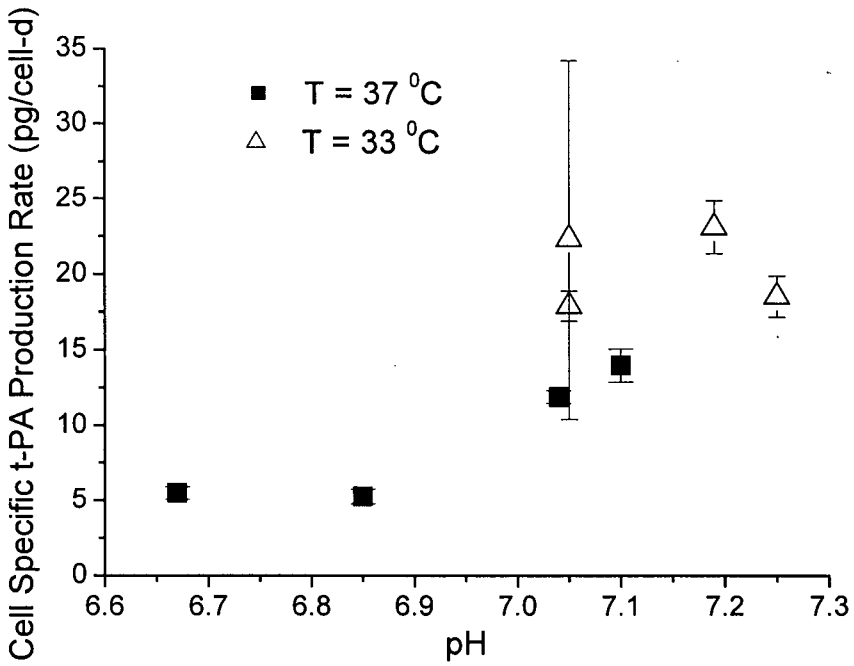

Figure 4.13 Cell specific t-PA production as a function of pH at 37 ^{0}C (closed squares) and 33 ^{0}C (open triangles).

Setpoints used: DO = 70% air saturation, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values at pH 7.10, 37 ^{0}C taken from run P44, all others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state, except value with large error bar at pH 7.05 (n = 2).

There was a significant effect of low pH on specific protein production (Figure 4.13). The CStPAPR dropped over 50% when the pH was shifted from 7.05 to 6.85 ($p < 0.05$). This is in agreement to other studies on CHO and HEL cells (Borys et al., 1993; Takagi and Ueda, 1994). However, this effect is cell line dependent, as hybridoma cells have been reported to increase cell specific production at low pH (Miller et al., 1988a; Ozturk

and Palsson, 1991). At higher pH values there was no significant effect on the cell specific t-PA production rate.

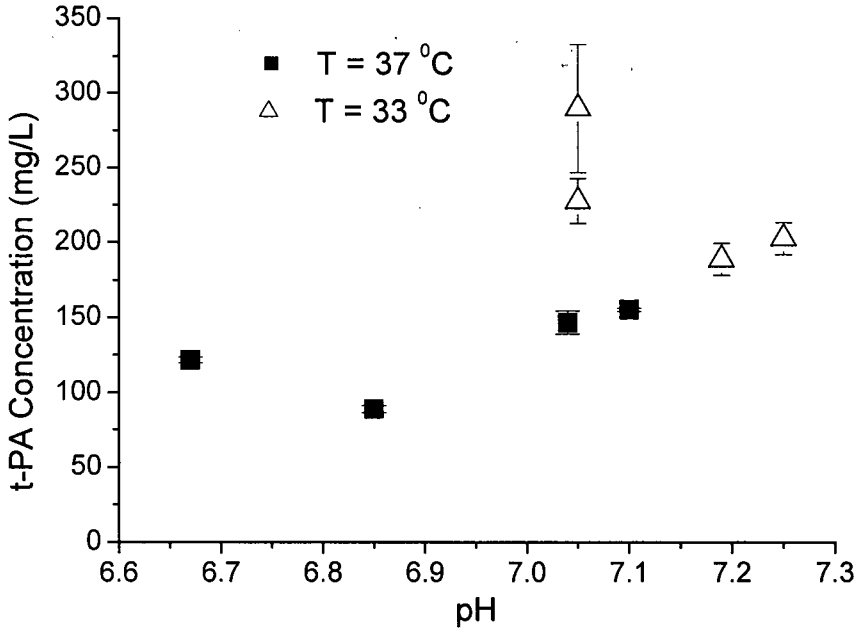

Figure 4.14 Concentration of t-PA as a function of pH at 37 ^{0}C (closed squares) and 33 ^{0}C (open triangles).

Setpoints used: DO = 70% air saturation, [glucose] = 5 mM, $X_v = 10^7$ cells/mL. Values at pH 7.10, 37 ^{0}C taken from run P44, all others from run P28. Each point represents mean of last 3 days ± SEM at pseudo steady state, except value with large error bar at pH 7.05 (n = 2).

Figure 4.14 shows t-PA concentration in response to pH changes. The t-PA concentration was lower at pH 6.85, due to the lower rate of cell specific t-PA production. The higher [t-PA] at 6.67 is likely due to the very low perfusion rate needed to keep the glucose concentration constant. Another reason for the increase in [t-PA] could be due to the drop in viability during this time. As noted previously, viability was

~55% at pH 6.67. During cell death, cells may have released any intracellular t-PA accumulated, which could account for the rise in t-PA concentration.

Volumetric productivity at 6.67 was 20 ± 2 mg/L·d, 88% lower than the volumetric productivity at pH 7.05 ($p < 0.05$). Higher pH values appeared to have a positive effect on the volumetric productivity, with a VtPAPR of 223 ± 15 mg/L·d at pH 7.19, 1.5-fold higher than the VtPAPR at pH 7.05, but the increase was not significant at a $p < 0.05$ level.

5 Results of Transient Method

5.1 Introduction to Transient Scanning

In the previous section, pseudo steady state results were obtained to determine the appropriate environmental conditions for production. The cell specific glucose uptake rate was used as a measure of steady state before changing operating conditions, due to easy and rapid analysis (~2 min) after sampling. Since determining t-PA concentration required a time-consuming assay, it was not feasible to perform these daily. Subsequently, it was apparent that the t-PA concentrations vary more than glucose, and the t-PA response may lag considerably behind the glucose response. In part, this was due to the need for adjusting perfusion rates to maintain glucose setpoint control. Figure 5.1 shows an example from perfusion run P28.

Even during the supposed steady state from day 17 to 19 at 34 ^{0}C, it is apparent that the CSGUR is fairly constant, but the [t-PA] has a marked upward trend over the last 2 days. Although the increase was not as sharp as at 34 ^{0}C, the t-PA concentration also increased during the last 3 days of the steady state at 37 ^{0}C. Paalme et al. (1995) have remarked that although three residence times can be used as the minimum time needed for steady state, high feed concentrations may require even longer run times due to the large difference between feed and reactor nutrient concentrations. It appears from the results in Figure 5.1 that to reach a steady state where [t-PA] is constant may require 10 or more days, perhaps double the 5-8 day intervals generally used based on the GUR steady state. Gathering steady state data for optimal conditions would then require substantially longer

process development times, but this delay would be highly undesirable in industry due to the increased expense and lost sales.

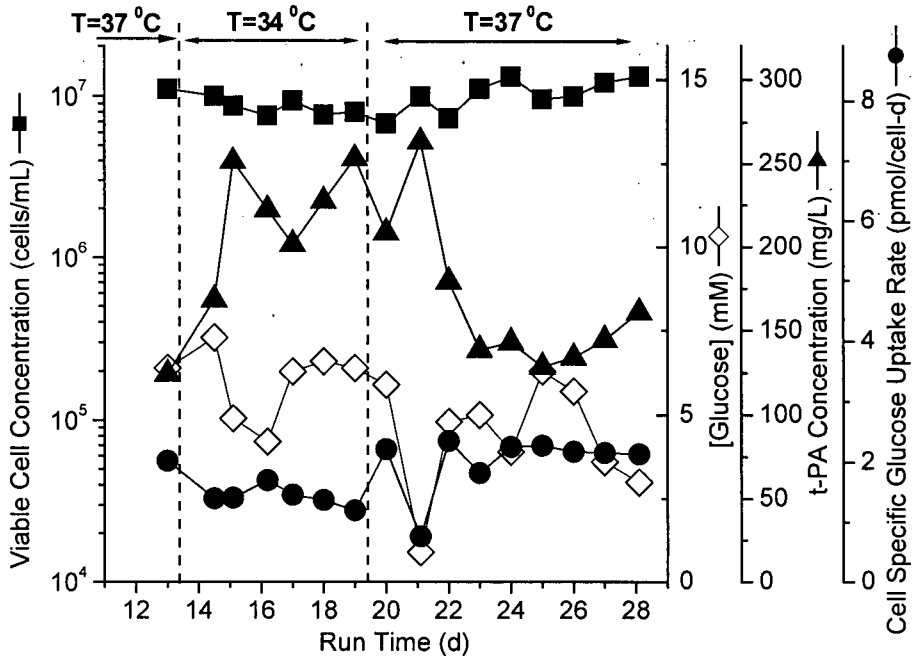

Figure 5.1 Viable cell concentration, [glucose], [t-PA] and cell specific glucose uptake rate during "steady-state" in perfusion run P28. pH: 7.04 ± 0.02, DO:71 ± 5% air saturation.

An alternative view of the data in Figure 5.1 could be used for a more rapid analysis. Overall, it appears that the t-PA concentration is higher at 34 ^{0}C than at 37 ^{0}C by roughly 1.5-fold. The trend of high concentration at 34 ^{0}C becomes apparent within 2 days of culture, and after the temperature change to 37 ^{0}C, within 3 days the t-PA concentration has dropped considerably. By using data from the transient phase of culture, it may then be possible to qualitatively predict whether or not a given environmental change to the system would be beneficial for production. In this way, more environmental conditions could be tested, and time-consuming steady state experiments would only be performed

on conditions close to the optimum state of productivity. To test this hypothesis, the transient response of a perfusion culture to step changes in temperature was investigated.

5.2 Transient Scanning of Temperature in Perfusion Culture

Temperature was used as the environmental parameter of concern chiefly due to the ease of control in the perfusion system used. Initially, the culture was run until a pseudo steady state was established at 37 ^{0}C, after approximately 20 days in culture. The temperature was then stepped down by 2 ^{0}C every 3 or 3.5 days to 31 ^{0}C, where the culture was maintained 3-5 days to reach a quasi steady state. Two perfusion runs were performed: P32 at pH 7.05 ± 0.01, 104 ± 5% air saturation, and P44 at pH 7.10 ± 0.01, 68 ± 7% air saturation. As in P28, the glucose concentration setpoint was 5mM (controlled by perfusion rate), while the viable cell concentration setpoint was 10^7 cells/mL (controlled by cell bleed rate).

There was a marked decrease in the cell specific glucose uptake rate as the temperature was scanned down from 37 to 35 ^{0}C, and then to 33 ^{0}C (Figure 5.2). After the first day drop at the new temperatures, the CSGUR increased slightly, but not to former levels (at the previous temperature). When the temperature was changed from 33 to 31 ^{0}C, the decrease in the CSGUR took at least 2 days longer than the other temperature shifts, increasing slightly on day 4. The difficulty in maintaining the cell concentration at 31 ^{0}C could be a reason for this delay. At this temperature, cell growth was inhibited, and sampling had a significant influence on the cell concentration, which decreased to 5 x 10^6 cells/mL by day 35.

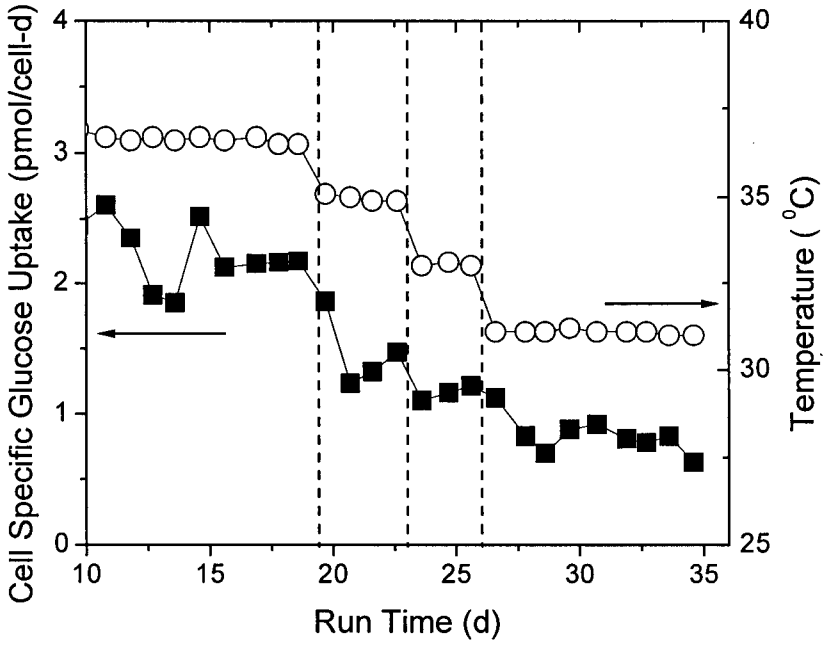

Figure 5.2 Time course of cell specific glucose uptake rate (closed squares) and temperature (open circles) during run P32.

The cell specific glucose uptake rate time profile was fairly consistent in its response to decreasing temperature. However, consistency was not observed in the cell specific t-PA production rate response (Figure 5.3). Fluctuations were apparent throughout the culture, regardless of temperature. This suggests evaluation of the cell specific productivity could be problematic with this transient scanning method.

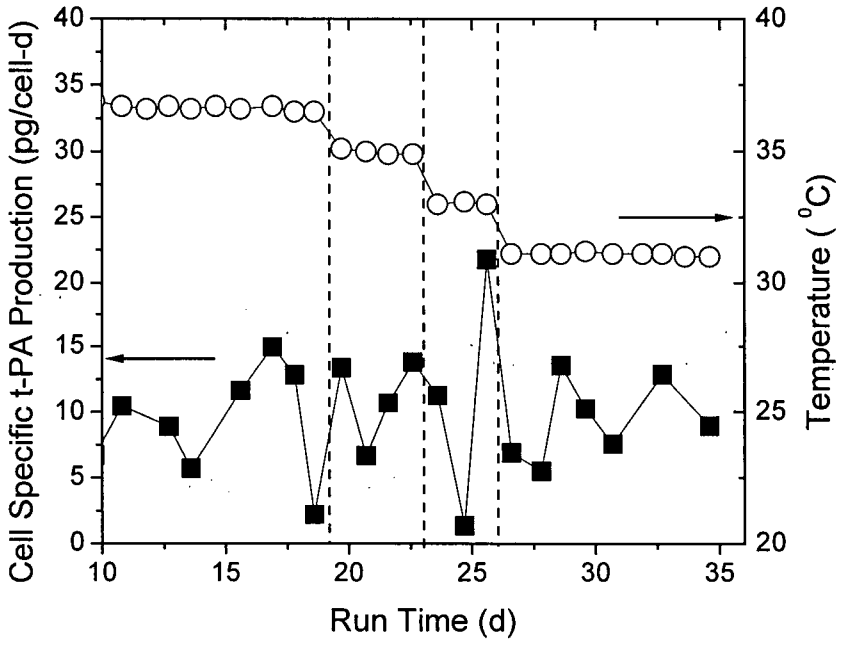

Figure 5.3 Time course of cell specific t-PA production rate (closed squares) and temperature (open circles) during run P32.

The t-PA response (Figure 5.4) during the culture was not as erratic as the cell specific t-PA response. There was a decrease in the t-PA concentration during the last day of pseudo steady state at 37 ^{0}C, reconfirming the difficulties in using the CSGUR as an indicator of steady state. The t-PA concentration increased steadily after the temperature was shifted to 35 ^{0}C, and continued to increase with lower temperatures, although not as consistently. This increase was also observed in previous pseudo steady state temperature experiments (Chapter 4). The decreases in t-PA concentration on the first day after the step changes to 33 and 31 ^{0}C were due to overestimation of the perfusion feed rate required at the lower temperature. The cells had a lower degree of glucose utilization than predicted, and consequently, the culture was over-diluted. By the second day, a better prediction of the feed rate was determined. The overall profile suggests that

evaluation of t-PA production dependence on temperature may be achieved with transient scanning with 3 day steps.

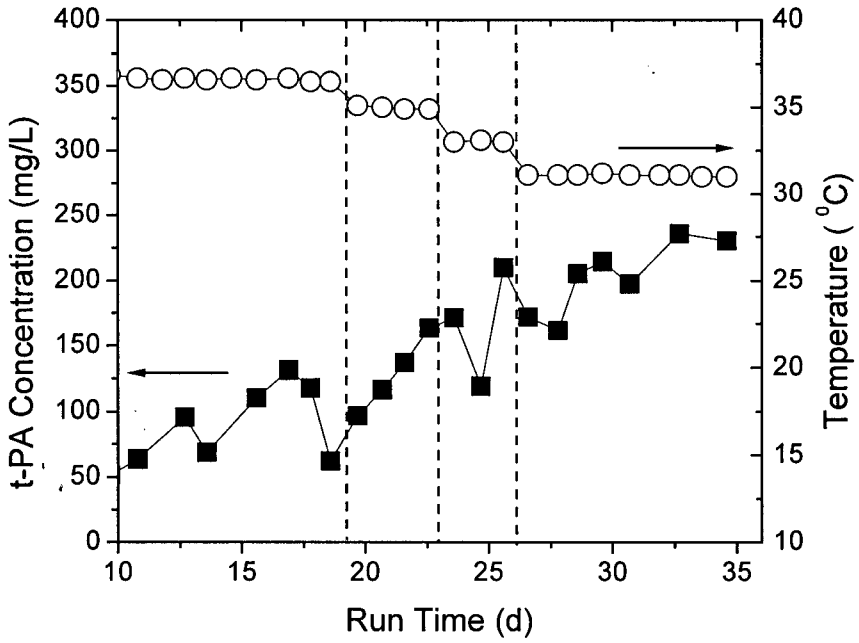

Figure 5.4 Time course of t-PA concentration (closed squares) and temperature (open circles) during run P32.

To obtain a clearer idea of how the transient method could be used to qualitatively evaluate environmental effects, graphs of transient values (3 days after a change in temperature) against temperature were produced. In the following graphs, vertical arrows indicate the trend during transient phase, while horizontal arrows represent the step change in temperature.

The transient profile of the cell specific glucose uptake rate shows a clear decrease at lower temperature (Figure 5.5). Each step change to a lower temperature produced a

lower CSGUR after 3 days (Figure 5.2), although the magnitude of the change was not the same.

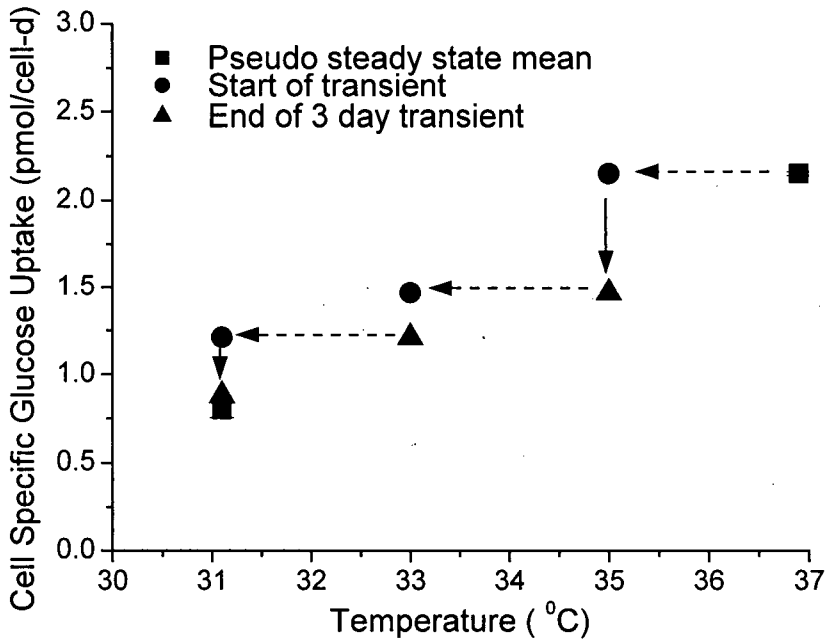

Figure 5.5 Transient profile of cell specific glucose uptake rate as a function of a 3 day scan down of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 5). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Data from run P32.

The cell specific lactate production rate transient profile also showed a decrease at low temperatures (Figure 5.6). There was a marked decrease when the temperature was changed from 37 to 35 ^{0}C, where the CSLPR decreased by 59% from 2.02 to 0.82 pmol/cell·d. A subsequent change in temperature to 33 ^{0}C did not alter the CSLPR much, but a change to 31 ^{0}C resulted in a further decrease to 0.56 pmol/cell·d. The larger decreases in the CSGUR and CSLPR at 35 ^{0}C suggest that in terms of cell adaptation to

environmental changes, there is a larger difference between 37 and 35 ^{0}C than between 35 and 33 or 31 ^{0}C.

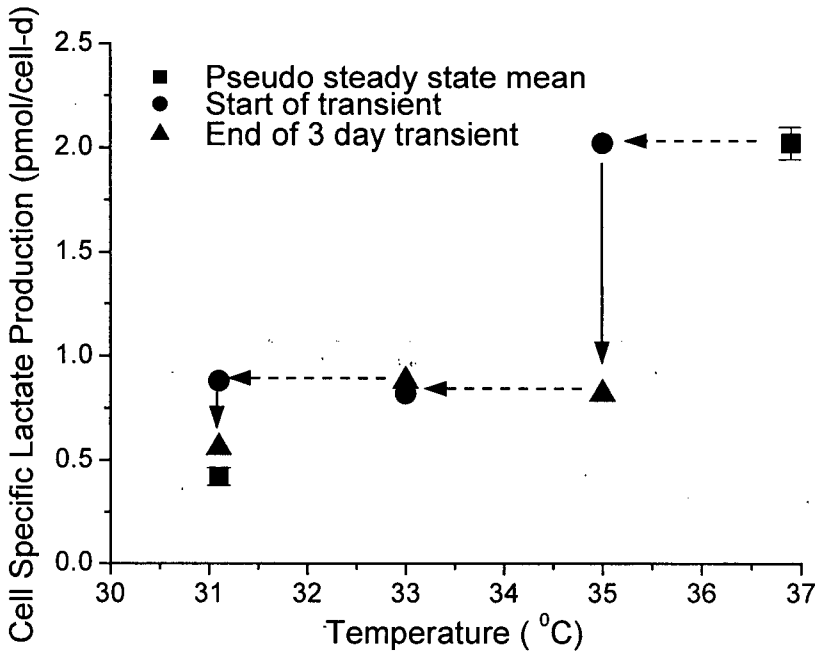

Figure 5.6 Transient profile of cell specific lactate production rate as a function of a 3 day scan down of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 5). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Data from run P32.

Cell specific t-PA productivity appeared to increase at lower temperatures, with a maximum 3 day transient value of 21.8 pg/cell·d (Figure 5.7). However, there were frequent day-to-day fluctuations in the CStPAPR (Figure 5.3). By focusing only on the 3 day transient value, inaccurate assumptions are a possibility when evaluating cell specific productivity. Comparison of the pseudo steady state values at 37 versus 31 ^{0}C show no significant change in CStPAPR due to temperature at the $p < 0.05$ level.

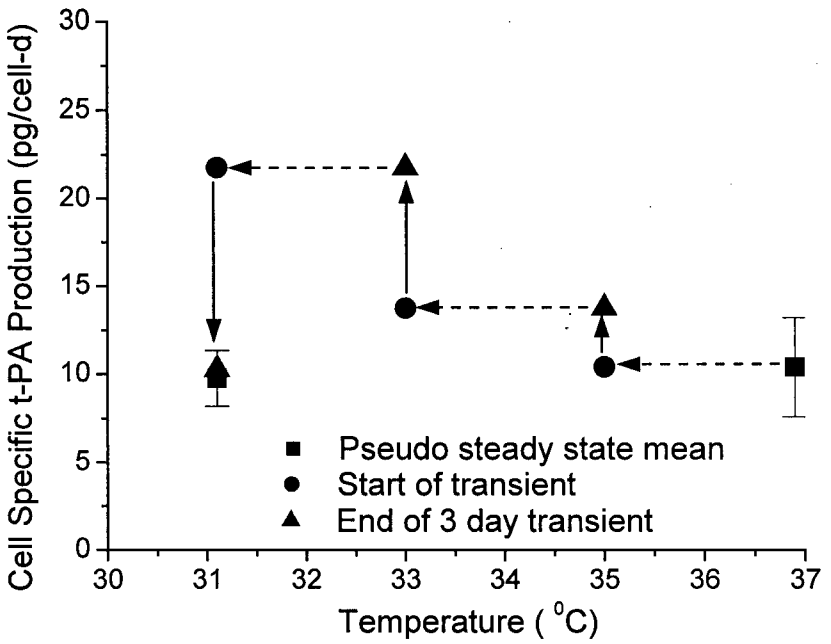

Figure 5.7 Transient profile of cell specific t-PA production rate as a function of a 3 day scan down of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 5). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Data from run P32.

Evaluation of the transient t-PA concentration in response to a decrease in temperature showed higher t-PA concentrations at lower temperature (Figure 5.8), 2-fold higher at 33 ^{0}C than at 37 ^{0}C. The high t-PA concentration is attributed to the lowered glucose uptake, and consequently, lowered medium feed rate. Although the concentration was even higher at 31 ^{0}C, growth at this temperature was so low ($\mu = 0.002 \pm 0.001$ h^{-1}) that cell concentration was not maintained at 10^7 cells/mL, and dropped to 5 million cells/mL by the end of the culture. Therefore, from a sustained operation point of view, 31 ^{0}C is likely not the best choice for production purposes.

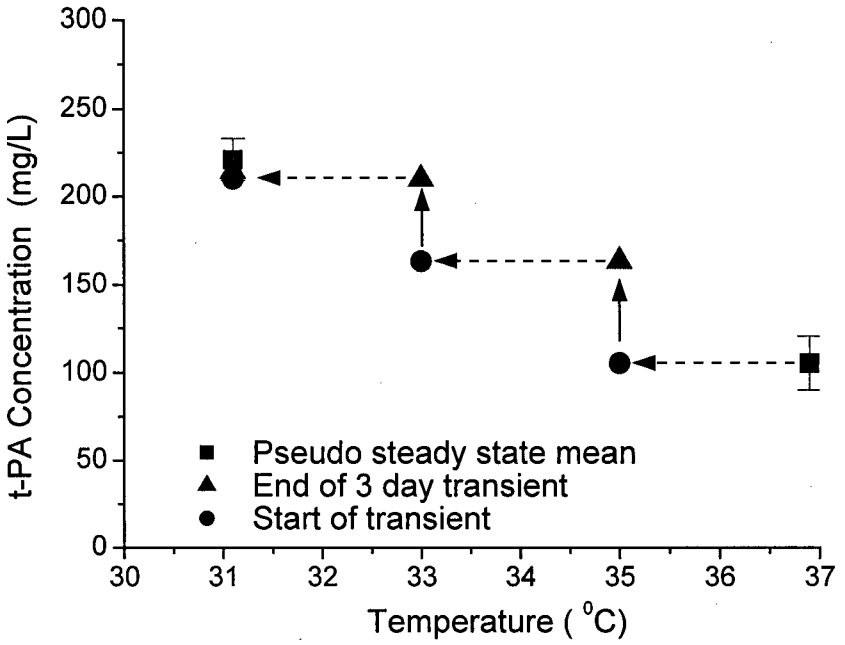

Figure 5.8 Transient profile of t-PA concentration as a function of a 3 day scan down of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 5). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Data from run P32.

One point of interest to note from all these results was the close concordance between the value at pseudo steady state and the 3 day transient value at 31 ^{0}C (Table 5.1). This seems to confirm the value of the transient method, where 3 days was sufficient to evaluate the benefit of an environmental change. However, recovery time was greater when the culture was subjected to more drastic changes, so this concordance may not be always observed.

Table 5.1 Comparison of Pseudo Steady State and Transient Values at 31 ^{0}C.

Response Variable	3 day Transient Value	Steady State Value
CSGUR (pmol/cell·d)	0.88	0.80 ± 0.05
CSLPR (pmol/cell·d)	0.56	0.42 ± 0.04
CStPAPR (pg/cell·d)	10.3	9.8 ± 1.6
[t-PA] (mg/L)	214	221 ± 12

Values last n days at pseudo steady state ± SEM (n = 3 to 5). Data from run P32, pH 7.05 ± 0.01, 104 ± 5% air saturation.

A similar experiment was conducted in another run (P44), with the added aspect that after a pseudo steady state at 31 ^{0}C was obtained, the temperature was increased by steps, again every 3 days. The purpose of this experiment was to check whether the direction of the environmental shift had an effect on the response. Again in the transient graphs, the horizontal dashed lines and arrows show the step change in temperature, and vertical arrows the transient trend direction. The dotted lines and arrows represent transient changes during the temperature scan up.

There was good agreement between the results of the two separate transient perfusion runs (P32 and P44). Overall trends were the same, although actual values obtained, especially for the CSLPR and CSGUR, differed (Table 5.2). This was probably because run P32 was operated at higher oxygen levels. Previous experiments (Chapter 4) suggested that higher oxygen conditions cause an increase in glucose and lactate metabolism. This was observed at 37 ^{0}C, but at 31 ^{0}C, the CSGUR at 104% air saturation was lower than observed at 68% air saturation. This may be due to the lower cell concentration at 31 ^{0}C in P32. The culture at 68% air saturation (P44) was growth inhibited as well, but the cell concentration was maintained, mainly because the problem was anticipated from the earlier run P32. The pH difference between the two runs of 0.1

units was not large enough to significantly affect cell productivity or metabolism (Figure 4.11).

Table 5.2 Comparison of Pseudo Steady State Values at 31 and 37 ^{0}C for P32 (104% air saturation) and P44 (70% air saturation).

Response Variable	31 ^{0}C		37 ^{0}C	
	P32	P44	P32	P44
CSGUR (pmol/cell·d)	0.80 ± 0.05	1.15 ± 0.07	2.15 ± 0.01	1.81 ± 0.05
CSLPR (pmol/cell·d)	0.42 ± 0.04	0.11 ± 0.04	2.02 ± 0.08	1.41 ± 0.18
CStPAPR (pg/cell·d)	9.8 ± 1.6	12.9 ± 0.4	10.4 ± 2.8	14.0 ± 1.1
[t-PA] (mg/L)	221 ± 12	238 ± 5	105 ± 15	155 ± 1

P32: pH 7.05 ± 0.01, 104 ± 5% air saturation. P44: pH 7.10 ± 0.01, 68 ± 7% air saturation. Values last n days of pseudo steady state ± SEM (n = 2 to 5).

Overall, the cell specific glucose uptake rate decreased in response to a scan down in temperature (Figure 5.9), similar to the data from P32 (Figure 5.5). At 35 ^{0}C, the CSGUR increased, in contrast to previous observations. This change in the CSGUR was likely due to a pH upset in the system during the first 20 h of the temperature change. The scan up results show agreement with the scan down results. For example, the CSGUR at 33 ^{0}C during scan down was 1.51 pmol/cell·d, compared to 1.75 pmol/cell·d during scan up. Unfortunately, the run was stopped after 2 days at 35 ^{0}C due to contamination, so no pseudo steady state value at 37 ^{0}C could be obtained for comparison.

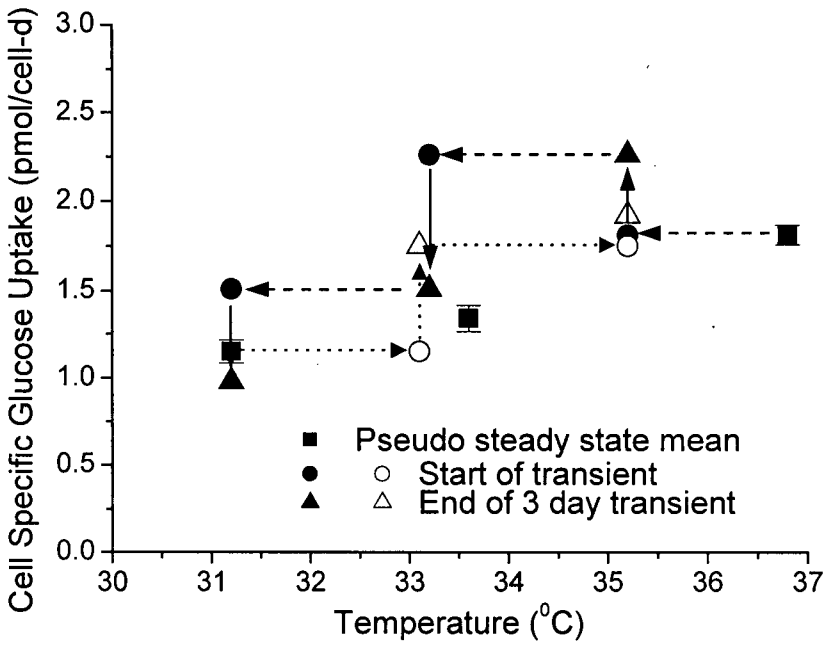

Figure 5.9 Transient profile of cell specific glucose uptake rate during 3 day scan down (closed symbols) and scan up (open symbols) of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 2 to 5). Transient value at 35 ^{0}C scan up was 2 day transient only. Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Data from run P44, except steady state value at 33.6 ^{0}C from P28 (Section 4.4.1).

The cell specific lactate production profile changed dramatically, as shown in Figure 5.10A, since lactate production increased little during the temperature scan up. This was correlated with a shift in metabolism to increased ammonium production, as shown in Figure 5.10B. At 31 ^{0}C pseudo steady state, the cell specific ammonium production rate was 2.7-fold higher than the 3 day transient value at the same temperature. As the temperature was scanned up, the ammonium production rate further increased. This shift to ammonium production has been reported before in response to very low dilution rates (Hayter et al., 1993), such as those resulting from low temperatures in the CHO t-PA

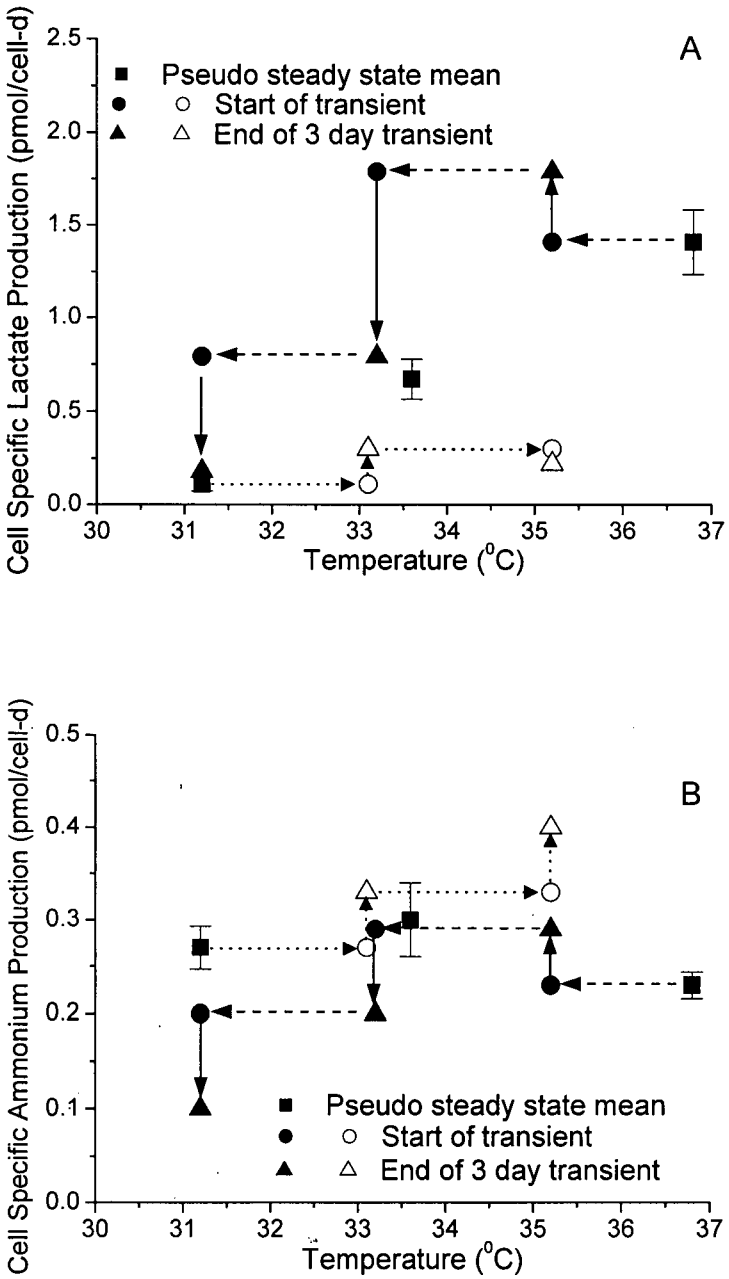

Figure 5.10 Transient profile of cell specific (A) lactate and (B) ammonium production rate during 3 day scan down (closed symbols) and scan up (open symbols) of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 2 to 5). Transient value at 35 ^{0}C scan up was 2 day transient only. Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Data from run P44, except steady state value at 33.6 ^{0}C from P28 (Section 4.4.1).

cultures (0.02 h^{-1} at 31 ^{0}C). It is possible that the cell metabolism would have shifted back to lactate production for longer culture times and at 37 ^{0}C once the dilution rate was increased, with glucose and other nutrients being added to the culture at higher rates.

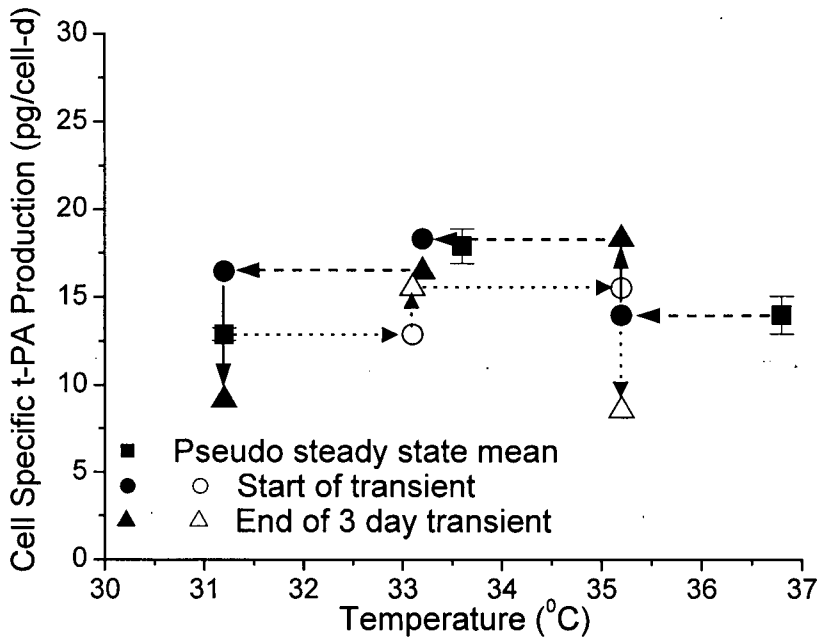

Figure 5.11 Transient profile of cell specific t-PA production rate during 3 day scan down (closed symbols) and scan up (open symbols) of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 2 to 5). Transient value at 35 ^{0}C scan up was 2 day transient only. Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Data from run P44, except steady state value at 33.6 ^{0}C from P28 (Section 4.4.1).

The shift in metabolism did not seem to have a marked effect on cell t-PA productivity and concentration as shown in Figure 5.11 and Figure 5.12. The cell specific t-PA production rate during scan up transiently increased and decreased at 33 and 35 ^{0}C, respectively, indicating as in the scan down that intermediate temperatures may increase

cell productivity. However, the increase with respect to 31 ^{0}C was only 1.2-fold. The t-PA concentration (Figure 5.12) decreased at higher temperatures, as expected. The profile was similar to the profile when the temperature was scanned down.

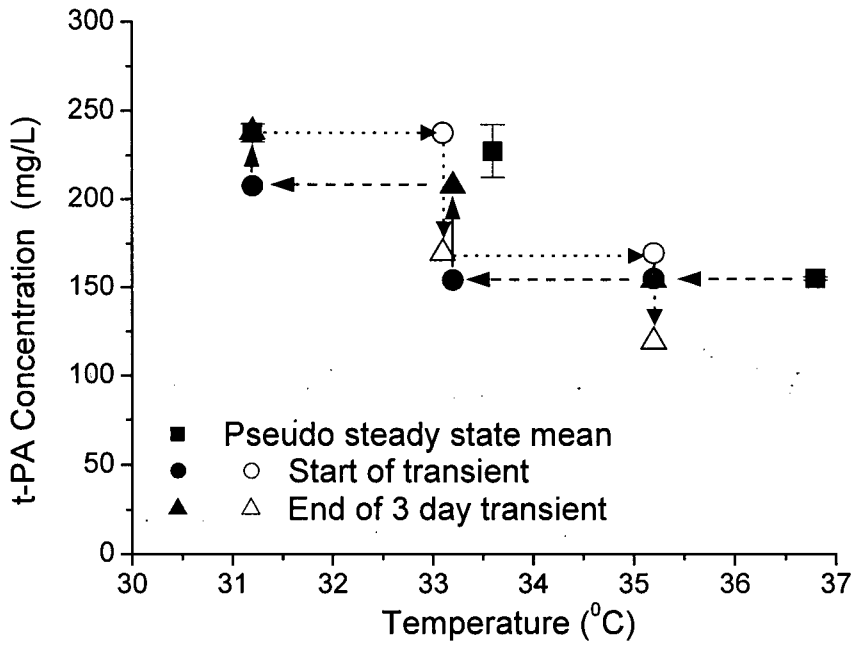

Figure 5.12 Transient profile of t-PA concentration during 3 day scan down (closed symbols) and scan up (open symbols) of temperature.

Squares indicate pseudo steady state mean obtained (± SEM, n = 2 to 5). Transient value at 35 ^{0}C scan up was 2 day transient only. Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Data from run P44, except steady state value at 33.6 ^{0}C from P28 (Section 4.4.1).

From the data presented in this section, transient scanning appears to be a promising method to accelerate process development. Variables such as the cell specific glucose uptake rate and t-PA concentration were evaluated more readily than variables such as

cell specific t-PA production rate, which underwent large fluctuations from day-to-day. As well, effects of extreme environmental changes may not be fully realized by transient scanning, as shown by the transition from lactate to ammonium production observed (Figure 5.10). If temperature scan up was the only experiment performed, the transition to ammonium production could be misleading.

The major advantage of the transient scanning method lies in the significant time saved. For example, to evaluate five different temperatures using the steady state method, with each steady state requiring 10-16 days, the time needed would be 50 to 80 days. With the 3 day transient method, only 15 days would be needed. Assuming both methods required the same perfusion startup time of ~2 weeks, scanning could cut process development time by at least half.

5.3 Transient Scanning of Temperature in Scale-down Cultures

5.3.1 3 Day Transient Scanning

Transient experiments in perfusion culture experiments showed promising results. However, most of the perfusion cultures required a roughly 2 week setup and startup period to reach high cell density (1-2x10^7 cells/mL). Also, the perfusion system itself is complex, including both cell retention and feedback control. The premature end of the experiments in Section 5.2 illustrates the difficulty there can be in obtaining perfusion culture results. To simplify the experimental design and reduce the complexity of the system, non-instrumented control in a small-scale spinner system was evaluated.

Semicontinuous cultures were used to simulate continuous cultures, operating at a much lower cell concentration. The dilution rate was adjusted so that the cell concentration after dilution was constant at 10^5 cells/mL. This low cell concentration was selected for a number of reasons, primarily to reduce the overall variability in the semicontinuous system, and so oxygen could be maintained in the culture. Another reason for maintaining a low cell concentration was to reduce fluctuations in pH from CO_2 produced by the cells. Earlier test experiments with a cell concentration setpoint of $5x10^5$ cells/mL caused the pH to fluctuate from 7.4 to 6.8 within 8 days. Once scale-down (SD) cultures were in operation and a pseudo steady state was obtained, transient scanning experiments similar to the experiments detailed in Section 5.2 were performed. Pseudo steady state was assumed after 6 days, based on previous perfusion experiments.

As was the case for transient scanning in perfusion cultures (Section 5.2), the objective with the SD system was to see if qualitative results similar to those in the perfusion system could be obtained. Quantitatively, t-PA concentrations would be much lower, while cell specific glucose uptake would likely be higher, due to the reduced degree of medium utilization. If useful results could be obtained in a small scale system, then process development time could be cut from 30 days in a transient perfusion system with a 2 week startup (assuming 5 different temperatures to evaluate), to 16 days in a small scale system with a 2 day startup.

SD cultures also have the advantage that more replicates can be run in parallel, since setup and operation is less time-consuming than a fully controlled system. However,

there were some drawbacks to the non-instrumented system used. The control of pH and DO was not robust. For instance, for DO control sample pO_2 was measured off-line. If the DO was too high, the gas mix into the culture was adjusted manually. This was only done during the time of daily sampling, so process upsets at other times would have been undetected and could have influenced the results. However, the DO profile during the course of a scale-down culture (Figure 5.13) did not vary appreciably (± 8%) between day 2 and 12 based on the sample data, so it is unlikely that large process upsets would have occurred with no indications.

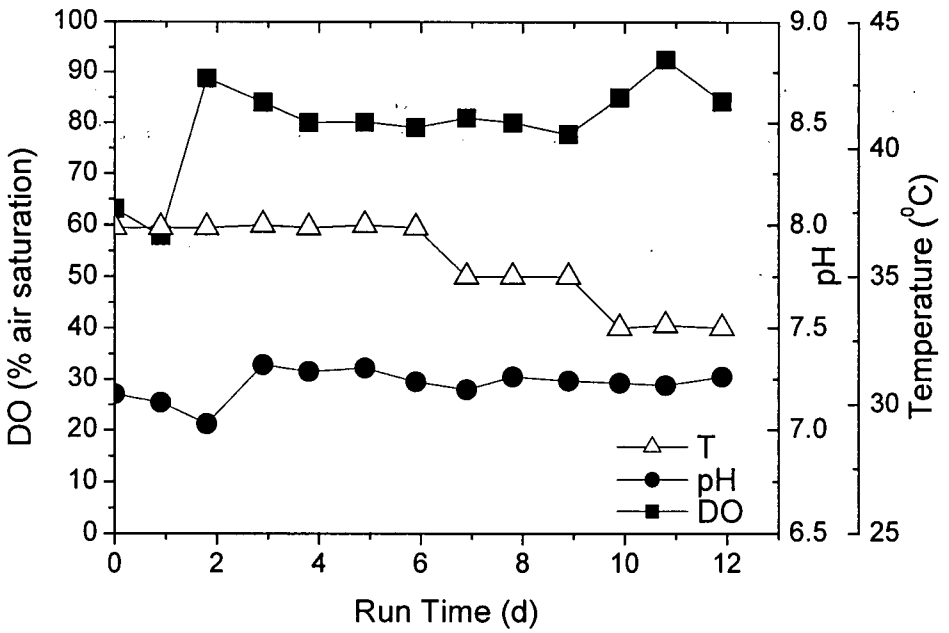

Figure 5.13 T, DO and pH profile during a typical SD culture.
Data from SD-D5: pH 7.3 ± 0.1, 82 ± 5% air saturation.

Two parallel spinner cultures investigated a scan down in temperature. Values were not obtained for 31 ^{0}C due to contamination, so another spinner run was performed to analyse

the effect of scanning up the temperature. No pseudo steady state value for 37 ^{0}C was obtained.

The cell specific glucose uptake rate decreased at every temperature step change from 37 to 33 ^{0}C (Figure 5.14). There was very good agreement between the two replicate cultures, with respect to direction of the transient response and also actual transient values. The CSGUR decreased approximately 55% at 33 ^{0}C from the pseudo steady state value at 37 ^{0}C. This was larger than the decrease observed in the two perfusion runs in Section 5.2. The cell specific glucose uptake rates in the SD cultures were about 5 times higher than in the perfusion system. This was likely due to the relatively less utilized medium, including increased availability of glucose (21 mM) compared to 5 mM in the perfusion system. Maintaining low glucose concentrations in the SD cultures was not practical because the corresponding high cell concentrations would require higher oxygen transfer and more frequent feeding than feasible in this simple spinner system.

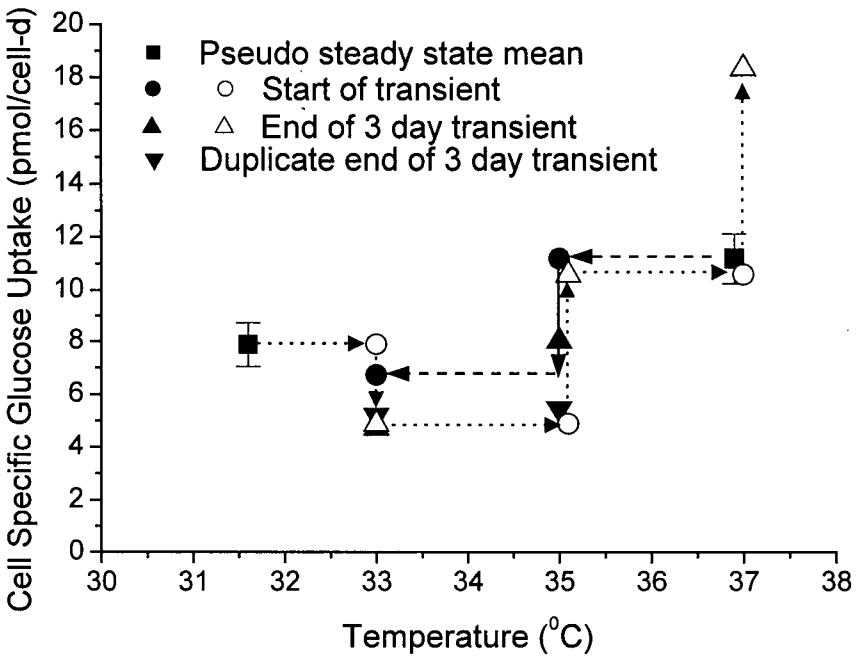

Figure 5.14 Transient profile of cell specific glucose uptake rate versus 3 day scan down (closed symbols) and scan up (open symbols) of temperature in SD culture.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 6). Scan down transient value at 33 ^{0}C for duplicate run used value on 2nd day. Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Results for scan down from SD5: pH 7.3 ± 0.1, 82 ± 5% air saturation. Results for scan up from SD-A6: pH 7.4 ± 0.1, 74 ± 5% air saturation.

As the temperature was scanned up, the CSGUR decreased at 33 ^{0}C, before subsequently increasing at 35 and 37 ^{0}C. The transient values at 33 ^{0}C for both the scan down and scan up cultures were similar, with more deviations at the higher temperatures. The transient value at 37 ^{0}C from the temperature scan up was 18.3 pmol/cell·d, 1.5-fold higher than the pseudo steady state value of 11.1 ± 0.9 pmol/cell·d obtained at the start of the scan down experiment. This would seem to indicate a change over the course of the culture at lower temperature. However, large fluctuations in day-to-day cell specific glucose

uptake rates were frequently observed throughout all SD cultures (Figure 5.15). For instance, the CSGUR on the second day after the step change to 33 ^{0}C was 4.7 pmol/cell·d, compared with the 3 day value of 11.8 pmol/cell·d. These fluctuations are at least partly due to measurement problems with glucose concentration rather than only a cell physiological response. In the SD cultures, glucose was kept at a high value close to 21 mM. Dilution did not change this concentration very much, and frequently, the calculated change in glucose concentration after dilution would be within 0.5 mM of the concentration before dilution. This is within the range of error for the blood/gas analyser used for glucose analysis.

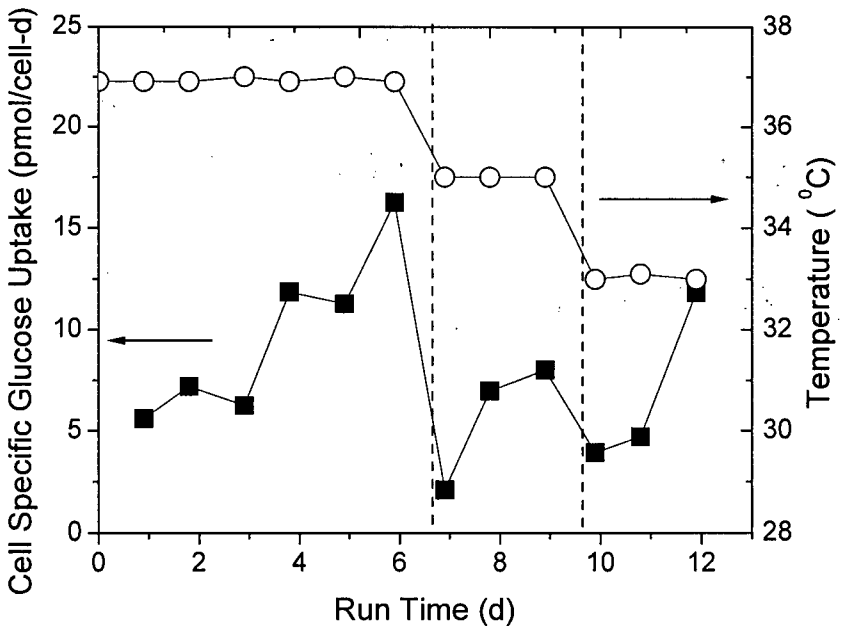

Figure 5.15 Time course of cell specific glucose uptake (closed squares) and temperature (open circles) for a typical SD culture.

Data from SD-D5: pH 7.3 ± 0.1, 82 ± 5% air saturation.

Examination of the t-PA response gives a more promising picture of the feasibility of using non-instrumented SD spinner cultures. Figure 5.16 shows the cell specific t-PA production rate with temperature step changes. When the temperature was scanned down (Figure 5.16A), the CStPAPR increased with decreasing temperature. There was more variability between the two replicates at 33 ^{0}C, but there was still an overall increase relative to 37 ^{0}C. Scanning up the temperature also showed increased productivity at 33 ^{0}C (Figure 5.16B). This is in agreement with the conclusions made from transient perfusion cultures (Figure 5.11), where it was determined that 33 ^{0}C may warrant further investigation.

The high CStPAPR at 33 ^{0}C observed for one of the duplicate runs during the temperature scan down may be due to problems in cell concentration determination. This particular CHO cell line tends to aggregate into cell clumps of roughly 30-50 cells. With the perfusion culture, the use of trypsin allowed accurate cell determination. In the SD cultures with low cell concentrations of 1 - 3 x 10^5 cells/mL, the clumps were smaller than observed in perfusion cultures, but more spherical, almost bead-like. Trypsin was not very helpful in separating these cells, and so counting error may have been amplified. Also, the spherical clumps tended to settle at the bottom of the spinner flask, so representative cell concentration samples may not have been consistently taken. These measurement errors would have an influence on the accuracy of cell specific rates. Cell washout was another concern at 31 ^{0}C, and batch operation had to be maintained until the cell concentration recovered, in some cases.

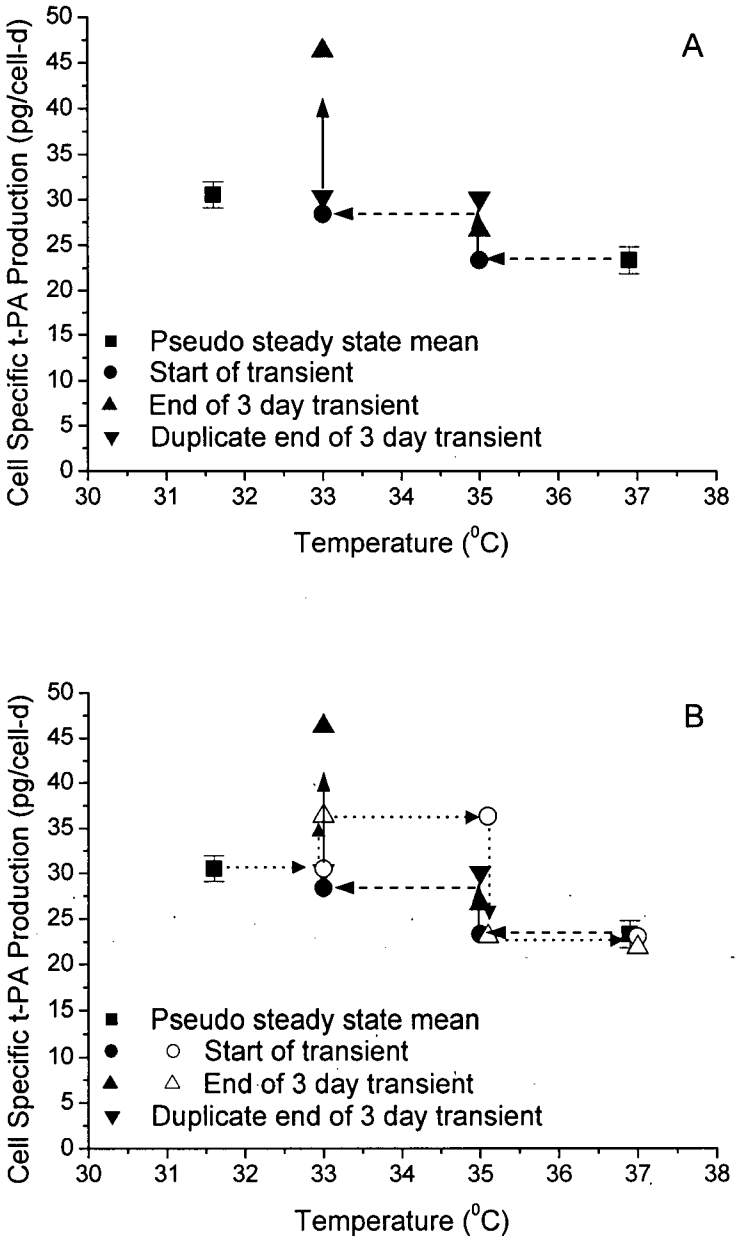

Figure 5.16 Transient profile of cell specific t-PA production rate versus (A) 3 day scan down (closed symbols) and (B) scan up (open symbols) of temperature in SD culture.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 6). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Results for scan down from SD5: pH 7.3 ± 0.1, 82 ± 5% air saturation. Results for scan up from SD6: pH 7.4 ± 0.1, 74 ± 5% air saturation.

The t-PA concentration profile follows the trends in the cell specific t-PA production rate (Figure 5.17). The t-PA concentration was about 15 - 30 times lower than obtained in perfusion culture, primarily due to the low cell concentration used (10^5 cells/mL in contrast to 10^7 cells/mL in perfusion).

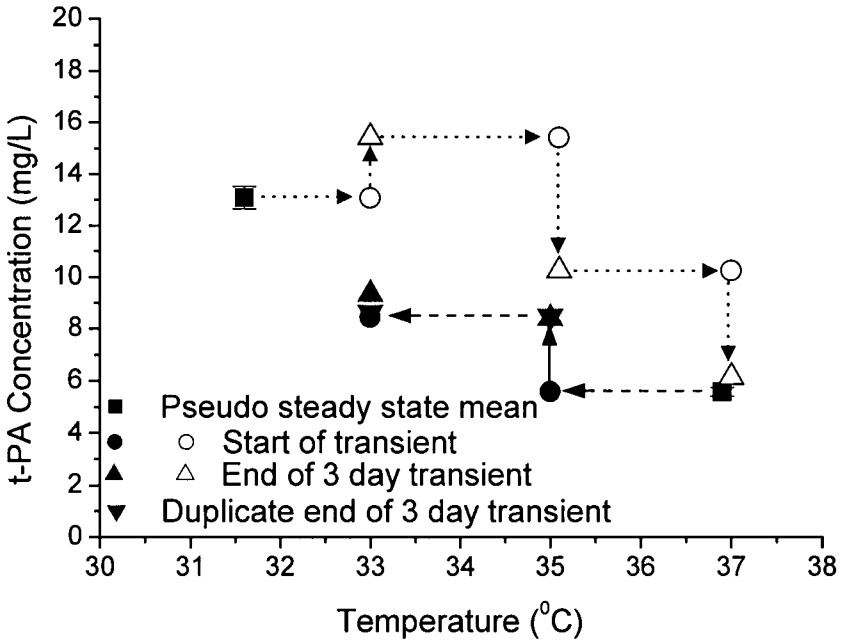

Figure 5.17 Transient profile of t-PA concentration versus 3 day scan down (closed symbols) and scan up (open symbols) of temperature in SD culture.

Squares indicate pseudo steady state mean obtained (± SEM, n = 3 to 6). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Dashed lines represent T scan down, dotted lines T scan up. Results for scan down from SD5: pH 7.3 ± 0.1, 82 ± 5% air saturation. Results for scan up from SD6: pH 7.4 ± 0.1, 74 ± 5% air saturation.

The two replicates for the scan down experiments are in agreement. The scan up results indicate the highest transient concentration reached was at 33 ^{0}C. There is also good

agreement between the pseudo steady state value at 37 ^{0}C of 5.6 ± 0.2 mg/L from the scan down experiment, and the 3 day transient value of 6.2 mg/L at 37 ^{0}C from the scan up experiment, indicating that there is consistency between small scale culture production.

5.3.2 1 day Transient Scanning

It is informative to evaluate how much time might be saved by even shorter transient scanning steps. For the case of evaluating 5 temperatures, shortening the step period to 1 day would reduce the time needed from 17 days in a 3 day small scale system to 7 days. This would be ~90% faster than a 50 day steady state perfusion experiment with a 2 week startup time to evaluate the same five temperatures. Table 5.3 shows the time period required for scanning experiments versus steady state culture.

Table 5.3 Time Advantage of Scanning versus Steady State Experiments.

	Steady State Perfusion	3d Scanning Perfusion	3d Scanning SD	1d Scanning SD
Startup Time (d)	~14	~14	~ 2	~ 2
Experimental Time (d)	50	15	15	5
Total Time (d)	64	29	17	7
% Time Saved relative to Steady State Case		55 %	73 %	89 %

Case presented assuming: 5 temperatures investigated, steady state requires 10-16 d with the minimum time 10 d used for calculation, no steady state was obtained in any scanning experiments.

Therefore to further accelerate the process, 1 day transient scan down experiments were conducted in small scale cultures. Two parallel experiments were performed. The cell specific t-PA production profile is shown in Figure 5.18.

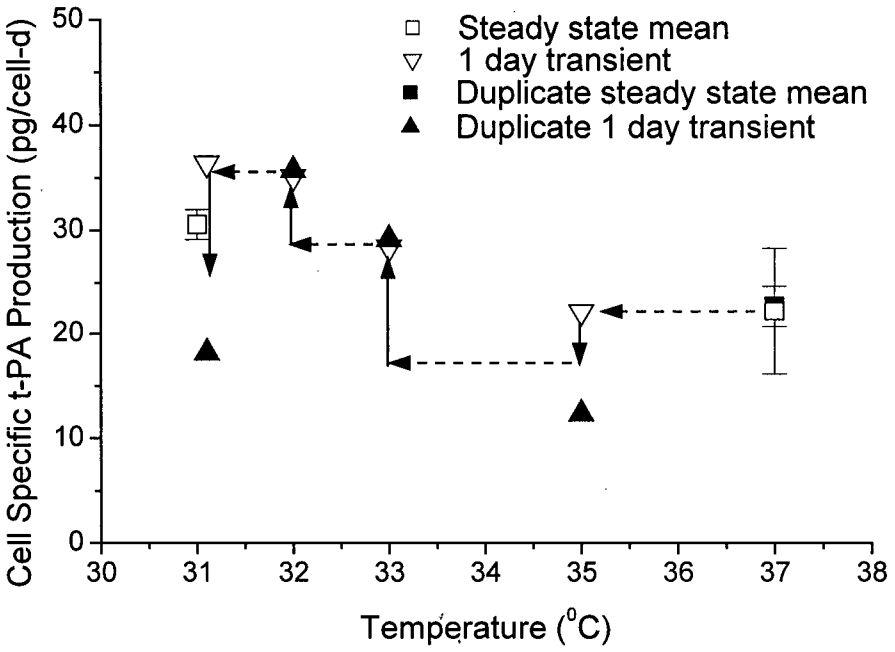

Figure 5.18 Transient profile of cell specific t-PA production rate versus 1 day scan down of temperature in SD culture.

Closed symbols from SD-B6. Open symbols from parallel run SD-A6. Squares indicate pseudo steady state mean obtained (± SEM, n = 3). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Results from SD6: pH 7.4 ± 0.1, 74 ± 5% air saturation.

There are greater differences in the replicate results here than observed previously in the 3 day scan-down experiments. At 35 ^{0}C, culture SD-B6 (closed symbols) showed a decreasing trend in the CStPAPR, while the other culture SD-A6 (open symbols) showed no change. It should be noted that only a 1-degree step change from 33 to 32 ^{0}C was obtained initially due to incubation temperature problems; a second day allowed for 31 ^{0}C. Both cultures showed an increase in productivity at 33 ^{0}C, but at 31 ^{0}C, SD-B6 showed an almost 50% drop in the CStPAPR from the previous day, while SD-A6 productivity remained high with only a 3% change. A temperature of 33 ^{0}C was

demonstrated to be a high CStPAPR operating temperature, similar to results obtained in the 3d perfusion culture (Figure 5.11).

The large drop in the CStPAPR at 31 ^{0}C in SD-B6 could be partially due to cell concentration counting problems, as described previously. Another reason could be the transient period chosen. The t-PA concentration profile also showed variability at each temperature change (Figure 5.19). Although the overall trend still showed higher [t-PA] at lower temperatures, the concentration decreased at 35 and 32 ^{0}C, in contrast to 3 d SD cultures (Figure 5.17). This suggests that that there may be a minimum time required at one temperature before a reliable transient response is obtained. Newland et al. (1994) describe an early transient stage where fluctuations are observed, and a late transient stage where the new steady state is approached. During 1 day temperature shifts, the response may not give a reliable indication of the new steady state since the transient stage may be too early.

Variability could also be attributed to inaccurate estimation of the feed rate required at the new temperature. If it is unknown how cell metabolism will be affected by a given change, it is possible that the predicted medium feed rate on the first day would be over- or underestimated, as previously seen with the 3 d transient perfusion experiments (Figure 5.4). This could lead to a change in t-PA concentration that may be contrary to what would actually occur if glucose utilization were kept constant.

The large increase at 31 ^{0}C in the pseudo steady state t-PA concentration versus the 1 day transient value was likely due to the reduced need for medium feeding at low temperature. As mentioned previously, batch culture was initiated in certain cases when cell concentration dropped and cell growth was inhibited.

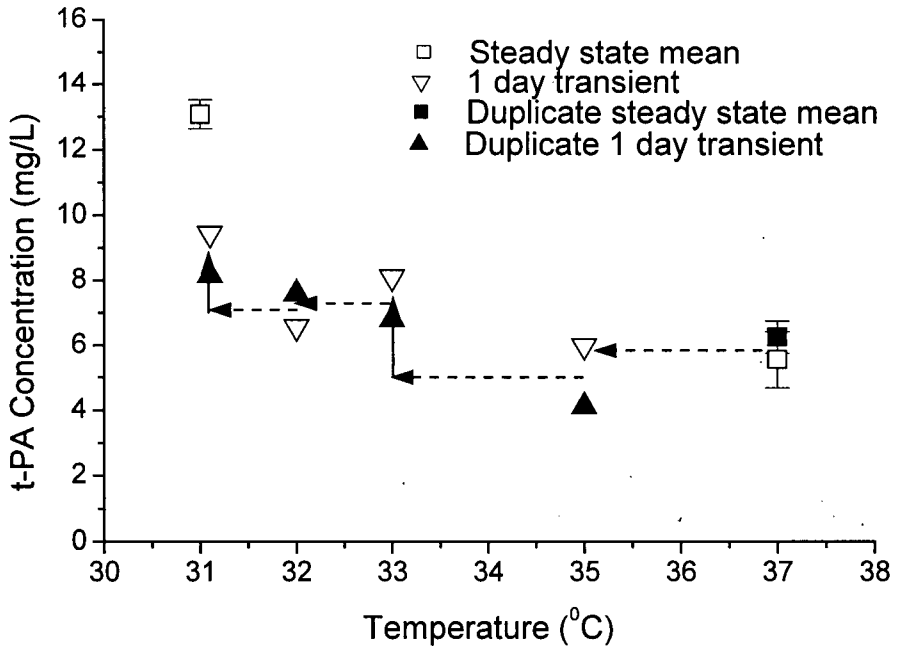

Figure 5.19 Transient profile of t-PA concentration versus 1 day scan down of temperature in SD culture.

Closed symbols from SD-B6. Open symbols from parallel run SD-A6. Squares indicate pseudo steady state mean obtained (± SEM, n = 3). Horizontal arrows represent step change in temperature, vertical arrows represent trend during transient phase. Results from SD6: pH 7.4 ± 0.1, 74 ± 5% air saturation.

However, a qualitative conclusion drawn from the 1 d t-PA profile suggests that 33 and 31 ^{0}C would produce higher [t-PA], a conclusion reached in previous 3 d scanning experiments in both perfusion and SD culture (Figure 5.20). The 3 d perfusion response and the 1d SD response had slopes significantly greater than zero ($p < 0.05$). The 3 d SD response also showed the same increasing trend with low temperature, although the slope was not significantly greater than zero. This was likely due to the lesser number of points available for analysis.

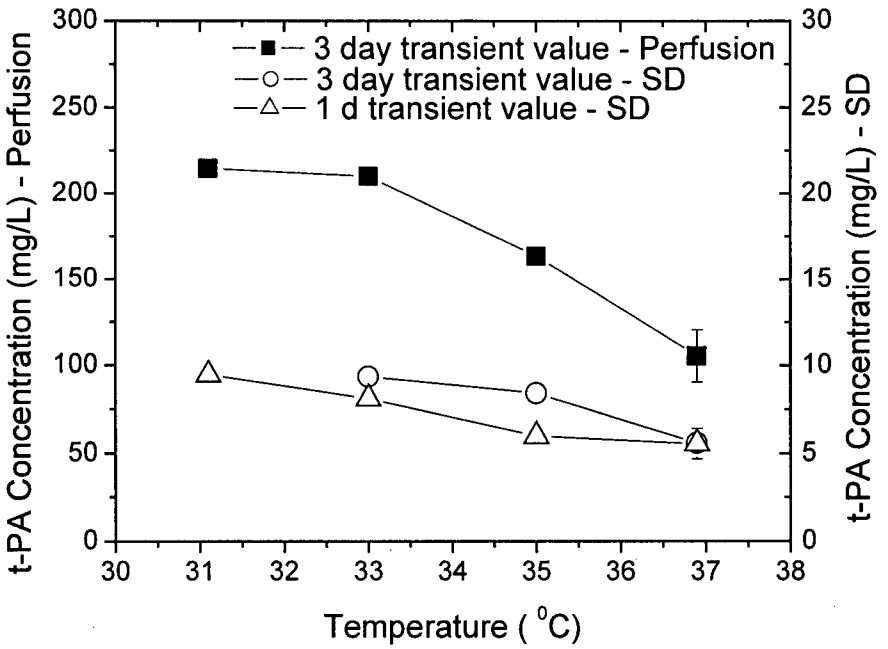

Figure 5.20 Comparison of t-PA concentration in temperature scanning cultures for 3 day perfusion (closed symbols), 3 day SD (open circles), and 1 day SD (open triangles).

Values represent transient value on the 1st or 3rd day after temperature change, except at 37 ^{0}C, where pseudo steady state mean (± SEM, n = 3) is presented. Closed symbols from P32 pH 7.05 ± 0.01, 104 ± 5% air saturation. Open circles from SD-D5 pH 7.3 ± 0.1, 82 ± 5% air saturation. Open triangles from SD-A6 pH 7.4 ± 0.1, 74 ± 5% air saturation.

Both 1 d and 3 d SD cultures show similar t-PA concentrations at each temperature, suggesting that using 3 day transients may not be necessary. The SD cultures also confirm the trend observed in perfusion cultures, although the increase in [t-PA] for SD is not as linear between 37 and 33 ^{0}C as in perfusion. This may result from a higher cell concentration and more frequent bleed adjustments in the perfusion culture. Although the cell specific t-PA production response to temperature appeared higher at 33 ^{0}C, the overall response was not significantly different from zero in the three Figure 5.20 cultures (data in Appendix).

Although 1 day transient results may not be ideal for picking a specific temperature that is the most advantageous, the rapid scanning does give the overall indication that low temperature conditions are likely to provide greater protein productivity and concentration than higher temperatures would. The 1 day scanning may be useful for parameters with many levels, for example, dissolved oxygen, where values from 10 to 120% air saturation have been investigated. A steady state experiment at the predicted parameter range could further refine the transient method, giving more conclusive results rather than just a narrowed optimal range. Taken together, the use of 1 day and 3 day scale-down cultures have the potential to simplify process optimization while significantly reducing process development time (Table 5.3).

6 Conclusions and Recommendations

In the first part of this work, the effect of temperature, dissolved oxygen and pH on t-PA productivity was determined. Results from batch experiments showed a significant increase in t-PA concentration and cell specific t-PA production at 33 ^{0}C compared to 37 ^{0}C. However, the cell specific growth rate was adversely affected at the lower temperature, dropping by 70% when compared to the growth rate at 37 ^{0}C. Perfusion temperature effects were similar to those in batch, with a maximum t-PA concentration obtained at 31 ^{0}C and maximum cell specific t-PA productivity obtained at 33 ^{0}C. The cell specific glucose uptake rate dropped with a decrease in temperature, in contrast to the batch cultures where no significant change was observed. A comparison of batch versus perfusion confirmed the advantages of perfusion culture in the model system used. Volumetric productivity was almost 14-fold higher in perfusion culture than in batch.

The effect of dissolved oxygen and pH were not as clear as the temperature effect. A DO concentration of 72% air saturation produced the highest t-PA concentration, with a significant decrease of 57% occurring when the oxygen was changed from 72 to 16%. The cell specific t-PA production rate also decreased significantly at the low DO value. Productivity and t-PA concentration was not significantly different at the higher DO of 100% air saturation, compared to 72%.

Cell specific protein production was negatively affected by low pH, decreasing over 50% when the pH was shifted from 7.05 to 6.85. The t-PA concentration was thereby significantly lower. Higher pH values between 7.05 and 7.25 had no significant effect on productivity or protein concentration. It is suggested that a more extensive range of pH values be examined to determine the threshold where alkaline pH values have a negative effect on cell productivity.

The second part of this work evaluated a method of using transient responses to qualitatively predict optimal process settings. Transient scanning has the potential to reduce process development times by at least half through eliminating the need for steady state experiments at every setpoint. Temperature was used as the model environmental parameter, and 3 day step changes of temperature in perfusion culture were investigated. Transient results showed that the CSGUR and CSLPR dropped with decreasing temperature, while the t-PA concentration increased. The transient results were similar to pseudo steady state results. The highest t-PA concentration was predicted at 31 ^{0}C, but growth inhibition at this temperature makes it impractical for production purposes. Interestingly, a shift from lactate to ammonium production occurred during the 31 ^{0}C pseudo steady state period. However, this was not predicted by the transient response, indicating that the method may have some limitations at extreme conditions. Glutamine may play an increasingly important role at 31 ^{0}C, as suggested by the increased ammonia production. Investigation of the role of glutamine at low temperature in future experiments is suggested.

Further confirmation of the transient method was investigated in small scale semicontinuous cultures that would simplify startup and operation. These experiments also evaluated whether non-instrumented scale-down reactors could be used to estimate perfusion culture results. The 3 day transient experiments in SD cultures yielded similar results as the perfusion cultures. However, the t-PA concentration in SD cultures was 15-30 times lower than perfusion due to the low cell concentration maintained, while the CStPAPR was two times higher in SD cultures, likely due to the high amount of glucose available. Experiments with 1 day changes in temperature were also carried out in SD culture and showed promising overall trends, but more variability from day-to-day. Using 1 day scans in SD cultures would take only 10% of the time needed for full steady state experiments, highlighting the potential value of this method. To minimize erroneous conclusions from 1 day experiments, the transient scanning method could be further refined to include a steady state experiment in the vicinity of the optimum range, in order to better determine actual productivity and growth characteristics at the operating conditions predicted. Though this would increase process development time, the overall time needed would still be approximately 30-70% shorter than required for full steady state experiments.

Although the SD cultures were simple to operate, the non-instrumented control implemented for these cultures did not work as well as expected. Cell concentration was the most problematic. It is recommended that further experiments with scale-down cultures use a higher cell concentration setpoint of at least $2x10^5$ cells/mL. To keep the pH and DO from fluctuating at this higher cell concentration, real time DO and pH

control may need to be implemented. Another recommendation is to adjust the feed medium glucose concentration in order to provide a more significant change in glucose uptake, outside the range of analysis error. A more precise glucose determination method is another alternative.

Finally, to extend the proof of concept, it is also recommended that the transient scanning method be used with another environmental parameter such as DO, in order to provide proof of general applicability. The method could also be used with another cell line producing a different protein other than t-PA in order to verify transferability to other systems.

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Appendix – Listing of Data

Table A. 1 Data for Batch Run at 37 ^{0}C.

Sample No	RunTime (h)	RunTime (day)	DeltaTime (h)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	[NH4] (mM)
B37/0	-0.2	0.0	-0.25	25.7	0.4	3.1	2.3
B37/1	1.5	0.1	1.75	23.7	2.7	3.6	2.6
B37/2	25.5	1.1	24.00	22.2	4.8	4.4	3.2
B37/3	44.5	1.9	19.00	20.1	6.9	5.3	3.8
B37/4	77.0	3.2	32.50	14.2	13.9	5.5	3.9
B37/5	104.7	4.4	27.67	8.1	16.1	5.0	3.6
B37/6	123.7	5.2	19.00	5.0	15.1	3.9	2.8
B37/7	146.0	6.1	22.33	2.3	17.3	3.6	2.6
B37/8	167.5	7.0	21.50	0.9	13.7	2.5	1.9
B37/9	192.0	8.0	24.50	1.0	9.0	3.8	2.8
B37/10	216.0	9.0	24.00	0.8	5.4	5.5	3.9
B37/11	243.0	10.1	27.00	1.8	3.1	7.3	5.2
B37/12	267.0	11.1	24.00	1.3	2.7	8.6	6.1

Sample No	Temperature (C)	pH	DO (%air sat)	Viab w/o tryp (%)	Total Xn (cells/mL)	Viab w/trypsin (%)	Xv (w/o trypsin) (cells/mL)
B37/0	36.9	7.17	74.1		#DIV/0!	#DIV/0!	#DIV/0!
B37/1	36.8	7.05	70.6	90.4	2.75E+05	92.3	2.48E+05
B37/2	36.8	7.05	69.5	93.4	4.48E+05	92.9	4.19E+05
B37/3	36.8	7.05	69.6	91.3	8.81E+05	91.9	8.05E+05
B37/4	36.8	7.07	77.6	94.9	1.20E+06	94.1	1.13E+06
B37/5	36.6	7.06	72.0	89.4	2.13E+06	94.7	1.90E+06
B37/6	36.8	7.06	69.7	98.9	2.55E+06	97.3	2.52E+06
B37/7	36.7	7.06	68.6	89.5	2.71E+06	96.7	2.42E+06
B37/8	36.8	7.05	72.3	89.4	2.79E+06	90.8	2.49E+06
B37/9	36.8	7.04	69.4	88.5	2.70E+06	88.5	2.39E+06
B37/10	36.7	7.06	64.9	80.6	3.24E+06	88.3	2.61E+06
B37/11	36.9	7.03	71.9	70.7	2.76E+06	69.6	1.95E+06
B37/12	36.8	7.05	74.9	0.0	3.93E+06	0.0	0.00E+00

Table A. 1 Data for Batch Run at 37 ^{0}C. (continued)

Sample No	X_v log mean (cells/mL)	GUR (mmol/h)	LPR (mmol/h)	APR (mmol/h)	CSGUR (pmol/cell-d)	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)
B37/0	#DIV/0!						
B37/1	#DIV/0!	0.674	0.775	0.115			
B37/2	3.26E+05	0.036	0.051	0.013	4.60	6.44	1.68
B37/3	5.91E+05	0.063	0.063	0.018	4.49	4.49	1.32
B37/4	9.60E+05	0.102	0.121	0.002	4.54	5.39	0.11
B37/5	1.49E+06	0.121	0.044	-0.007	3.56	1.28	-0.20
B37/6	2.20E+06	0.088	-0.028	-0.021	1.78	-0.57	-0.43
B37/7	2.47E+06	0.064	0.052	-0.005	1.17	0.96	-0.09
B37/8	2.46E+06	0.034	-0.086	-0.018	0.64	-1.63	-0.34
B37/9	2.44E+06	-0.002	-0.097	0.018	-0.04	-1.89	0.36
B37/10	2.50E+06	0.004	-0.074	0.024	0.08	-1.44	0.47
B37/11	2.27E+06	-0.018	-0.041	0.022	-0.39	-0.90	0.48
B37/12	#NUM!	0.010	-0.008	0.018	#NUM!	#NUM!	#NUM!

Sample No	tPA (U/mL)	tPA (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
B37/0					
B37/1	7720	13.31			
B37/2	11120	19.17	0.14	5.86	17.98
B37/3	16187	27.91	0.26	11.04	18.68
B37/4	22564	38.90	0.19	8.12	8.46
B37/5	29121	50.21	0.22	9.81	6.60
B37/6	34659	59.76	0.27	12.06	5.49
B37/7	37937	65.41	0.13	6.07	2.46
B37/8	38611	66.57	0.03	1.30	0.53
B37/9	45503	78.45	0.24	11.64	4.77
B37/10	39317	67.79	-0.22	-10.67	-4.27
B37/11	37735	65.06	-0.05	-2.42	-1.07
B37/12	35496	61.20	-0.08	-3.86	#NUM!

Table A. 2 Data for Batch Run at 33 ^{0}C.

Sample No	RunTime (h)	RunTime (day)	DeltaTime (h)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)
B33/0	-0.8	0.0	-0.75	24.8	0.1	2.2
B33/1	0.2	0.0	0.92	22.1	3.1	2.7
B33/2	25.4	1.1	25.25	21.7	5.5	3.2
B33/3	49.4	2.1	24.00	19.5	8.6	3.9
B33/4	72.9	3.0	23.50	18.0	10.8	4.2
B33/5	104.9	4.4	32.00	15.3	13.1	5.0
B33/6	145.9	6.1	41.00	12.1	17.4	4.8
B33/7	165.4	6.9	19.50	9.8	17.2	4.8
B33/8	195.9	8.2	30.50	6.5	18.9	4.3
B33/9	219.9	9.2	24.00	5.3	19.8	4.1
B33/10	243.9	10.2	24.00	4.2	17.7	3.7
B33/11	272.9	11.4	29.00	2.7	15.8	2.4
B33/12	295.4	12.3	22.50	2.2	16.6	3.4
B33/13	315.9	13.2	21.50	1.8	14.8	3.6
B33/14	332.9	13.9	17.00	1.1	14.5	3.6
B33/15	364.4	15.2	31.50	0.9	13.7	4.1
B33/16	386.7	16.1	22.25	0.7	13.3	4.5
B33/17	409.9	17.1	23.25	0.6	13.0	5.0
B33/18	440.9	18.4	31.00	0.5	11.6	6.2
B33/19	459.4	19.1	18.50	0.6	11.1	6.9
B33/20	479.2	20.0	19.75	0.5	11.3	7.5

Sample No	[NH4] (mM)	Temperature (C)	pH	DO (% air sat)	Osmolality (mOsm/kg)	Viab w/o tryp (%)
B33/0	1.7	33.0	6.95	83.6	398	
B33/1	2.0	33.6	7.03	81.1	403	92
B33/2	2.4	33.1	7.02	79.3	398	90
B33/3	2.8	33.0	7.02	81.0	396	70
B33/4	3.0	32.9	7.01	80.6	447	94
B33/5	3.6	33.0	7.04	83.7	415	74
B33/6	3.5	33.0	6.97	84.6	406	88
B33/7	3.5	33.1	6.87	83.2	408	87
B33/8	3.1	33.0	7.10	85.1	409	89
B33/9	3.0	33.1	7.10	85.0	381	88
B33/10	2.7	33.1	7.12	84.0	392	90
B33/11	1.8	33.1	7.28	83.2	386	72
B33/12	2.5	33.1	6.94	83.0	410	91
B33/13	2.6	33.1	6.97	80.3	384	89
B33/14	2.6	33.0	6.96	79.4	385	78
B33/15	3.0	33.0	7.01	80.4	384	81
B33/16	3.3	33.0	7.08	84.0	375	74
B33/17	3.6	33.1	7.02	82.4	409	72
B33/18	4.4	33.1	7.03	82.5	375	71
B33/19	4.9	33.1	7.04	83.9	379	61
B33/20	5.3	33.0	7.04	83.6	376	59

Table A. 2 Data for Batch Run at 33 ^{0}C. (continued)

Sample No	Total Xn (cells/mL)	Viab w/ trypsin (%)	Xv w/o trypsin (cells/mL)	Xv log mean (cells/mL)	GUR (mmol/h)	LPR (mmol/h)
B33/0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
B33/1	3.36E+05	95.5	3.10E+05	#DIV/0!	2.150	2.389
B33/2	3.75E+05	89.5	3.38E+05	3.23E+05	0.011	0.068
B33/3	3.83E+05	91.2	3.49E+05	3.43E+05	0.065	0.092
B33/4	2.42E+05	90.7	2.28E+05	2.84E+05	0.045	0.066
B33/5	6.55E+05	92.4	6.05E+05	3.86E+05	0.058	0.050
B33/6	8.96E+05	94.6	7.89E+05	6.93E+05	0.053	0.071
B33/7	9.30E+05	77.4	8.11E+05	8.00E+05	0.079	-0.007
B33/8	9.64E+05	91.4	8.59E+05	8.35E+05	0.071	0.037
B33/9	1.16E+06	87.4	1.02E+06	9.36E+05	0.033	0.024
B33/10	1.13E+06	85.7	1.02E+06	1.02E+06	0.029	-0.056
B33/11	1.25E+06	85.5	1.07E+06	1.04E+06	0.033	-0.041
B33/12	1.36E+06	92.6	1.24E+06	1.15E+06	0.013	0.021
B33/13	1.04E+06	81.7	9.31E+05	1.08E+06	0.011	-0.048
B33/14	9.90E+05	83.3	7.76E+05	8.51E+05	0.023	-0.010
B33/15	9.94E+05	82.3	8.05E+05	7.90E+05	0.003	-0.014
B33/16	1.12E+06	84.6	8.25E+05	8.15E+05	0.005	-0.010
B33/17	8.66E+05	90.9	6.24E+05	7.20E+05	0.002	-0.007
B33/18	9.90E+05	73.5	6.99E+05	6.61E+05	0.002	-0.023
B33/19	9.23E+05	63.0	5.64E+05	6.29E+05	-0.003	-0.014
B33/20	1.13E+06	62.9	6.72E+05	6.16E+05	0.002	0.005

Sample No	APR (mmol/h)	CSGUR (pmol/cell-d)	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)
B33/0				
B33/1	0.273			
B33/2	0.010	1.18	7.05	1.01
B33/3	0.014	6.41	9.04	1.40
B33/4	0.006	5.40	7.91	0.74
B33/5	0.012	5.25	4.47	1.06
B33/6	-0.002	2.70	3.63	-0.12
B33/7	0.000	3.54	-0.31	0.00
B33/8	-0.007	3.11	1.60	-0.32
B33/9	-0.004	1.28	0.96	-0.15
B33/10	-0.007	1.08	-2.06	-0.27
B33/11	-0.019	1.19	-1.51	-0.71
B33/12	0.018	0.46	0.74	0.64
B33/13	0.004	0.41	-1.87	0.14
B33/14	0.000	1.16	-0.50	0.00
B33/15	0.006	0.19	-0.77	0.33
B33/16	0.007	0.26	-0.53	0.36
B33/17	0.008	0.14	-0.43	0.49
B33/18	0.014	0.12	-1.64	0.96
B33/19	0.013	-0.21	-1.03	0.99
B33/20	0.010	0.20	0.39	0.81

Table A. 2 Data for Batch Run at 33 ^{0}C. (continued)

Sample No	tPA (U/mL)	tPA (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
B33/0					
B33/1	9584	16.52			
B33/2	10904	18.80	0.06	2.16	6.69
B33/3	14963	25.80	0.21	7.00	20.40
B33/4					
B33/5	23211	40.02	0.18	6.19	16.05
B33/6	29119	50.21	0.17	5.96	8.61
B33/7	32192	55.50	0.18	6.52	8.15
B33/8	37606	64.84	0.20	7.35	8.80
B33/9	39711	68.47	0.10	3.63	3.88
B33/10	45363	78.21	0.26	9.74	9.58
B33/11	44338	76.44	-0.04	-1.46	-1.41
B33/12	45586	78.60	0.06	2.30	2.00
B33/13	48519	83.65	0.13	5.64	5.24
B33/14	46721	80.55	-0.10	-4.38	-5.14
B33/15	47570	82.02	0.03	1.12	1.41
B33/16	47051	81.12	-0.02	-0.97	-1.18
B33/17	48432	83.50	0.05	2.46	3.41
B33/18	48483	83.59	0.00	0.07	0.10
B33/19	48590	83.78	0.01	0.24	0.38
B33/20	42971	74.09	-0.23	-11.77	-19.11

Table A. 3 Cell Specific Calculation Data used for 37 ^{0}C Batch Culture.

Time (d)	Cell Conc (cells/mL)	Int cell d (cells-d/mL)	Int cell d 10^6 (cells-d/mL)	[tPA] (ug/mL)	[Gluc] (mM)	[Lac] (mM)	[NH4] (mM)
0	0	0	0	0			
0.1	248148	7755	0.01	13.31	23.7	2.7	2.6
1.1	418549	341103	0.34	19.17	22.2	4.8	3.2
1.9	804581	825259	0.83	27.91	20.1	6.9	3.8
3.2	1134055	2137877	2.14	38.90	14.2	13.9	3.9
4.4	1904220	3889105	3.89	50.21	8.1	16.1	3.6
5.2	2521950	5641131	5.64	59.76	5.0	15.1	2.8
6.1	2423213	7942005	7.94	65.41	2.3	17.3	2.6
7.0	2494260	10144623	10.14	66.57	0.9	13.7	1.9
8.0	2389500	12637375	12.64	78.45	1.0	9.0	2.8
9.0	2611440	15137845	15.14	67.79	0.8	5.4	3.9
10.1	1951320	17704398	17.70	65.06	1.8	3.1	5.2
11.1	0	18680058	18.68	61.20	1.3	2.7	6.1

Table A. 4 Cell Specific Calculation Data used for 33 ^{0}C Batch Culture.

Time (d)	Cell Conc (cells/mL)	Int cell d (cells-d/mL)	Int cell d 10^6 (cells-d/mL)	[tPA] (ug/mL)	[Gluc] (mM)	[Lac] (mM)	[NH4] (mM)
0.0	0	0	0.00	0.00			
0.0	309782	1076	0.00	16.52	22.1	3.1	2.0
1.1	337500	341573	0.34	18.80	21.7	5.5	2.4
2.1	348750	684698	0.68	25.80	19.5	8.6	2.8
3.0	227604	966871	0.97		18.0	10.8	3.0
4.4	605000	1521941	1.52	40.02	15.3	13.1	3.6
6.1	788700	2712393	2.71	50.21	12.1	17.4	3.5
6.9	810960	3362255	3.36	55.50	9.8	17.2	3.5
8.2	858701	4423185	4.42	64.84	6.5	18.9	3.1
9.2	1017383	5361227	5.36	68.47	5.3	19.8	3.0
10.2	1017004	6378420	6.38	78.21	4.2	17.7	2.7
11.4	1065000	7636298	7.64	76.44	2.7	15.8	1.8
12.3	1237376	8715537	8.72	78.60	2.2	16.6	2.5
13.2	930953	9641594	9.64	83.65	1.8	14.8	2.6
13.9	776160	10246196	10.25	80.55	1.1	14.5	2.6
15.2	804938	11283791	11.28	82.02	0.9	13.7	3.0
16.1	824715	12039203	12.04	81.12	0.7	13.3	3.3
17.1	623700	12740779	12.74	83.50	0.6	13.0	3.6
18.4	698940	13594984	13.59	83.59	0.5	11.6	4.4
19.1	563648	14081606	14.08	83.78	0.6	11.1	4.9
20.0	671573	14589848	14.59	74.09	0.5	11.3	5.3

Table A. 5 Average T, pH, and DO settings (last three days) of each pseudo steady state experiment from Perfusion Run P28.

Cond Set	Mean T (C)	Mean pH	Mean DO (% air sat)	St Dev T (C)	St Dev pH	St Dev DO (% air sat)
A	37.0	7.04	70.5	0.36	0.010	12.20
B	33.6	7.05	70.2	0.42	0.010	1.31
C	36.9	7.04	72.0	0.36	0.021	4.08
D	33.4	7.19	70.9	0.42	0.015	6.43
E	33.2	7.05	72.6	0.32	0.010	2.42
F	33.1	7.25	68.5	0.20	0.006	1.50
G	37.1	7.09	65.3	0.46	0.012	4.95
H	37.0	7.04	16.3	0.40	0.010	2.00
I	36.9	6.97	6.7	0.35	0.010	2.65
J	36.9	7.04	11.8	0.42	0.164	6.30
K	36.5	7.00	72.2	0.12	0.006	0.38
L	37.4	7.04	74.8	0.15	0.017	1.19
M	36.8	6.87	70.9	0.31	0.012	0.46
N	36.7	6.85	72.3	0.23	0.021	0.72
O	36.0	6.67	74.9	0.68	0.000	1.50

Table A. 6 Data from Pseudo Steady State Perfusion Run P28.

Cond Set	Sample No	RunTime (h)	RunTime (d)	DeltaTime (h)	total Bleed (mL)	total bleed rate (mL/h)	total Feed (mL)
A	P28/0	0.0	0.0	0.00	15	#DIV/0!	0
	P28/1	0.6	0.0	0.58	15	25.71	0
	P28/2	27.5	1.1	26.92	15	0.56	0.00
	P28/3	51.5	2.1	24.00	15	0.63	0.00
	P28/4	74.5	3.1	23.00	15	0.65	0.00
	P28/5	98.0	4.1	23.50	15	0.64	0.00
	P28/6	122.0	5.1	24.00	15	0.63	0.00
	P28/7	146.0	6.1	24.00	15	0.63	0.00
	P28/8	170.5	7.1	24.50	15	0.61	0.00
	P28/9	193.5	8.0	23.00	15	0.65	238.00
	P28/10	219.5	9.1	26.00	15	0.58	279.00
	P28/11	241.5	10.0	22.00	15	0.68	408.00
	P28/12	265.5	11.0	24.00	15	0.63	502.00
	P28/13	290.0	12.1	24.50	15	0.61	348.00
A	P28/14	313.5	13.0	23.50	30	1.28	571.00
B	P28/15	347.5	14.5	34.00	15	0.44	700.00
	P28/16	364.0	15.1	16.50	15	0.91	210.00
	P28/17	389.0	16.2	25.00	135	5.40	400.00
	P28/18	408.5	17.0	19.50	15	0.77	366.00
	P28/19	433.0	18.0	24.50	80	3.27	391.00
B	P28/20	457.5	19.0	24.50	15	0.61	304.00
C	P28/21	481.5	20.0	24.00	15	0.63	501.00
	P28/22	507.5	21.1	26.00	15	0.58	50.00
	P28/22-1	516.5	21.5	9.00	15	1.67	236.00
	P28/23	529.5	22.0	22.00	15	0.68	325.00
	P28/24	553.0	23.0	23.50	183	7.79	480.00
	P28/25	576.5	24.0	23.50	164	6.98	720.00
	P28/26	600.5	25.0	24.00	147	6.13	825.00
	P28/27	625.0	26.0	24.50	303	12.37	660.00
	P28/28	647.5	27.0	22.50	208	9.24	588.00
C	P28/29	675.5	28.1	28.00	257	9.18	844.00
D	P28/30	704.5	29.3	29.00	231	7.97	368.00
	P28/31	720.5	30.0	16.00	15	0.94	551.00
	P28/32	744.5	31.0	24.00	175	7.29	499.00
	P28/33	769.0	32.0	24.50	148	6.04	647.00
	P28/34	793.0	33.0	24.00	213	8.88	749.00
D	P28/35	816.5	34.0	23.50	340	14.47	672.00
E	P28/36	843.5	35.1	27.00	15	0.56	824.00
	P28/37	872.5	36.3	29.00	276	9.52	780.00
	P28/38	888.5	37.0	16.00	151	9.44	214.00
	P28/39	912.5	38.0	24.00	219	9.13	536.00
	P28/40	937.0	39.0	24.50	219	8.94	226.00
	P28/41	961.0	40.0	24.00	134	5.58	370.00
	P28/42	985.0	41.0	24.00	142	5.92	382.00
E	P28/43	1016.0	42.3	31.00	100	3.23	496.00

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Feed rate used (mL/h)	Perfusion Rate (L/L-d)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	Temperature (C)	pH
A	P28/0	#VALUE!	#VALUE!	23.1	0.3	1.5	37.7	7.47
	P28/1	0.00	0.00	20.0	6.2	2.4	37.0	7.42
	P28/2	0.00	0.00	17.2	10.3	3.1	37.1	7.22
	P28/3	0.00	0.00	11.8	18.7	4.2	37.3	7.05
	P28/4	0.00	0.00	17.2	25.7	4.5	37.0	7.04
	P28/5	0.00	0.00	15.9	10.7	3.9	33.6	7.25
	P28/6	0.00	0.00	16.8	9.2	4.1	33.3	7.05
	P28/7	0.00	0.00	11.5	13.0	4.9	35.7	7.05
	P28/8	0.00	0.00	5.6	14.1	3.9	37.7	7.05
	P28/9	10.35	0.41	6.1	11.5	3.4	37.2	7.05
	P28/10	10.73	0.43	4.8	10.6	3.5	37.3	7.05
	P28/11	18.55	0.74	6.2	10.2	3.6	36.5	7.05
	P28/12	20.92	0.84	8.7	9.9	3.6	37.4	7.05
	P28/13	14.20	0.57	5.6	11.4	3.5	36.9	7.03
A	P28/14	24.30	0.97	6.4	12.2	3.5	36.7	7.04
B	P28/15	20.59	0.82	7.3	9.9	4.0	32.9	7.06
	P28/16	12.73	0.51	4.9	9.8	3.6	33.0	7.06
	P28/17	16.00	0.64	4.2	9.1	3.9	33.7	7.05
	P28/18	18.77	0.75	6.3	8.7	4.2	33.1	7.06
	P28/19	15.96	0.64	6.6	8.7	4.0	33.9	7.05
B	P28/20	12.41	0.50	6.4	9.4	4.2	33.7	7.04
C	P28/21	20.88	0.84	5.9	12.6	3.4	37.0	7.05
	P28/22	1.92	0.08	0.9	uc	7.4	37.2	7.04
	P28/22-1	26.22	1.05	4.5	uc	4.8	37.2	7.04
	P28/23	25.00	1.00	4.8	11.5	3.7	36.8	7.05
	P28/24	20.43	0.82	5.0	12.0	3.3	36.4	7.04
	P28/25	30.64	1.23	3.9	11.4	3.0	36.5	7.00
	P28/26	34.38	1.38	6.3	13.2	3.3	37.4	7.00
	P28/27	26.94	1.08	5.7	14.3	3.1	37.0	7.03
	P28/28	26.13	1.05	3.6	14.3	3.0	36.5	7.02
C	P28/29	30.14	1.21	3.0	16.9	3.0	37.2	7.06
D	P28/30	12.69	0.51	6.7	11.9	3.6	34.7	7.23
	P28/31	34.44	1.38	3.2	18.1	3.1	35.5	7.10
	P28/32	20.79	0.83	2.0	15.5	2.7	33.6	7.25
	P28/33	26.41	1.06	3.8	16.0	2.8	33.3	7.20
	P28/34	31.21	1.25	5.5	17.3	3.0	33.1	7.19
D	P28/35	28.60	1.14	3.8	17.5	2.9	33.9	7.17
E	P28/36	30.52	1.22	8.2	13.7	3.3	33.6	7.05
	P28/37	26.90	1.08	7.7	13.0	3.1	33.3	7.04
	P28/38	13.38	0.54	5.9	9.6	2.7	33.6	7.05
	P28/39	22.33	0.89	9.5	10.7	3.4	33.1	7.06
	P28/40	9.22	0.37	4.6	11.7	3.1	33.1	7.13
	P28/41	15.42	0.62	4.4	9.5	3.3	33.1	7.05
	P28/42	15.92	0.64	5.1	8.9	3.5	33.6	7.06
E	P28/43	16.00	0.64	4.5	10.1	3.6	33.0	7.04

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	DO (% air sat)	Viab w/o Tryp (%)	comment	Total Xn (cells/mL)	Viab w/ tryp (%)
A	P28/0	99.9			0.0E+00	
	P28/1	82.2			5.9E+05	85.9
	P28/2	80.3			4.7E+05	85.6
	P28/3	62.1			1.1E+06	94.3
	P28/4	63.0			1.8E+06	98.3
	P28/5	69.1			1.6E+06	95.0
	P28/6	68.4			1.1E+06	97.2
	P28/7	68.8			1.4E+06	94.4
	P28/8	65.3			2.5E+06	93.5
	P28/9	75.0			3.1E+06	94.8
	P28/10	66.0			3.5E+06	96.3
	P28/11	73.0			5.5E+06	93.1
	P28/12	83.9			4.8E+06	96.7
	P28/13	67.7			7.3E+06	98.4
A	P28/14	60.0		RNA Sample	1.1E+07	96.2
B	P28/15	77.0			1.0E+07	96.2
	P28/16	74.0			9.5E+06	91.8
	P28/17	60.5	81		8.0E+06	94.4
	P28/18	71.6	84		9.5E+06	97.5
	P28/19	70.0	86	RNA + Sial Sample	8.3E+06	93.1
B	P28/20	69.0			8.7E+06	91.4
C	P28/21	75.7			7.2E+06	93.4
	P28/22	70.0		feed pump crashed	1.1E+07	89.7
	P28/22-1	70.0			#DIV/0!	
	P28/23	71.8			7.8E+06	93.4
	P28/24	73.6			1.1E+07	94.5
	P28/25	73.7		RNA + Sial Sample	1.3E+07	96.3
	P28/26	65.6			9.8E+06	96.9
	P28/27	76.5			1.0E+07	96.2
	P28/28	71.1		RNA + Sial Sample	1.2E+07	96.4
C	P28/29	68.5			1.4E+07	96.5
D	P28/30	82.5			7.0E+06	93.5
	P28/31	68.1		feed pump crashed	8.7E+06	93.8
	P28/32	74.9			1.1E+07	94.8
	P28/33	76.0			9.3E+06	97.7
	P28/34	63.7			1.1E+07	97.8
D	P28/35	73.1		RNA + Sial Sample	9.0E+06	96.3
E	P28/36	75.7			1.1E+07	93.7
	P28/37	70.1			9.5E+06	95.2
	P28/38	67.6		feed pump crashed	1.0E+07	98.5
	P28/39	66.3			9.7E+06	95.1
	P28/40	72.4	93		8.6E+06	97.6
	P28/41	74.8		RNA + Sial Sample	9.8E+06	97.8
	P28/42	73.0			9.5E+06	97.8
E	P28/43	70.0		RNA + Sial Sample	9.6E+06	96.9

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Xn viable (cells/mL)	Xv log mean (cells/mL)	GUR (mmol/h)	CSGUR (pmol/cell-d)	LPR (mmol/h)	CSLPR (pmol/cell-d)	APR (mmol/h)	CSAPR (pmol/cell-d)
A	P28/0	0.0E+00							
	P28/1	5.0E+05	#DIV/0!	3.189	#DIV/0!	-6.069	#DIV/0!	-1.054	#DIV/0!
	P28/2	4.0E+05	4.49E+05	0.062	5.56	-0.091	-8.14	-0.018	-1.60
	P28/3	9.9E+05	6.51E+05	0.135	8.29	-0.210	-12.90	-0.032	-1.95
	P28/4	1.8E+06	1.33E+06	-0.141	-4.22	-0.183	-5.48	-0.009	-0.27
	P28/5	1.5E+06	1.63E+06	0.033	0.82	0.383	9.42	0.018	0.43
	P28/6	1.0E+06	1.25E+06	-0.023	-0.72	0.038	1.20	-0.006	-0.18
	P28/7	1.4E+06	1.18E+06	0.133	4.48	-0.095	-3.21	-0.023	-0.78
	P28/8	2.3E+06	1.78E+06	0.144	3.24	-0.027	-0.60	0.028	0.63
	P28/9	2.9E+06	2.59E+06	0.185	2.86	0.200	3.09	-0.018	-0.27
	P28/10	3.4E+06	3.14E+06	0.240	3.05	0.139	1.77	-0.034	-0.43
	P28/11	5.1E+06	4.21E+06	0.323	3.07	0.204	1.94	-0.060	-0.57
	P28/12	4.7E+06	4.90E+06	0.305	2.49	0.218	1.78	-0.065	-0.53
	P28/13	7.2E+06	5.83E+06	0.329	2.26	0.115	0.79	-0.041	-0.28
A	P28/14	1.1E+07	8.73E+06	0.441	2.02	0.266	1.22	-0.073	-0.33
B	P28/15	1.0E+07	1.02E+07	0.358	1.40	0.268	1.05	-0.078	-0.30
	P28/16	8.7E+06	9.33E+06	0.328	1.41	0.129	0.55	-0.026	-0.11
	P28/17	7.6E+06	8.12E+06	0.344	1.70	0.168	0.83	-0.061	-0.30
	P28/18	9.3E+06	8.39E+06	0.306	1.46	0.179	0.86	-0.079	-0.38
	P28/19	7.7E+06	8.45E+06	0.289	1.37	0.139	0.66	-0.053	-0.25
B	P28/20	8.0E+06	7.81E+06	0.234	1.20	0.095	0.49	-0.051	-0.26
C	P28/21	6.8E+06	7.33E+06	0.406	2.21	0.150	0.82	-0.047	-0.25
	P28/22	9.9E+06	8.22E+06	0.157	0.76	#VALUE!		-0.116	-0.57
	P28/22-1		#NUM!	0.345				0.043	
	P28/23	7.3E+06	8.51E+06	0.501	2.35			-0.062	-0.29
	P28/24	1.1E+07	8.91E+06	0.405	1.82	0.227	1.02	-0.049	-0.22
	P28/25	1.3E+07	1.17E+07	0.658	2.25	0.374	1.28	-0.071	-0.24
	P28/26	9.5E+06	1.10E+07	0.624	2.27	0.378	1.38	-0.098	-0.36
	P28/27	9.9E+06	9.70E+06	0.527	2.17	0.343	1.42	-0.066	-0.27
	P28/28	1.2E+07	1.09E+07	0.588	2.15	0.374	1.37	-0.062	-0.23
C	P28/29	1.3E+07	1.25E+07	0.667	2.13	0.415	1.32	-0.073	-0.23
D	P28/30	6.5E+06	9.40E+06	0.179	0.76	0.286	1.22	-0.049	-0.21
	P28/31	8.2E+06	7.30E+06	0.822	4.50	0.284	1.56	-0.076	-0.41
	P28/32	1.0E+07	9.24E+06	0.496	2.15	0.414	1.79	-0.036	-0.16
	P28/33	9.1E+06	9.72E+06	0.540	2.22	0.404	1.66	-0.059	-0.24
	P28/34	1.0E+07	9.75E+06	0.593	2.43	0.487	2.00	-0.078	-0.32
D	P28/35	8.6E+06	9.53E+06	0.625	2.63	0.492	2.07	-0.065	-0.27
E	P28/36	1.0E+07	9.40E+06	0.482	2.05	0.561	2.39	-0.088	-0.37
	P28/37	9.0E+06	9.59E+06	0.469	1.96	0.374	1.56	-0.066	-0.28
	P28/38	9.9E+06	9.46E+06	0.311	1.32	0.279	1.18	-0.014	-0.06
	P28/39	9.2E+06	9.58E+06	0.296	1.24	0.199	0.83	-0.075	-0.32
	P28/40	8.4E+06	8.81E+06	0.286	1.30	0.079	0.36	-0.017	-0.07
	P28/41	9.5E+06	8.96E+06	0.321	1.43	0.218	0.98	-0.047	-0.21
	P28/42	9.3E+06	9.42E+06	0.305	1.29	0.161	0.69	-0.052	-0.22
E	P28/43	9.3E+06	9.31E+06	0.335	1.44	0.129	0.55	-0.051	-0.22

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	μ bleed (1/h)	μ apparent (1/h)	μ real (1/h)	tPA (U/mL)	[t-PA] (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
A	P28/0	#DIV/0!		#DIV/0!	5416	9.34			
	P28/1	4.3E-02	#DIV/0!	#DIV/0!	6791	11.71	2.44	97.54	#DIV/0!
	P28/2	9.3E-04	-8.48E-03	-7.55E-03	8903	15.35	0.08	3.25	7.23
	P28/3	1.0E-03	3.78E-02	3.88E-02	23035	39.72	0.61	24.37	37.43
	P28/4	1.1E-03	2.48E-02	2.59E-02	34115	58.82	0.50	19.93	14.94
	P28/5	1.1E-03	-6.28E-03	-5.21E-03	30279	52.21	-0.17	-6.75	-4.15
	P28/6	1.0E-03	-1.59E-02	-1.49E-02	30223	52.11	0.00	-0.10	-0.08
	P28/7	1.0E-03	1.13E-02	1.23E-02	82597	142.41	2.26	90.30	76.34
	P28/8	1.0E-03	2.17E-02	2.28E-02	107000	184.48	1.03	41.22	23.12
	P28/9	1.1E-03	1.01E-02	1.12E-02	85326	147.11	0.74	29.63	11.45
	P28/10	9.6E-04	6.12E-03	7.08E-03	83181	143.42	1.47	58.94	18.75
	P28/11	1.1E-03	1.88E-02	1.99E-02	64097	110.51	1.46	58.29	13.84
	P28/12	1.0E-03	-4.08E-03	-3.04E-03	52625	90.73	1.61	64.41	13.16
	P28/13	1.0E-03	1.76E-02	1.86E-02	61200	105.52	1.76	70.23	12.06
A	P28/14	2.1E-03	1.62E-02	1.84E-02	72199	124.48	3.28	131.14	15.02
B	P28/15	7.4E-04	-1.46E-03	-7.29E-04	97764	168.56	3.79	151.78	14.82
	P28/16	1.5E-03	-8.38E-03	-6.86E-03	146000	251.72	5.70	227.95	24.43
	P28/17	9.0E-03	-5.62E-03	3.38E-03	129000	222.41	3.09	123.59	15.23
	P28/18	1.3E-03	1.05E-02	1.17E-02	117000	201.72	3.34	133.75	15.95
	P28/19	5.4E-03	-7.68E-03	-2.24E-03	132000	227.59	4.06	162.36	19.21
B	P28/20	1.0E-03	1.41E-03	2.43E-03	147000	253.45	3.62	144.71	18.52
C	P28/21	1.0E-03	-6.82E-03	-5.78E-03	121000	208.62	3.70	148.09	20.19
	P28/22	9.6E-04	1.47E-02	1.57E-02	153000	263.79	1.73	69.10	8.40
	P28/22-1	2.8E-03	#NUM!	#NUM!					
	P28/23	1.1E-03	#DIV/0!	#DIV/0!	104000	179.31	4.04	161.56	18.98
	P28/24	1.3E-02	1.69E-02	2.99E-02	80412	138.64	2.21	88.35	9.91
	P28/25	1.2E-02	6.66E-03	1.83E-02	83012	143.12	4.43	177.23	15.16
	P28/26	1.0E-02	-1.20E-02	-1.75E-03	74649	128.71	4.31	172.46	15.71
	P28/27	2.1E-02	1.89E-03	2.25E-02	77783	134.11	3.67	146.89	15.14
	P28/28	1.5E-02	8.42E-03	2.38E-02	83858	144.58	3.92	156.84	14.35
C	P28/29	1.5E-02	3.00E-03	1.83E-02	93306	160.87	4.95	198.11	15.83
D	P28/30	1.3E-02	-2.40E-02	-1.07E-02	111000	191.38	2.87	114.65	12.19
	P28/31	1.6E-03	1.41E-02	1.57E-02	71808	123.81	2.89	115.73	15.84
	P28/32	1.2E-02	1.01E-02	2.23E-02	96989	167.22	4.11	164.44	17.80
	P28/33	1.0E-02	-5.67E-03	4.40E-03	110000	189.66	5.26	210.46	21.65
	P28/34	1.5E-02	6.03E-03	2.08E-02	98709	170.19	5.13	205.13	21.04
D	P28/35	2.4E-02	-8.17E-03	1.59E-02	120000	206.90	6.33	253.15	26.58
E	P28/36	9.3E-04	6.15E-03	7.07E-03	106000	182.76	5.41	216.38	23.02
	P28/37	1.6E-02	-4.32E-03	1.15E-02	133000	229.31	6.50	260.19	27.14
	P28/38	1.6E-02	6.15E-03	2.19E-02	106000	182.76	1.01	40.40	4.27
	P28/39	1.5E-02	-3.00E-03	1.22E-02	116000	200.00	4.71	188.21	19.64
	P28/40	1.5E-02	-3.89E-03	1.10E-02	108000	186.21	1.44	57.74	6.55
	P28/41	9.3E-03	5.30E-03	1.46E-02	193000	332.76	7.66	306.57	34.22
	P28/42	9.9E-03	-1.06E-03	8.80E-03	143000	246.55	2.46	98.21	10.43
E	P28/43	5.4E-03	1.04E-04	5.48E-03					

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	RunTime (h)	RunTime (d)	DeltaTime (h)	total Bleed (mL)	total bleed rate (mL/h)	total Feed (mL)
F	P28/44	1037.0	43.2	21.00	31	1.48	348.00
	P28/45	1057.0	44.0	20.00	165	8.25	630.00
	P28/46	1081.0	45.0	24.00	253	10.54	550
	P28/47	1105.0	46.0	24.00	270	11.25	576
	P28/48	1129.0	47.0	24.00	79.5	3.31	487.2
	P28/49	1153.0	48.0	24.00	219	9.13	576
F	P28/50	1180.0	49.1	27.00	322	11.93	742
G	P28/51	1207.0	50.3	27.00	112	4.15	812
	P28/52	1225.0	51.0	18.00	249	13.83	590
	P28/53	1252.0	52.1	27.00	366	13.56	882
	P28/54	1276.0	53.1	24.00	344	14.33	792
	P28/55	1297.0	54.0	21.00	356.4	16.97	776
	P28/56	1321.0	55.0	24.00	351	14.63	885
	P28/57	1348.0	56.1	27.00	312	11.56	1050
	P28/58	1378.0	57.4	30.00	435	14.50	1157
G	P28/59	1393.0	58.0	15.00	225	15.00	585
H	P28/60	1417.0	59.0	24.00	338	14.08	960
	P28/61	1445.0	60.2	28.00	485	17.32	1063
	P28/62	1465.0	61.0	20.00	267	13.35	694
	P28/63	1489.0	62.0	24.00	339	14.13	912
	P28/64	1515.0	63.1	26.00	388	14.92	975
H	P28/65	1540.0	64.1	25.00	451	18.04	942.5
I	P28/66	1561.0	65.0	21.00	228	10.86	791.7
	P28/67	1585.0	66.0	24.00	71	2.96	792
	P28/68	1609.0	67.0	24.00	177	7.38	833
I	P28/69	1633.0	68.0	24.00	230	9.58	866
J	P28/70	1657.0	69.0	24.00	154.5	6.44	912
	P28/71	1684.0	70.1	27.00	217.5	8.06	1055
J	P28/72	1713.5	71.4	29.50	400	13.56	1172
K	P28/73	1732.7	72.2	19.25	40	2.08	702
	P28/74	1753.0	73.0	20.25	76	3.75	680
	P28/75	1777.0	74.0	24.00	218	9.08	806.4
	P28/76	1801.0	75.0	24.00	328	13.67	878
	P28/77	1825.0	76.0	24.00	328	13.67	950
	P28/78	1849.0	77.0	24.00	447.5	18.65	986
	P28/79	1872.0	78.0	23.00	266.5	11.59	952
K	P28/80	1897.0	79.0	25.00	295	11.80	1064
L	P28/81	1920.0	80.0	23.00	200	8.70	1015
	P28/82	1945.0	81.0	25.00	260	10.40	1101
	P28/83	1969.0	82.0	24.00	403	16.79	1067
	P28/84	1993.0	83.0	24.00	243	10.13	1056
	P28/85	2019.0	84.1	26.00	280	10.77	1139
	P28/86	2045.0	85.2	26.00	332	12.77	960
	P28/87	2065.0	86.0	20.00	280	14.00	592
	P28/88	2089.0	87.0	24.00	431	17.96	696
L	P28/89	2113.0	88.0	24.00	317.6	13.23	703

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Feed rate used (mL/h)	Perfusion Rate (L/L-d)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	Temperature (C)	pH
F	P28/44	16.57	0.66	2.5	12.6	3.0	33.5	7.20
	P28/45	31.50	1.26	7.3	13.3	2.8	33.5	7.23
	P28/46	22.92	0.92	5.5	14.2	2.7	33.4	7.25
	P28/47	24.00	0.96	7.0	15.3	3.0	33.3	7.24
	P28/48	20.30	0.81	5.4	18.8	3.2	32.9	7.25
	P28/49	24.00	0.96	4.3	16.6	2.9	33.1	7.24
F	P28/50	27.48	1.10	3.8	17.8	3.1	33.3	7.25
G	P28/51	30.07	1.20	1.8	20.0	2.9	37.3	7.13
	P28/52	32.78	1.31	2.5	16.4	2.8	37.3	7.23
	P28/53	32.67	1.31	4.1	14.6	2.9	37.2	7.15
	P28/54	33.00	1.32	3.3	22.7	3.0	37.3	7.17
	P28/55	36.95	1.48	4.1	21.0	3.1	37.3	7.13
	P28/56	36.88	1.48	4.4	21.3	3.1	36.5	7.11
	P28/57	38.89	1.56	4.3	14.5	3.3	37.4	7.08
	P28/58	38.57	1.54	4.3	20.7	3.4	37.4	7.10
G	P28/59	39.00	1.56	4.5	20.1	3.2	36.6	7.08
H	P28/60	40.00	1.60	5.7	18.4	3.7	36.8	7.03
	P28/61	37.96	1.52	5.7	17.4	3.6	37.4	7.00
	P28/62	34.70	1.39	5.0	22.4	3.3	37.3	7.02
	P28/63	38.00	1.52	5.5	20.4	3.4	37.1	7.04
	P28/64	37.50	1.50	5.0	22.1	3.5	37.4	7.03
H	P28/65	37.70	1.51	5.1	18.0	3.4	36.6	7.05
I	P28/66	37.70	1.51	6.0	19.0	3.9	36.4	7.04
	P28/67	33.00	1.32	4.3	27.9	3.5	36.5	6.97
	P28/68	34.71	1.39	3.9	27.7	4.0	37.2	6.96
I	P28/69	36.08	1.44	3.5	27.7	3.5	36.9	6.98
J	P28/70	38.00	1.52	4.2	26.3	3.6	37.0	6.98
	P28/71	39.07	1.56	3.3	12.8	3.7	37.2	6.92
J	P28/72	39.73	1.59	8.2	11.3	4.9	36.4	7.23
K	P28/73	36.47	1.46	6.9	20.0	4.1	36.5	7.05
	P28/74	33.58	1.34	5.0	19.1	3.6	36.5	7.04
	P28/75	33.60	1.34	3.4	22.6	3.3	36.8	7.00
	P28/76	36.58	1.46	3.4	22.2	3.2	36.5	7.01
	P28/77	39.58	1.58	3.9	21.5	3.7	36.5	7.00
	P28/78	41.08	1.64	4.6	21.1	3.6	36.6	7.00
	P28/79	41.39	1.66	4.4	22.4	3.5	36.6	7.00
K	P28/80	42.56	1.70	3.9	22.3	3.4	36.4	6.99
L	P28/81	44.13	1.77	4.1	22.8	3.7	36.5	6.98
	P28/82	44.04	1.76	4.6	19.3	3.5	36.5	6.99
	P28/83	44.46	1.78	5.6	19.6	3.4	36.9	6.99
	P28/84	44.00	1.76	5.2	17.8	3.3	36.7	6.99
	P28/85	43.81	1.75	6.3	24.8	3.9	36.7	7.03
	P28/86	36.92	1.48	7.9	17.9	3.7	37.1	7.05
	P28/87	29.60	1.18	5.4	23.5	3.5	37.2	7.02
	P28/88	29.00	1.16	4.7	23.2	3.4	37.4	7.05
L	P28/89	29.29	1.17	4.1	22.4	3.2	37.5	7.05

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	DO (% air sat)	Viab w/o Tryp (%)	comment	Total Xn (cells/mL)	Viab w/ tryp (%)
F	P28/44	70.2			1.1E+07	96.9
	P28/45	76.2			9.8E+06	98.5
	P28/46	73.2			1.2E+07	98.0
	P28/47	69.5	96		7.5E+06	97.2
	P28/48	70.0			1.0E+07	96.2
	P28/49	68.5			1.0E+07	96.4
F	P28/50	67.0		RNA + Sial Sample	1.0E+07	93.7
G	P28/51	37.3			1.5E+07	98.0
	P28/52	73.3			1.1E+07	98.1
	P28/53	77.3			1.2E+07	96.6
	P28/54	66.4	95		1.0E+07	98.3
	P28/55	71.5			1.2E+07	96.7
	P28/56	66.9			9.1E+06	98.4
	P28/57	70.7			1.2E+07	95.2
	P28/58	61.0			1.1E+07	93.3
G	P28/59	64.1			1.0E+07	97.7
H	P28/60	23.7	87		1.2E+07	95.1
	P28/61	17.4			9.3E+06	97.7
	P28/62	12.9			1.0E+07	97.7
	P28/63	18.6			9.8E+06	94.2
	P28/64	15.3			1.2E+07	94.5
H	P28/65	15.0	89		9.8E+06	94.5
I	P28/66	17.7	83		8.1E+06	95.5
	P28/67	9.7	89	pH probe read 6.93	9.6E+06	96.3
	P28/68	4.6	87		1.1E+07	95.2
I	P28/69	5.9	84.5		1.1E+07	95.4
J	P28/70	11.8	84		1.0E+07	92.6
	P28/71	5.5	87		1.4E+07	95.1
J	P28/72	18.1	70		5.3E+06	86.4
K	P28/73	68.6	80		7.2E+06	90.8
	P28/74	49.2	79		9.3E+06	93.5
	P28/75	72.0	85		1.1E+07	94.3
	P28/76	69.3	88		1.1E+07	97.2
	P28/77	70.4	90		1.2E+07	95.5
	P28/78	71.8	86		1.0E+07	96.4
	P28/79	72.4	87		1.0E+07	97.7
K	P28/80	72.5	91		1.1E+07	95.3
L	P28/81	75.2	88	actually at 10 am; back calc from GUR	1.3E+07	96.1
	P28/82	69.4	90	SE experiments	1.4E+07	93.9
	P28/83	75.2	90		1.3E+07	94.1
	P28/84	70.0	91.5	big sample sialylase	1.7E+07	95.5
	P28/85	72.0	91		1.6E+07	95.2
	P28/86	80.0	91		1.1E+07	94.0
	P28/87	74.0	90		1.2E+07	96.7
	P28/88	74.3	87		1.1E+07	95.2
L	P28/89	76.2	86.5		1.1E+07	95.2

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Xn viable (cells/mL)	Xv log mean (cells/mL)	GUR (mmol/h)	CSGUR (pmol/cell-d)	LPR (mmol/h)	CSLPR (pmol/cell-d)	APR (mmol/h)	CSAPR (pmol/cell-d)
F	P28/44	1.0E+07	9.76E+06	0.413	1.69	0.117	0.48	-0.026	-0.11
	P28/45	9.7E+06	9.93E+06	0.489	1.97	0.387	1.56	-0.066	-0.26
	P28/46	1.2E+07	1.06E+07	0.471	1.78	0.293	1.11	-0.046	-0.17
	P28/47	7.3E+06	9.24E+06	0.412	1.78	0.327	1.41	-0.063	-0.27
	P28/48	9.9E+06	8.53E+06	0.422	1.98	0.259	1.21	-0.057	-0.27
	P28/49	9.7E+06	9.81E+06	0.511	2.08	0.480	1.96	-0.051	-0.21
F	P28/50	9.4E+06	9.57E+06	0.587	2.45	0.446	1.86	-0.072	-0.30
G	P28/51	1.5E+07	1.20E+07	0.712	2.37	0.520	1.73	-0.068	-0.23
	P28/52	1.1E+07	1.29E+07	0.726	2.25	0.717	2.22	-0.070	-0.22
	P28/53	1.1E+07	1.11E+07	0.673	2.42	0.546	1.97	-0.076	-0.27
	P28/54	1.0E+07	1.07E+07	0.723	2.71	0.413	1.55	-0.081	-0.30
	P28/55	1.1E+07	1.08E+07	0.764	2.84	0.856	3.18	-0.095	-0.35
	P28/56	9.0E+06	1.02E+07	0.758	2.98	0.772	3.04	-0.093	-0.37
	P28/57	1.1E+07	1.01E+07	0.805	3.18	0.847	3.34	-0.108	-0.43
	P28/58	1.1E+07	1.09E+07	0.798	2.92	0.555	2.03	-0.111	-0.41
G	P28/59	1.0E+07	1.03E+07	0.795	3.08	0.820	3.17	-0.099	-0.38
H	P28/60	1.1E+07	1.06E+07	0.766	2.88	0.813	3.05	-0.132	-0.50
	P28/61	9.1E+06	1.01E+07	0.733	2.91	0.701	2.79	-0.118	-0.47
	P28/62	1.0E+07	9.62E+06	0.703	2.92	0.541	2.25	-0.092	-0.38
	P28/63	9.2E+06	9.70E+06	0.738	3.04	0.863	3.56	-0.110	-0.45
	P28/64	1.1E+07	1.03E+07	0.752	2.93	0.758	2.95	-0.113	-0.44
H	P28/65	9.3E+06	1.03E+07	0.750	2.91	0.854	3.31	-0.108	-0.42
I	P28/66	7.7E+06	8.48E+06	0.708	3.34	0.669	3.16	-0.136	-0.64
	P28/67	9.2E+06	8.45E+06	0.698	3.30	0.551	2.61	-0.095	-0.45
	P28/68	1.0E+07	9.65E+06	0.735	3.05	0.970	4.02	-0.128	-0.53
I	P28/69	1.1E+07	1.03E+07	0.779	3.03	1.000	3.89	-0.104	-0.40
J	P28/70	9.4E+06	9.93E+06	0.786	3.17	1.061	4.27	-0.119	-0.48
	P28/71	1.4E+07	1.13E+07	0.850	3.00	1.064	3.76	-0.126	-0.45
J	P28/72	4.6E+06	8.24E+06	0.665	3.23	0.509	2.47	-0.184	-0.89
K	P28/73	6.5E+06	5.48E+06	0.677	4.94	0.300	2.19	-0.122	-0.89
	P28/74	8.7E+06	7.54E+06	0.696	3.69	0.683	3.62	-0.097	-0.51
	P28/75	1.0E+07	9.51E+06	0.739	3.11	0.613	2.58	-0.090	-0.38
	P28/76	1.0E+07	1.04E+07	0.790	3.03	0.829	3.18	-0.096	-0.37
	P28/77	1.2E+07	1.10E+07	0.833	3.03	0.882	3.21	-0.131	-0.48
	P28/78	9.7E+06	1.06E+07	0.835	3.15	0.885	3.34	-0.127	-0.48
	P28/79	1.0E+07	9.93E+06	0.854	3.44	0.866	3.49	-0.123	-0.50
K	P28/80	1.1E+07	1.05E+07	0.899	3.41	0.954	3.62	-0.122	-0.46
L	P28/81	1.3E+07	1.18E+07	0.922	3.13	0.982	3.34	-0.144	-0.49
	P28/82	1.3E+07	1.28E+07	0.897	2.81	1.011	3.16	-0.131	-0.41
	P28/83	1.2E+07	1.27E+07	0.860	2.71	0.857	2.70	-0.128	-0.40
	P28/84	1.6E+07	1.42E+07	0.872	2.46	0.868	2.44	-0.121	-0.34
	P28/85	1.5E+07	1.55E+07	0.818	2.11	0.772	1.99	-0.152	-0.39
	P28/86	9.9E+06	1.23E+07	0.624	2.03	0.948	3.09	-0.118	-0.38
	P28/87	1.1E+07	1.06E+07	0.618	2.34	0.445	1.68	-0.085	-0.32
	P28/88	1.0E+07	1.06E+07	0.596	2.24	0.685	2.57	-0.082	-0.31
L	P28/89	1.0E+07	1.00E+07	0.618	2.46	0.688	2.74	-0.075	-0.30

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	μ bleed (1/h)	μ apparent (1/h)	μ real (1/h)	tPA (U/mL)	[t-PA] (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
F	P28/44	2.5E-03	4.25E-03	6.71E-03					
	P28/45	1.4E-02	-2.72E-03	1.10E-02	218000	375.86	7.72	308.96	31.12
	P28/46	1.8E-02	7.34E-03	2.49E-02	191000	329.31	6.92	276.65	26.19
	P28/47	1.9E-02	-1.91E-02	-3.16E-04	169000	291.38	6.50	260.00	28.13
	P28/48	5.5E-03	1.28E-02	1.83E-02	127000	218.97	3.37	134.79	15.80
	P28/49	1.5E-02	-7.65E-04	1.44E-02	120000	206.90	4.81	192.34	19.61
F	P28/50	2.0E-02	-1.16E-03	1.87E-02	106000	182.76	4.82	192.71	20.14
G	P28/51	6.9E-03	1.72E-02	2.41E-02	128000	220.69	6.91	276.38	23.04
	P28/52	2.3E-02	-1.73E-02	5.72E-03	107000	184.48	5.43	217.34	16.87
	P28/53	2.3E-02	9.00E-04	2.35E-02	93537	161.27	5.13	205.26	18.47
	P28/54	2.4E-02	-4.45E-03	1.94E-02	78061	134.59	4.21	168.58	15.80
	P28/55	2.8E-02	5.97E-03	3.43E-02	70842	122.14	4.39	175.51	16.29
	P28/56	2.4E-02	-1.02E-02	1.42E-02	77862	134.24	5.03	201.19	19.79
	P28/57	1.9E-02	8.88E-03	2.81E-02	46959	80.96	3.00	120.02	11.84
	P28/58	2.4E-02	-2.74E-03	2.14E-02	47221	81.42	3.14	125.61	11.48
G	P28/59	2.5E-02	-2.13E-03	2.29E-02	47488	81.88	3.20	128.10	12.40
H	P28/60	2.3E-02	3.76E-03	2.72E-02	53699	92.58	3.76	150.28	14.12
	P28/61	2.9E-02	-7.35E-03	2.15E-02	44817	77.27	2.90	115.84	11.52
	P28/62	2.2E-02	5.93E-03	2.82E-02	49585	85.49	3.07	122.82	12.77
	P28/63	2.4E-02	-4.25E-03	1.93E-02	30052	51.81	1.77	70.67	7.29
	P28/64	2.5E-02	8.20E-03	3.31E-02	31202	53.80	2.03	81.04	7.89
H	P28/65	3.0E-02	-8.14E-03	2.19E-02	48475	83.58	3.30	132.17	12.81
I	P28/66	1.8E-02	-8.93E-03	9.17E-03	44478	76.69	2.82	112.96	13.32
	P28/67	4.9E-03	7.54E-03	1.25E-02	56056	96.65	3.36	134.36	15.90
	P28/68	1.2E-02	3.63E-03	1.59E-02	58705	101.22	3.55	141.92	14.70
I	P28/69	1.6E-02	1.70E-03	1.77E-02	59715	102.96	3.73	149.09	14.49
J	P28/70	1.1E-02	-4.66E-03	6.07E-03	54216	93.48	3.50	139.81	14.07
	P28/71	1.3E-02	1.34E-02	2.69E-02	57699	99.48	3.90	156.13	13.79
J	P28/72	2.3E-02	-3.68E-02	-1.42E-02	48010	82.78	3.28	131.23	15.93
K	P28/73	3.5E-03	1.85E-02	2.20E-02	39957	68.89	2.33	93.31	17.04
	P28/74	6.3E-03	1.41E-02	2.04E-02	49260	84.93	3.06	122.32	16.23
	P28/75	1.5E-02	7.62E-03	2.28E-02	56816	97.96	3.40	135.93	14.29
	P28/76	2.3E-02	2.39E-04	2.30E-02	52470	90.47	3.26	130.37	12.49
	P28/77	2.3E-02	3.98E-03	2.68E-02	42004	72.42	2.77	110.91	10.09
	P28/78	3.1E-02	-7.08E-03	2.40E-02	58157	100.27	4.24	169.74	16.02
	P28/79	1.9E-02	1.84E-03	2.12E-02	50107	86.39	3.50	140.04	14.10
K	P28/80	2.0E-02	3.07E-03	2.27E-02	52171	89.95	3.84	153.52	14.57
L	P28/81	1.4E-02	6.21E-03	2.07E-02	33086	57.04	2.39	95.40	8.11
	P28/82	1.7E-02	9.39E-04	1.83E-02	31475	54.27	2.38	95.38	7.46
	P28/83	2.8E-02	-1.48E-03	2.65E-02	26395	45.51	2.00	79.96	6.29
	P28/84	1.7E-02	1.06E-02	2.74E-02	37611	64.85	2.91	116.45	8.20
	P28/85	1.8E-02	-2.67E-03	1.53E-02	64548	111.29	4.93	197.19	12.69
	P28/86	2.1E-02	-1.60E-02	5.30E-03	36988	63.77	2.14	85.41	6.96
	P28/87	2.3E-02	6.52E-03	2.99E-02	33212	57.26	1.60	63.84	6.04
	P28/88	3.0E-02	-4.94E-03	2.50E-02	39448	68.01	2.09	83.41	7.84
L	P28/89	2.2E-02	2.49E-04	2.23E-02	36112	62.26	1.76	70.57	7.02

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	RunTime (h)	RunTime (d)	DeltaTime (h)	total Bleed (mL)	total bleed rate (mL/h)	total Feed (mL)
M	P28/90	2137.0	89.0	24.00	222.5	9.27	741
	P28/91	2161.0	90.0	24.00	204	8.50	405
	P28/92	2187.0	91.1	26.00	152	5.85	210
	P28/93	2217.0	92.4	30.00	200	6.67	450
	P28/94	2233.0	93.0	16.00	20	1.25	210
	P28/95	2257.0	94.0	24.00	188	7.83	312
	P28/96	2281.0	95.0	24.00	20	0.83	350
	P28/97	2308.0	96.1	27.00	20	0.74	431
M	P28/98	2330.0	97.1	22.00	236	10.73	461
N	P28/99	2353.0	98.0	23.00	135	5.87	408
	P28/100	2379.0	99.1	26.00	20	0.77	345
	P28/101	2408.0	100.3	29.00	20	0.69	351
	P28/102	2433.0	101.4	25.00	20	0.80	347
	P28/103	2457.0	102.4	24.00	20	0.83	399.5
	P28/104	2481.0	103.4	24.00	20	0.83	463
	P28/105	2505.0	104.4	24.00	291	12.13	506
	P28/106	2521.0	105.0	16.00	20	1.25	214.5
	P28/107	2545.0	106.0	24.00	164	6.83	345
	P28/108	2577.0	107.4	32.00	92.5	2.89	499
N	P28/109	2593.0	108.0	16.00	185	11.56	327
O	P28/110	2616.0	109.0	23.00	100	4.35	476
	P28/111	2641.0	110.0	25.00	20	0.80	128
	P28/112	2665.0	111.0	24.00	68	2.83	126
	P28/113	2689.0	112.0	24.00	20	0.83	95
	P28/114	2715.0	113.1	26.00	20	0.77	156
	P28/115	2737.0	114.0	22.00	20	0.91	159.6
	P28/116	2761.0	115.0	24.00	20	0.83	132
	P28/117	2785.0	116.0	24.00	20	0.83	144
	P28/118	2809.0	117.0	24.00	20	0.83	121
O	P28/119	2833.0	118.0	24.00	20	0.83	64

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Feed rate used (mL/h)	Perfusion Rate (L/L-d)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	Temperature (C)	pH
M	P28/90	30.88	1.24	8.9	11.7	3.9	37.0	6.87
	P28/91	16.88	0.68	9.3	9.1	4.2	36.5	6.91
	P28/92	8.08	0.32	5.1	7.7	4.8	36.5	6.85
	P28/93	15.00	0.60	5.7	5.0	5.2	37.3	6.88
	P28/94	13.13	0.53	5.5	5.0	5.8	37.4	6.90
	P28/95	13.00	0.52	4.4	5.1	5.8	36.8	6.83
	P28/96	14.58	0.58	3.7	5.5	5.1	36.7	6.86
	P28/97	15.96	0.64	3.5	5.4	5.9	36.5	6.88
M	P28/98	20.95	0.84	5.3	5.6	5.9	37.1	6.86
N	P28/99	17.74	0.71	6.7	5.9	5.6	36.4	6.88
	P28/100	13.27	0.53	5.5	5.7	5.3	36.9	6.88
	P28/101	12.10	0.48	4.1	5.6	5.5	37.5	6.85
	P28/102	13.88	0.56	3.2	5.4	5.4	37.0	6.85
	P28/103	16.65	0.67	3.7	5.6	5.5	36.9	6.85
	P28/104	19.29	0.77	3.9	6.1	5.3	37.4	6.85
	P28/105	21.08	0.84	6.6	6.7	5.0	37.3	6.84
	P28/106	13.41	0.54	5.3	7.0	5.4	36.8	6.85
	P28/107	14.38	0.58	4.2	6.9	5.4	36.6	6.87
	P28/108	15.59	0.62	3.4	6.2	5.3	37.0	6.86
N	P28/109	20.44	0.82	4.3	6.5	5.5	36.6	6.83
O	P28/110	20.70	0.83	10.6	3.5	6.2	37.5	6.64
	P28/111	5.12	0.20	7.6	2.8	7.4	37.4	6.67
	P28/112	5.25	0.21	5.7	2.2	8.2	37.0	6.68
	P28/113	3.96	0.16	3.9	1.7	9.0	37.3	6.69
	P28/114	6.00	0.24	4.4	1.2	9.7	37.2	6.68
	P28/115	7.25	0.29	5.6	1.0	9.0	36.7	6.68
	P28/116	5.50	0.22	6.3	1.1	9.7	36.9	6.68
	P28/117	6.00	0.24	7.3	0.9	9.6	36.8	6.67
	P28/118	5.04	0.20	7.5	1.1	10.0	35.8	6.67
O	P28/119	2.67	0.11	6.3	1.0	10.9	35.5	6.67

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	DO (% air sat)	Viab w/o Tryp (%)	comment	Total Xn (cells/mL)	Viab w/ tryp (%)
M	P28/90	69.5	85		9.9E+06	94.0
	P28/91	72.6	83		9.2E+06	93.8
	P28/92	71.6	87		9.5E+06	94.3
	P28/93	70.8	82		7.0E+06	88.5
	P28/94	70.7	79		1.1E+07	88.0
	P28/95	72.4	70		7.3E+06	88.9
	P28/96	71.2	75		7.7E+06	85.3
	P28/97	70.4	78		1.3E+07	82.7
M	P28/98	71.2	84		9.0E+06	90.6
N	P28/99	74.1	79		8.9E+06	84.2
	P28/100	74.3	84		8.9E+06	84.1
	P28/101	73.6	79		8.3E+06	92.8
	P28/102	71.1	76		9.5E+06	88.3
	P28/103	73.4	80		9.7E+06	87.7
	P28/104	58.1	78		1.3E+07	89.5
	P28/105	76.7	78		9.0E+06	89.3
	P28/106	72.7	83		1.1E+07	90.9
	P28/107	72.9	79		9.2E+06	89.6
	P28/108	72.5	77		1.3E+07	89.4
N	P28/109	71.5	67		1.3E+07	89.3
O	P28/110	70.7	76		8.1E+06	84.5
	P28/111	68.4	56		9.5E+06	75.5
	P28/112	75.6	66		7.5E+06	81.1
	P28/113	74.4	42		6.9E+06	65.9
	P28/114	72.9	49		7.4E+06	60.6
	P28/115	75.8	43		1.0E+07	63.0
	P28/116	74.8	36		7.0E+06	58.1
	P28/117	73.3	38		6.5E+06	58.5
	P28/118	75.0	35		7.1E+06	54.7
O	P28/119	76.3	25	end of cultivation	5.3E+06	52.6

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	Xn viable (cells/mL)	Xv log mean (cells/mL)	GUR (mmol/h)	CSGUR (pmol/cell-d)	LPR (mmol/h)	CSLPR (pmol/cell-d)	APR (mmol/h)	CSAPR (pmol/cell-d)
M	P28/90	9.3E+06	9.70E+06	0.451	1.86	0.794	3.27	-0.114	-0.47
	P28/91	8.6E+06	8.98E+06	0.258	1.15	0.241	1.07	-0.070	-0.31
	P28/92	9.0E+06	8.82E+06	0.241	1.09	0.100	0.45	-0.049	-0.22
	P28/93	6.2E+06	7.52E+06	0.282	1.50	0.149	0.79	-0.080	-0.43
	P28/94	9.4E+06	7.70E+06	0.262	1.36	0.066	0.34	-0.095	-0.50
	P28/95	6.5E+06	7.86E+06	0.288	1.47	0.063	0.32	-0.073	-0.37
	P28/96	6.6E+06	6.54E+06	0.323	1.98	0.067	0.41	-0.056	-0.34
	P28/97	1.0E+07	8.37E+06	0.346	1.65	0.089	0.43	-0.105	-0.50
M	P28/98	8.1E+06	9.24E+06	0.383	1.66	0.110	0.48	-0.121	-0.52
N	P28/99	7.5E+06	7.81E+06	0.301	1.54	0.094	0.48	-0.090	-0.46
	P28/100	7.5E+06	7.48E+06	0.278	1.49	0.082	0.44	-0.062	-0.33
	P28/101	7.7E+06	7.57E+06	0.273	1.44	0.070	0.37	-0.067	-0.36
	P28/102	8.4E+06	8.03E+06	0.318	1.58	0.081	0.40	-0.070	-0.35
	P28/103	8.5E+06	8.46E+06	0.346	1.64	0.087	0.41	-0.090	-0.43
	P28/104	1.1E+07	9.92E+06	0.404	1.63	0.100	0.40	-0.094	-0.38
	P28/105	8.0E+06	9.65E+06	0.349	1.45	0.120	0.50	-0.095	-0.39
	P28/106	9.6E+06	8.78E+06	0.304	1.39	0.081	0.37	-0.084	-0.38
	P28/107	8.3E+06	8.89E+06	0.319	1.43	0.102	0.46	-0.075	-0.34
	P28/108	1.2E+07	9.95E+06	0.346	1.39	0.115	0.46	-0.078	-0.31
N	P28/109	1.1E+07	1.15E+07	0.399	1.38	0.119	0.41	-0.115	-0.40
O	P28/110	6.9E+06	8.87E+06	0.199	0.90	0.182	0.82	-0.139	-0.63
	P28/111	7.2E+06	7.03E+06	0.153	0.87	0.033	0.19	-0.068	-0.39
	P28/112	6.1E+06	6.61E+06	0.144	0.87	0.028	0.17	-0.065	-0.39
	P28/113	4.5E+06	5.26E+06	0.125	0.95	0.020	0.15	-0.058	-0.44
	P28/114	4.5E+06	4.50E+06	0.114	1.01	0.020	0.18	-0.077	-0.68
	P28/115	6.5E+06	5.44E+06	0.112	0.83	0.013	0.10	-0.049	-0.36
	P28/116	4.1E+06	5.21E+06	0.087	0.67	0.003	0.03	-0.074	-0.57
	P28/117	3.8E+06	3.94E+06	0.084	0.85	0.011	0.11	-0.058	-0.58
	P28/118	3.9E+06	3.84E+06	0.084	0.87	0.000	0.00	-0.063	-0.66
O	P28/119	2.8E+06	3.28E+06	0.078	0.95	0.005	0.06	-0.055	-0.67

Table A. 6 Data from Pseudo Steady State Perfusion Run P28. (continued)

Cond Set	Sample No	μ bleed (1/h)	μ apparent (1/h)	μ real (1/h)	tPA (U/mL)	[t-PA] (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
M	P28/90	1.5E-02	-3.22E-03	1.22E-02	32275	55.65	1.65	66.19	6.82
	P28/91	1.4E-02	-3.20E-03	1.10E-02					
	P28/92	9.7E-03	1.57E-03	1.13E-02	68315	117.78	1.83	73.10	8.29
	P28/93	1.1E-02	-1.24E-02	-1.26E-03	65533	112.99	1.63	65.39	8.70
	P28/94	2.1E-03	2.60E-02	2.81E-02	60319	104.00	1.09	43.47	5.64
	P28/95	1.3E-02	-1.56E-02	-2.53E-03	59852	103.19	1.33	53.06	6.75
	P28/96	1.4E-03	7.65E-04	2.15E-03	51352	88.54	1.03	41.27	6.31
	P28/97	1.2E-03	1.70E-02	1.82E-02	41659	71.83	0.91	36.34	4.34
M	P28/98	1.8E-02	-1.14E-02	6.51E-03					
N	P28/99	9.8E-03	-3.51E-03	6.28E-03	55752	96.12	1.95	77.95	9.98
	P28/100	1.3E-03	-1.54E-04	1.13E-03	54531	94.02	1.21	48.52	6.48
	P28/101	1.1E-03	9.56E-04	2.11E-03					
	P28/102	1.3E-03	3.58E-03	4.92E-03	58085	100.15	1.33	53.17	6.62
	P28/103	1.4E-03	5.91E-04	1.98E-03					
	P28/104	1.4E-03	1.24E-02	1.37E-02	52914	91.23	1.61	64.32	6.49
	P28/105	2.0E-02	-1.48E-02	5.44E-03	50596	87.23	1.78	71.26	7.38
	P28/106	2.1E-03	1.09E-02	1.30E-02	55933	96.44	1.58	63.05	7.18
	P28/107	1.1E-02	-6.18E-03	5.20E-03	50064	86.32	1.06	42.42	4.77
	P28/108	4.8E-03	1.14E-02	1.62E-02					
N	P28/109	1.9E-02	-3.57E-03	1.57E-02	52914	91.23	1.66	66.43	5.75
O	P28/110	7.2E-03	-2.13E-02	-1.41E-02	44951	77.50	1.39	55.51	6.26
	P28/111	1.3E-03	1.88E-03	3.21E-03	69018	119.00	1.50	59.96	8.52
	P28/112	4.7E-03	-7.18E-03	-2.46E-03	68418	117.96	0.60	23.85	3.61
	P28/113	1.4E-03	-1.21E-02	-1.07E-02	79860	137.69	1.00	39.97	7.60
	P28/114	1.3E-03	-5.13E-04	7.69E-04	82945	143.01	0.96	38.59	8.58
	P28/115	1.5E-03	1.73E-02	1.88E-02	75294	129.82	0.63	25.19	4.63
	P28/116	1.4E-03	-1.97E-02	-1.83E-02	75385	129.97	0.72	28.73	5.51
	P28/117	1.4E-03	-2.85E-03	-1.46E-03	71959	124.07	0.61	24.58	6.23
	P28/118	1.4E-03	6.51E-04	2.04E-03	68418	117.96	0.46	18.30	4.77
O	P28/119	1.4E-03	-1.41E-02	-1.27E-02	71311	122.95	0.45	17.84	5.43

Table A. 7 Data from Transient Perfusion Run P32.

Cond Set	Sample No	RunTime (h)	RunTime (d)	DeltaTime (h)	total Bleed (mL)	total bleed rate (mL/h)	total Feed (mL)
	P32/Inoc	0.0	0.0	0.00	0	#DIV/0!	0
	P32/0	-0.5	0.0	-0.50	0	0.00	0
	P32/1	0.3	0.0	0.83	20	24.00	0
	P32/2	19.0	0.8	18.67	25	1.34	0.00
	P32/3	43.0	1.8	24.00	15	0.63	0.00
	P32/4	63.0	2.6	20.00	30	1.50	0.00
	P32/5	88.5	3.7	25.50	30	1.18	215.00
	P32/6	111.5	4.6	23.00	30	1.30	184.00
	P32/7	136.5	5.7	25.00	20	0.80	390.00
	P32/8	158.5	6.6	22.00	30	1.36	410.10
	P32/9	183.5	7.6	25.00	20	0.80	500.00
	P32/10	212.5	8.9	29.00	30	1.03	646.20
	P32/11	233.5	9.7	21.00	20	0.95	578.50
	P32/12	258.5	10.8	25.00	30	1.20	881.00
	P32/13	282.5	11.8	25.00	150	6.00	734.00
	P32/14	304.5	12.7	22.00	177	8.05	630.00
	P32/15	327.5	13.6	23.00	319	13.87	621.00
	P32/16	351.5	14.6	24.00	30	1.25	609.00
	P32/17	375.5	15.6	24.00	20	0.83	614.00
	P32/18	404.5	16.9	29.00	378	13.03	841.00
	P32/19	426.5	17.8	22.00	262	11.91	616.00
37sstate	P32/20	447.5	18.6	21.00	230	10.95	525.00
	P32/21	473.5	19.7	26.00	172	6.62	676.00
	P32/22	496.5	20.7	23.00	145	6.30	260.00
	P32/23	518.5	21.6	22.00	195	8.86	416.00
35trans	P32/24	543.5	22.6	25.00	190	7.60	475.00
	P32/25	567.5	23.6	24.00	172.7	7.20	350.00
	P32/26	592.5	24.7	25.00	40	1.60	286.00
33trans	P32/27	615.5	25.6	23.00	151	6.57	304.50
	P32/28	639.5	26.6	24.00	40	1.67	286.70
	P32/29	666.5	27.8	27.00	50	1.85	200.00
	P32/30	686.5	28.6	20.00	25	1.25	104.00
31trans	P32/31	711.5	29.6	25.00	15	0.60	167.50
	P32/32	737.5	30.7	26.00	19	0.73	201.00
	P32/33	766.5	31.9	29.00	24	0.83	188.50
	P32/34	784.5	32.7	18.00	8	0.44	90.00
	P32/35	807.5	33.6	23.00	9	0.39	123.50
31sstate	P32/36	831.5	34.6	24.00	11	0.46	133.50
	P32/37	857.5	35.7	26.00	9	0.35	110.00
	P32/38	877.5	36.6	20.00	9	0.45	82.50

Table A. 7 Data from Transient Perfusion Run P32. (continued)

Cond Set	Sample No	Feed rate used (mL/h)	Perfusion Rate (L/L-d)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	[NH4] (mM)
	P32/Inoc	#VALUE!	#VALUE!	16.7	10.8	3.5	2.8
	P32/0	0.00	0.00	24.9	0.4	2.4	2.0
	P32/1	0.00	0.00	22.3	3.8	2.6	2.1
	P32/2	0.00	0.00	21.2	6.5	2.9	2.3
	P32/3	0.00	0.00	16.1	13.4	3.8	3.0
	P32/4	0.00	0.00	9.6	22.1	4.4	3.4
	P32/5	8.43	0.31	7.8	24.6	4.2	3.3
	P32/6	8.00	0.30	4.5	27.5	4.3	3.3
	P32/7	15.60	0.58	6.3	23.3	4.4	3.4
	P32/8	18.64	0.69	6.6	21.0	4.4	3.4
	P32/9	20.00	0.74	6.5	19.7	3.6	2.8
	P32/10	22.28	0.82	4.2	19.8	3.6	2.8
	P32/11	27.55	1.02	6.5	21.2	3.4	2.7
	P32/12	36.71	1.36	8.2	16.8	3.5	2.8
	P32/13	29.36	1.08	5.6	19.8	3.3	2.6
	P32/14	28.64	1.06	4.8	17.5	3.3	2.6
	P32/15	27.00	1.00	6.6	17.1	3.7	2.9
	P32/16	25.38	0.94	6.1	17.8	3.6	2.8
	P32/17	25.58	0.94	5.2	17.5	3.6	2.8
	P32/18	29.00	1.07	5.3		3.6	2.8
	P32/19	28.00	1.03	6.0	18.1	3.7	2.9
37sstate	P32/20	25.00	0.92	5.3	16.9	3.3	2.6
	P32/21	26.00	0.96	8.0	15.2	3.5	2.8
	P32/22	11.30	0.42	4.1	15.8	3.2	2.5
	P32/23	18.91	0.70	4.7	13.6	3.5	2.8
35trans	P32/24	19.00	0.70	4.9	16.4	3.8	3.0
	P32/25	14.58	0.54	5.9	13.7	3.9	3.0
	P32/26	11.44	0.42	4.6	13.7	4.0	3.1
33trans	P32/27	13.24	0.49	4.8	13.2	3.7	2.9
	P32/28	11.95	0.44	6.0	12.1	4.0	3.1
	P32/29	7.41	0.27	5.8	9.8	4.3	3.3
	P32/30	5.20	0.19	5.1	9.7	4.7	3.6
31trans	P32/31	6.70	0.25	4.8	8.6	4.6	3.5
	P32/32	7.73	0.29	5.3	7.5	5.1	3.9
	P32/33	6.50	0.24	5.6	6.8	5.7	4.3
	P32/34	5.00	0.18	5.3	6.2	5.1	3.9
	P32/35	5.37	0.20	4.9	5.6	5.5	4.2
31sstate	P32/36	5.56	0.21	5.8	5.0	5.8	4.4
	P32/37	4.23	0.16	6.0	4.4	6.6	5.0
	P32/38	4.13	0.15	5.2	4.0	6.9	5.2

Table A. 7 Data from Transient Perfusion Run P32. (continued)

Cond Set	Sample No	Temperature (C)	pH	DO (% air sat)	Osmolality (mOsm/kg)	Viab w/o Tryp (%)
	P32/Inoc	37.0	7.38	120.3pO2	396	
	P32/0	36.7	7.23	110.0	400	
	P32/1	36.9	7.20	109.5	409	
	P32/2	36.3	7.25	84.5	401	
	P32/3	36.7	7.23	86.5	399	
	P32/4	36.7	7.15	105.2	394	93
	P32/5	36.7	7.09	92.4	385	95
	P32/6	36.7	6.89	107.0	391	96
	P32/7	36.7	7.04	101.0	386	94
	P32/8	36.5	7.03	101.5	388	95
	P32/9	36.6	7.05	97.0	380	96
	P32/10	36.8	7.05	96.7	392	95
	P32/11	36.9	7.03	96.1	375	95
	P32/12	36.7	7.05	100.5	370	87
	P32/13	36.6	7.04	98.7	361	93
	P32/14	36.7	7.05	99.7	353	89
	P32/15	36.6	7.05	105.1	368	92
	P32/16	36.7	7.05	102.6	359	89
	P32/17	36.6	7.03	98.3	356	91
	P32/18	36.7	7.05	105.7	397	90
	P32/19	36.5	7.05	111.5	362	93
37sstate	P32/20	36.5	7.04	104.0	359	92
	P32/21	35.1	7.05	102.4	362	96
	P32/22	35.0	7.05	104.2	370	95
	P32/23	34.9	7.05	109.1	365	94
35trans	P32/24	34.9	7.05	101.6	357	95
	P32/25	33.0	7.05	100.5	359	92
	P32/26	33.1	7.05	100.0	378	94
33trans	P32/27	33.0	7.08	104.2	370	94
	P32/28	31.1	7.04	101.5	361	93
	P32/29	31.1	7.05	104.7	358	94
	P32/30	31.1	7.05	103.0	365	89
31trans	P32/31	31.2	7.05	108.3	357	91
	P32/32	31.1	7.05	110.0	402	90
	P32/33	31.1	7.05	104.2	372	92
	P32/34	31.1	7.05	107.9	392	87
	P32/35	31.0	7.05	111.0	371	89
31sstate	P32/36	31.0	7.05	114.0	395	88
	P32/37	31.1	7.05	108.6	365	87
	P32/38	33.0	7.05	110.4	374	88

Table A. 7 Data from Transient Perfusion Run P32. (continued)

Cond Set	Sample No	comment	Total Xn (cells/mL)	Viab w/ tryp (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)
	P32/Inoc	inoculum nova	#DIV/0!		#DIV/0!	#DIV/0!
	P32/0	pre-inoc	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	P32/1	post-inoc	2.6E+05	88.0	2.3E+05	#DIV/0!
	P32/2		3.3E+05	91.1	3.0E+05	2.60E+05
	P32/3		1.2E+06	93.9	1.1E+06	6.19E+05
	P32/4	started perf evening	1.7E+06	97.8	1.6E+06	1.35E+06
	P32/5		2.0E+06	96.2	1.9E+06	1.75E+06
	P32/6		3.1E+06	97.6	3.0E+06	2.40E+06
	P32/7		4.4E+06	97.3	4.1E+06	3.54E+06
	P32/8		5.1E+06	97.8	4.8E+06	4.48E+06
	P32/9	cell count w/o T	6.0E+06	95.5	5.7E+06	5.25E+06
	P32/10		6.3E+06	95.0	6.0E+06	5.85E+06
	P32/11		8.7E+06	96.4	8.2E+06	7.08E+06
	P32/12	daylight savings add 1h	1.0E+07	96.5	8.8E+06	8.54E+06
	P32/13	much debris (noteb)	1.1E+07	98.1	1.0E+07	9.40E+06
	P32/14		1.5E+07	95.7	1.3E+07	1.14E+07
	P32/15		7.1E+06	94.5	6.6E+06	9.39E+06
	P32/16		8.7E+06	96.4	7.7E+06	7.12E+06
	P32/17		1.2E+07	90.9	1.1E+07	9.05E+06
	P32/18		1.0E+07	97.4	9.1E+06	9.80E+06
	P32/19		9.4E+06	97.9	8.7E+06	8.91E+06
37sstate	P32/20	RNA/sial Tchange35C at 5pm	9.2E+06	97.8	8.5E+06	8.61E+06
	P32/21		8.1E+06	96.7	7.8E+06	8.12E+06
	P32/22		1.3E+07	94.7	1.2E+07	9.71E+06
	P32/23		9.5E+06	98.1	8.9E+06	1.04E+07
35trans	P32/24	RNA Tchange33C at 11am	1.1E+07	95.8	1.0E+07	9.51E+06
	P32/25		8.0E+06	94.0	7.4E+06	8.69E+06
	P32/26		9.8E+06	96.2	9.2E+06	8.29E+06
33trans	P32/27	RNA Tchange31C at 6pm	7.4E+06	96.3	7.0E+06	8.05E+06
	P32/28		6.9E+06	93.5	6.4E+06	6.67E+06
	P32/29		7.3E+06	95.9	6.8E+06	6.59E+06
	P32/30		6.9E+06	93.5	6.2E+06	6.48E+06
31trans	P32/31	took RNA samp 12pm	6.3E+06	91.4	5.7E+06	5.93E+06
	P32/32	continue 31C sstate	6.3E+06	94.6	5.7E+06	5.69E+06
	P32/33		5.7E+06	95.0	5.2E+06	5.46E+06
	P32/34		5.8E+06	91.2	5.0E+06	5.13E+06
	P32/35		6.1E+06	92.4	5.5E+06	5.23E+06
31sstate	P32/36		5.1E+06	90.7	4.5E+06	4.94E+06
	P32/37	T to 33C 2pm** T upset see noteb	4.7E+06	88.8	4.1E+06	4.28E+06
	P32/38		4.9E+06	88.3	4.3E+06	4.21E+06

Table A. 7 Data from Transient Perfusion Run P32. (continued)

Cond Set	Sample No	GUR (mmol/h)	CSGUR (pmol/cell-d)	LPR (mmol/h)	CSLPR (pmol/cell-d)	APR (mmol/h)	CSAPR (pmol/cell-d)	μ bleed (1/h)
	P32/Inoc							
	P32/0		#DIV/0!		#DIV/0!		#DIV/0!	0.0E+00
	P32/1		#DIV/0!		#DIV/0!		#DIV/0!	3.7E-02
	P32/2	0.038	5.44	0.094	13.35	0.007	1.06	2.1E-03
	P32/3	0.138	8.24	0.187	11.15	0.017	1.04	9.6E-04
	P32/4	0.211	5.76	0.283	7.71	0.014	0.38	2.3E-03
	P32/5	0.183	3.86	0.133	2.80	0.032	0.67	1.8E-03
	P32/6	0.244	3.75	0.126	1.94	0.024	0.37	2.0E-03
	P32/7	0.259	2.70	0.505	5.28	0.051	0.53	1.2E-03
	P32/8	0.337	2.78	0.481	3.96	0.063	0.52	2.1E-03
	P32/9	0.372	2.61	0.441	3.10	0.077	0.54	1.2E-03
	P32/10	0.489	3.09	0.438	2.76	0.063	0.40	1.6E-03
	P32/11	0.470	2.45	0.521	2.72	0.080	0.42	1.5E-03
	P32/12	0.604	2.61	0.812	3.51	0.098	0.42	1.8E-03
	P32/13	0.599	2.35	0.459	1.80	0.082	0.32	9.2E-03
	P32/14	0.591	1.91	0.602	1.95	0.075	0.24	1.2E-02
	P32/15	0.470	1.85	0.478	1.88	0.066	0.26	2.1E-02
	P32/16	0.487	2.52	0.424	2.20	0.075	0.39	1.9E-03
	P32/17	0.519	2.12	0.460	1.87	0.072	0.29	1.3E-03
	P32/18	0.571	2.15			0.082	0.31	2.0E-02
	P32/19	0.521	2.16	0.515	2.13	0.078	0.32	1.8E-02
37sstate	P32/20	0.505	2.17	0.475	2.04	0.078	0.33	1.7E-02
	P32/21	0.410	1.86	0.460	2.09	0.066	0.30	1.0E-02
	P32/22	0.324	1.23	0.158	0.60	0.036	0.14	9.7E-03
	P32/23	0.372	1.32	0.343	1.22	0.044	0.16	1.4E-02
35trans	P32/24	0.379	1.47	0.212	0.82	0.049	0.19	1.2E-02
	P32/25	0.259	1.10	0.293	1.24	0.042	0.18	1.1E-02
	P32/26	0.260	1.16	0.157	0.70	0.033	0.15	2.5E-03
33trans	P32/27	0.263	1.21	0.192	0.88	0.046	0.21	1.0E-02
	P32/28	0.202	1.12	0.181	1.00	0.030	0.17	2.6E-03
	P32/29	0.147	0.83	0.136	0.77	0.019	0.10	2.8E-03
	P32/30	0.123	0.70	0.054	0.31	0.009	0.05	1.9E-03
31trans	P32/31	0.142	0.88	0.090	0.56	0.026	0.16	9.2E-04
	P32/32	0.142	0.92	0.090	0.58	0.020	0.13	1.1E-03
	P32/33	0.120	0.81	0.062	0.42	0.017	0.12	1.3E-03
	P32/34	0.109	0.78	0.054	0.39	0.036	0.26	6.8E-04
	P32/35	0.118	0.83	0.049	0.34	0.014	0.10	6.0E-04
31sstate	P32/36	0.085	0.63	0.046	0.34	0.018	0.13	7.1E-04
	P32/37	0.076	0.65	0.035	0.30	0.006	0.05	5.3E-04
	P32/38	0.106	0.93	0.030	0.27	0.014	0.12	6.9E-04

Table A. 7 Data from Transient Perfusion Run P32. (continued)

Cond Set	Sample No	μ apparent (1/h)	μ real (1/h)	tPA (U/mL)	[t-PA] (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	P32/Inoc		#DIV/0!					
	P32/0	#DIV/0!	#DIV/0!					
	P32/1	#DIV/0!	#DIV/0!					
	P32/2	1.43E-02	1.64E-02					
	P32/3	5.54E-02	5.63E-02					
	P32/4	1.85E-02	2.08E-02					
	P32/5	6.16E-03	7.97E-03	19808	34.15			
	P32/6	1.99E-02	2.19E-02					
	P32/7	1.29E-02	1.42E-02	23192	39.99	0.52	19.07	5.39
	P32/8	7.10E-03	9.20E-03					
	P32/9	6.45E-03	7.68E-03	22759	39.24	0.76	27.88	5.31
	P32/10	2.00E-03	3.59E-03					
	P32/11	1.49E-02	1.64E-02	29753	51.30	1.28	47.43	6.70
	P32/12	2.76E-03	4.61E-03	36730	63.33	2.42	89.23	10.45
	P32/13	4.90E-03	1.41E-02					
	P32/14	1.18E-02	2.42E-02	55488	95.67	2.75	101.63	8.91
	P32/15	-2.95E-02	-8.20E-03	39807	68.63	1.45	53.69	5.71
	P32/16	6.70E-03	8.63E-03					
	P32/17	1.31E-02	1.43E-02	64039	110.41	2.85	105.11	11.61
	P32/18	-5.14E-03	1.49E-02	76051	131.12	3.97	146.45	14.95
	P32/19	-1.79E-03	1.65E-02	68322	117.80	3.09	114.13	12.81
37sstate	P32/20	-1.38E-03	1.55E-02	35946	61.98	0.52	19.18	2.23
	P32/21	-3.38E-03	6.80E-03	56118.5	96.76	2.93	108.30	13.34
	P32/22	1.87E-02	2.84E-02	67479	116.34	1.76	64.91	6.68
	P32/23	-1.32E-02	4.73E-04	79464	137.01	3.01	110.98	10.69
35trans	P32/24	4.83E-03	1.65E-02	94782	163.42	3.54	130.73	13.75
	P32/25	-1.29E-02	-1.79E-03	99240	171.10	2.65	97.75	11.25
	P32/26	8.77E-03	1.12E-02	69039	119.03	0.31	11.29	1.36
33trans	P32/27	-1.22E-02	-2.10E-03	121727	209.87	4.74	175.18	21.76
	P32/28	-3.78E-03	-1.22E-03	99610	171.74	1.25	46.03	6.90
	P32/29	2.48E-03	5.33E-03	93583	161.35	0.98	36.31	5.51
	P32/30	-4.95E-03	-3.02E-03	118986	205.15	2.38	87.74	13.53
31trans	P32/31	-3.17E-03	-2.25E-03	124343	214.38	1.65	60.76	10.25
	P32/32	-1.19E-04	1.01E-03	114427	197.29	1.16	42.97	7.55
	P32/33	-2.81E-03	-1.54E-03	96339				
	P32/34	-2.34E-03	-1.66E-03	136834	235.92	1.78	65.71	12.82
	P32/35	3.61E-03	4.21E-03					
31sstate	P32/36	-8.31E-03	-7.61E-03	133483	230.14	1.19	44.08	8.92
	P32/37	-3.29E-03	-2.76E-03					
	P32/38	2.51E-03	3.20E-03					

Table A. 8 Data from Transient Perfusion Run P44.

Cond Set	Sample No	RunTime (h)	RunTime (d)	DeltaTime (h)	total Bleed (mL)	total bleed rate (mL/h)	total Feed (mL)
	P44/Inoc	0.0	0.0	0.00	0	#DIV/0!	0
	P44/0	-1.0	0.0	-1.00	0	0.00	0
	P44/1	0.5	0.0	1.50	10	6.67	0
	P44/2	20.5	0.9	20.00	15	0.75	0
	P44/3	46.7	1.9	26.25	15	0.57	0
	P44/4	64.5	2.7	17.75	30	1.69	149
	P44/5	85.5	3.6	21.00	15	0.71	450.7
	P44/6	110.5	4.6	25.00	30	1.20	777.5
	P44/7	133.5	5.6	23.00	20	0.87	911.00
	P44/8	156.5	6.5	23.00	629.1	27.35	779.00
	P44/9	180.5	7.5	24.00	20	0.83	241.50
	P44/10	208.5	8.7	28.00	234	8.36	765.00
	P44/11	229.5	9.6	21.00	20	0.95	442.00
	P44/12	254.5	10.6	25.00	37	1.48	525.00
	P44/13	277.5	11.6	23.00	490	21.30	570.00
	P44/14	303.5	12.6	26.00	30	1.15	600.00
	P44/15	324.5	13.5	21.00	180	8.57	441.00
	P44/16	348.5	14.5	24.00	30	1.25	478.00
	P44/17	377.5	15.7	29.00	115.5	3.98	815.00
	P44/18	396.5	16.5	19.00	180	9.47	453.90
	P44/19	421.5	17.6	25.00	190	7.60	674.00
37 st	P44/20	448.5	18.7	27.00	305	11.30	824.00
	P44/21	474.5	19.8	26.00	152	5.85	650.00
	P44/22	493.5	20.6	19.00	214	11.26	604.00
35t	P44/23	518.5	21.6	25.00	120	4.80	675.00
	P44/24	541.5	22.6	23.00	12	0.52	529.00
	P44/25	568.5	23.7	27.00	78	2.89	546.00
33t	P44/26	590.5	24.6	22.00	52	2.36	469.00
	P44/27	613.5	25.6	23.00	94.6	4.11	414.00
	P44/28	638.5	26.6	25.00	10	0.40	315.00
31t	P44/29	664.5	27.7	26.00	73	2.81	259.00
	P44/30	688.5	28.7	24.00	10	0.42	300.00
31s	P44/31	708.5	29.5	20.00	12	0.60	316.00
	P44/32	734.5	30.6	26.00	68	2.62	482.00
	P44/33	758.5	31.6	24.00	12	0.50	506.00
33t	P44/34	781.5	32.6	23.00	12	0.52	508.50
	P44/35	810.5	33.8	29.00	70	2.41	609.00
	P44/36	829.5	34.6	19.00	138	7.26	464.00
35t	P44/37	858.5	35.8	29.00	52	1.79	528.00
	P44/38	878.5	36.6	20.00	30	1.50	450.00

Table A. 8 Data from Transient Perfusion Run P44. (continued)

Cond Set	Sample No	Feed rate used (mL/h)	Perfusion Rate (L/L-d)	[Gluc] (mM)	[Lac] (mM)	[Bun] (mM)	[NH4] (mM)
	P44/Inoc	#VALUE!	#VALUE!	12.3	16.8	4.7	3.6
	P44/0	0.00	0.00	23.1	0.3	2.0	1.7
	P44/1	0.00	0.00	20.4	5.2	2.6	2.1
	P44/2	0.00	0.00	18.4	9.9	3.5	2.8
	P44/3	0.00	0.00	10.6	20.8	5.0	3.8
	P44/4	8.39	0.34	7.1	25.6	4.8	3.7
	P44/5	21.46	0.86	7.2	24.2	4.3	3.3
	P44/6	31.10	1.24	7.2	22.6	4.2	3.3
	P44/7	39.61	1.58	6.8	21.2	3.8	3.0
	P44/8	33.87	1.35	10.1	17.0	3.9	3.0
	P44/9	10.06	0.40	4.9	19.6	3.4	2.7
	P44/10	27.32	1.09	7.5	15.9	3.5	2.8
	P44/11	21.05	0.84	5.4	19.8	3.5	2.8
	P44/12	21.00	0.84	3.3	17.4	3.1	2.5
	P44/13	24.78	0.99	5.8	17.7	3.5	2.8
	P44/14	23.08	0.92	5.9	17.5	3.5	2.8
	P44/15	21.00	0.84	4.1	16.6	3.2	2.5
	P44/16	19.92	0.80	3.8	15.6	3.3	2.6
	P44/17	28.10	1.12	6.8	14.3	3.5	2.8
	P44/18	23.89	0.96	3.9	16.1	3.0	2.4
	P44/19	26.96	1.08	3.3	17.2	3.3	2.6
37 st	P44/20	30.52	1.22	5.6	15.9	3.4	2.7
	P44/21	25.00	1.00	1.2	19.2	2.7	2.2
	P44/22	31.79	1.27	5.6	15.0	3.2	2.5
35t	P44/23	27.00	1.08	5.7	14.3	3.4	2.7
	P44/24	23.00	0.92	6.2	10.7	3.6	2.8
	P44/25	20.22	0.81	4.7	9.2	3.5	2.8
33t	P44/26	21.32	0.85	5.0	7.7	3.8	3.0
	P44/27	18.00	0.72	7.2	4.6	4.1	3.2
	P44/28	12.60	0.50	5.9	3.0	5.1	3.9
31t	P44/29	9.96	0.40	3.5	2.0	6.1	4.6
	P44/30	12.50	0.50	3.5	2.4	5.9	4.5
31s	P44/31	15.80	0.63	4.1	2.4	5.6	4.3
	P44/32	18.54	0.74	3.7	3.1	4.6	3.5
	P44/33	21.08	0.84	4.4	2.9	4.7	3.6
33t	P44/34	22.11	0.88	5.8	2.5	5.0	3.8
	P44/35	21.00	0.84	4.0	2.7	5.4	4.1
	P44/36	24.42	0.98	3.9	3.1	4.8	3.7
35t	P44/37	18.21	0.73	19.2	6.4	3.5	2.8
	P44/38	22.50	0.90	10.3	5.8	4.1	3.2

Table A. 8 Data from Transient Perfusion Run P44. (continued)

Cond Set	Sample No	Temperature (C)	pH	DO (% air sat)	Osmolality (mOsm/kg)	Viab w/o Tryp (%)
	P44/Inoc	37.0	7.07	50.7		95
	P44/0	36.8	7.47	88.8	422	
	P44/1	36.9	7.46	83.4	449	89
	P44/2	36.8	7.26	78.7	469	94
	P44/3	36.8	7.25	76.3	383	98
	P44/4	36.7	7.11	80.2	416	93
	P44/5	36.8	7.13	77.1	392	97
	P44/6	36.7	7.06	77.5	380	93
	P44/7	36.9	7.09	62.6	389	90
	P44/8	36.9	7.10	88.2	403	94
	P44/9	36.7	7.05	65.0	381	94
	P44/10	36.6	7.10	74.5	414	95
	P44/11	36.7	7.08	70.0	388	94
	P44/12	36.7	7.09	66.0	374	98
	P44/13	36.7	7.10	73.4	388	94
	P44/14	36.8	7.10	72.4	383	96
	P44/15	36.8	7.10	73.3	372	93
	P44/16	35.0	7.10	50.7	422	91
	P44/17	36.7	7.10	59.8	399	94
	P44/18	36.9	7.10	79.5	371	95
	P44/19	36.7	7.10	69.2	403	98
37 st	P44/20	36.8	7.11	68.8	413	92
	P44/21	35.0	7.09	71.3	443	94
	P44/22	35.3	7.09	70.3	455	96
35t	P44/23	35.3	7.10	72.6	386	97
	P44/24	33.1	7.10	74.1	414	97
	P44/25	33.2	7.10	74.2	429	95
33t	P44/26	33.2	7.10	63.4	395	94
	P44/27	31.2	7.10	67.0	411	98
	P44/28	31.2	7.10	69.2	384	96
31t	P44/29	31.2	7.10	70.5	401	95
	P44/30	31.1	7.10	71.5	391	96
31s	P44/31	31.1	7.10	66.9	374	96
	P44/32	33.1	7.10	64.2	424	95
	P44/33	33.2	7.10	64.4	357	95
33t	P44/34	33.0	7.10	61.8	372	96
	P44/35	35.2	7.12	46.7	437	95
	P44/36	35.2	7.12	68.5	386	94
35t	P44/37	35.2	7.30	100.0		83
	P44/38	36.7	7.10	115.3		89

Table A. 8 Data from Transient Perfusion Run P44. (continued)

Cond Set	Sample No	comment	Total Xn (cells/mL)	Viab w/ tryp (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)
	P44/Inoc	inoculum nova	1.2E+06	95.0	1.2E+06	#VALUE !
	P44/0	pre-inoc	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	P44/1	post-inoc, rpm 100, pH sp 7.25, DO 70%	2.9E+05	95.5	2.6E+05	#DIV/0!
	P44/2		6.0E+05	98.4	5.6E+05	3.89E+05
	P44/3	started feed only 9pm, tech problems	1.3E+06	99.4	1.3E+06	8.80E+05
	P44/4	started perf 9am	2.3E+06	98.6	2.2E+06	1.71E+06
	P44/5		3.5E+06	96.2	3.5E+06	2.77E+06
	P44/6	DO dipped to 54 for 2h	8.3E+06	98.6	7.7E+06	5.30E+06
	P44/7	bleed did not work, open to air	1.7E+07	95.0	1.6E+07	1.12E+07
	P44/8	TOO MUCH BLEED!!!!	4.3E+06	89.5	4.0E+06	8.53E+06
	P44/9		7.8E+06	91.1	7.4E+06	5.54E+06
	P44/10		7.1E+06	87.8	6.7E+06	7.04E+06
	P44/11		8.0E+06	88.3	7.5E+06	7.11E+06
	P44/12		1.5E+07	95.5	1.5E+07	1.06E+07
	P44/13		7.6E+06	92.9	7.1E+06	1.04E+07
	P44/14		9.9E+06	91.5	9.6E+06	8.26E+06
	P44/15		8.9E+06	95.9	8.2E+06	8.86E+06
	P44/16		1.1E+07	97.0	9.9E+06	9.03E+06
	P44/17	changed T to 37 at 1:15pm, fed too much	1.4E+07	95.7	1.4E+07	1.17E+07
	P44/18	bled 100mL for VG at 4pm, see noteb	1.3E+07	97.6	1.2E+07	1.27E+07
	P44/19		1.4E+07	97.6	1.3E+07	1.26E+07
37 st	P44/20	change T to 35C at 2pm	1.1E+07	94.0	1.1E+07	1.20E+07
	P44/21	pH at 7.3 overnight, no CO2	1.3E+07	93.3	1.2E+07	1.12E+07
	P44/22		1.0E+07	92.6	9.7E+06	1.07E+07
35t	P44/23		9.0E+06	97.0	8.7E+06	9.19E+06
	P44/24		9.9E+06	96.7	9.6E+06	9.15E+06
	P44/25		1.1E+07	97.3	1.1E+07	1.01E+07
33t	P44/26		1.2E+07	98.5	1.2E+07	1.12E+07
	P44/27		1.0E+07	93.8	1.0E+07	1.10E+07
	P44/28		1.1E+07	96.7	1.1E+07	1.06E+07
31t	P44/29		1.1E+07	96.3	1.0E+07	1.05E+07
	P44/30	took 15mL out of harvest 10am, change T to 33C 12 pm	1.0E+07	97.4	9.9E+06	9.95E+06
31s	P44/31		1.2E+07	93.1	1.1E+07	1.05E+07
	P44/32		1.0E+07	94.6	9.5E+06	1.03E+07
	P44/33		9.5E+06	97.5	8.9E+06	9.22E+06
33t	P44/34		9.9E+06	95.5	9.5E+06	9.21E+06
	P44/35		1.2E+07	96.3	1.2E+07	1.05E+07
	P44/36		1.1E+07	97.2	1.0E+07	1.08E+07
35t	P44/37	harvested too much, go back to batch	3.3E+06		2.7E+06	5.61E+06
	P44/38		4.4E+06		3.9E+06	3.29E+06

Table A. 8 Data from Transient Perfusion Run P44. (continued)

Cond Set	Sample No	GUR (mmol/h)	CSGUR (pmol/cell-d)	LPR (mmol/h)	CSLPR (pmol/cell-d)	APR (mmol/h)	CSAPR (pmol/cell-d)	μ bleed (1/h)
	P44/Inoc							
	P44/0		#DIV/0!		#DIV/0!		#DIV/0!	0.0E+00
	P44/1		#DIV/0!		#DIV/0!		#DIV/0!	1.1E-02
	P44/2	0.060	6.17	0.141	14.50	0.019	1.98	1.3E-03
	P44/3	0.178	8.11	0.249	11.33	0.024	1.11	9.5E-04
	P44/4	0.254	5.95	0.357	8.37	0.027	0.63	2.8E-03
	P44/5	0.380	5.50	0.574	8.30	0.085	1.23	1.2E-03
	P44/6	0.554	4.18	0.766	5.79	0.104	0.79	2.0E-03
	P44/7	0.723	2.59	0.904	3.24	0.131	0.47	1.4E-03
	P44/8	0.474	2.22	0.756	3.55	0.100	0.47	4.6E-02
	P44/9	0.306	2.21	0.119	0.86	0.038	0.27	1.4E-03
	P44/10	0.458	2.60	0.564	3.21	0.073	0.41	1.4E-02
	P44/11	0.450	2.53	0.264	1.49	0.058	0.33	1.6E-03
	P44/12	0.484	1.82	0.448	1.68	0.062	0.23	2.5E-03
	P44/13	0.442	1.70	0.427	1.65	0.057	0.22	3.6E-02
	P44/14	0.440	2.13	0.411	1.99	0.064	0.31	1.9E-03
	P44/15	0.471	2.13	0.384	1.73	0.062	0.28	1.4E-02
	P44/16	0.427	1.89	0.346	1.53	0.050	0.22	2.1E-03
	P44/17	0.492	1.69	0.447	1.53	0.072	0.25	6.6E-03
	P44/18	0.561	1.77	0.306	0.96	0.073	0.23	1.6E-02
	P44/19	0.591	1.87	0.422	1.34	0.062	0.20	1.3E-02
37 st	P44/20	0.576	1.93	0.534	1.79	0.079	0.26	1.9E-02
	P44/21	0.642	2.30	0.363	1.30	0.072	0.26	9.7E-03
	P44/22	0.548	2.04	0.676	2.52	0.064	0.24	1.9E-02
35t	P44/23	0.520	2.26	0.412	1.79	0.067	0.29	8.0E-03
	P44/24	0.425	1.86	0.381	1.67	0.060	0.26	8.7E-04
	P44/25	0.429	1.69	0.235	0.93	0.058	0.23	4.8E-03
33t	P44/26	0.421	1.51	0.221	0.79	0.055	0.20	3.9E-03
	P44/27	0.283	1.03	0.192	0.70	0.050	0.18	6.9E-03
	P44/28	0.264	1.00	0.086	0.33	0.027	0.10	6.7E-04
31t	P44/29	0.258	0.98	0.048	0.18	0.026	0.10	4.7E-03
	P44/30	0.269	1.08	0.018	0.07	0.060	0.24	6.9E-04
31s	P44/31	0.317	1.21	0.038	0.15	0.075	0.29	1.0E-03
	P44/32	0.400	1.56	0.035	0.14	0.089	0.35	4.4E-03
	P44/33	0.424	1.84	0.068	0.30	0.074	0.32	8.3E-04
33t	P44/34	0.403	1.75	0.070	0.30	0.077	0.33	8.7E-04
	P44/35	0.459	1.75	0.050	0.19	0.077	0.29	4.0E-03
	P44/36	0.517	1.92	0.058	0.22	0.109	0.40	1.2E-02
35t	P44/37	-0.072	-0.51	0.018	0.13	0.078	0.55	3.0E-03
	P44/38	0.498	6.05	0.155	1.89	0.054	0.66	2.5E-03

Table A. 8 Data from Transient Perfusion Run P44. (continued)

Cond Set	Sample No	μ apparent (1/h)	μ real (1/h)	tPA (U/mL)	[t-PA] (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	P44/Inoc		#DIV/0!					
	P44/0	#DIV/0!	#DIV/0!					
	P44/1	#DIV/0!	#DIV/0!					
	P44/2	3.85E-02	3.98E-02	16847	29.05			
	P44/3	3.24E-02	3.34E-02					
	P44/4	2.89E-02	3.17E-02	29771	51.33	0.47	18.90	11.08
	P44/5	2.19E-02	2.31E-02					
	P44/6	3.21E-02	3.41E-02	39835	68.68	1.80	72.13	13.62
	P44/7	3.05E-02	3.20E-02					
	P44/8	-5.87E-02	-1.31E-02	39517	68.13	2.51	100.24	11.75
	P44/9	2.51E-02	2.65E-02					
	P44/10	-3.33E-03	1.06E-02	60455	104.23	2.03	81.10	11.53
	P44/11	5.42E-03	7.00E-03					
	P44/12	2.63E-02	2.88E-02	71871	123.92	2.66	106.20	9.98
	P44/13	-3.11E-02	4.36E-03					
	P44/14	1.14E-02	1.34E-02	70660	121.83	2.91	116.59	14.11
	P44/15	-7.30E-03	6.99E-03					
	P44/16	7.92E-03	1.00E-02	83219	143.48	3.00	120.10	13.31
	P44/17	1.09E-02	1.76E-02	91483	157.73	4.53	181.09	15.53
	P44/18	-7.34E-03	8.45E-03	88942	153.35	3.58	143.09	11.27
	P44/19	5.16E-03	1.78E-02	89510	154.33	4.17	166.84	13.21
37 st	P44/20	-8.94E-03	9.89E-03	90078	155.31	4.75	189.86	15.87
	P44/21	4.18E-03	1.39E-02	81900	141.21	3.38	135.24	12.10
	P44/22	-1.02E-02	8.53E-03	87089	150.15	4.91	196.54	18.34
35t	P44/23	-4.37E-03	3.63E-03	89448	154.22	4.21	168.27	18.30
	P44/24	4.36E-03	5.23E-03	101463	174.94	4.33	173.03	18.91
	P44/25	3.76E-03	8.58E-03	114880	198.07	4.29	171.42	16.93
33t	P44/26	4.23E-03	8.17E-03	120483	207.73	4.59	183.55	16.45
	P44/27	-5.61E-03	1.25E-03	115277	198.75	3.42	136.97	12.49
	P44/28	2.47E-03	3.14E-03	137651	237.33	3.67	146.93	13.87
31t	P44/29	-3.26E-03	1.42E-03	138240	238.34	2.39	95.71	9.14
	P44/30	-7.17E-04	-2.23E-05	140807	242.77	3.12	124.70	12.53
31s	P44/31	5.74E-03	6.74E-03	135080	232.90	3.46	138.46	13.24
	P44/32	-5.80E-03	-1.44E-03	120060	207.00	3.48	139.19	13.55
	P44/33	-2.66E-03	-1.83E-03	105282	181.52	3.46	138.35	15.00
33t	P44/34	2.70E-03	3.57E-03	98431	169.71	3.57	142.98	15.52
	P44/35	6.93E-03	1.10E-02	87570	150.98	2.98	119.19	11.32
	P44/36	-7.81E-03	4.30E-03	69311	119.50	2.31	92.35	8.55
35t	P44/37	-4.47E-02	-4.17E-02					
	P44/38	1.79E-02	2.04E-02					

Table A. 9 Data from Scale-down Transient Culture SD-C5.

Cond Set	Sample No	Run Time (h)	Run Time (d)	Delta Time (h)	T (deg C)	pH	pCO2 (mm Hg)	pO2 (mm Hg)
	C5/0	-4.5	-0.2	0.0	37.0	7.391	41.1	95.8
	C5/inoc	0.0	0.0	4.5	36.9	7.229	53.1	102.7
	C5/1	21.0	0.9	21.0	37.0	7.160	63.8	93.0
	C5/2	44.3	1.8	23.3	36.9	7.037	88.4	132.9
	C5/3	69.0	2.9	24.7	36.9	7.309	41.6	117.5
	C5/4	92.1	3.8	23.1	36.9	7.301	40.6	115.7
	C5/5	116.9	4.9	24.8	36.9	7.336	39.2	118.3
37 s	C5/6	141.7	5.9	24.8	36.9	7.317	38.3	116.5
	C5/7	165.1	6.9	23.4	35.0	7.285	40.5	115.9
	C5/8	187.3	7.8	22.3	35.0	7.319	40.8	113.8
35 t	C5/9	213.0	8.9	25.7	35.0	7.320	41.0	110.8
	C5/10	237.5	9.9	24.5	33.0	7.310	44.6	121.7
	C5/11	260.0	10.8	22.5	33.0	7.321	44.9	128.5
33 t	C5/12	286.0	11.9	26.0	33.1	7.363	40.9	124.3
	C5/13	309.4	12.9	23.4	31.2	7.347	42.8	125.4

Cond Set	Sample No	DO (% air sat)	[Gluc] (mM)	[Lac] (mM)	[BUN] (mM)	[NH4] (mM)	Osmolality (mOsm/kg)	[Na+] (mM)
	C5/0	68.0	24.8	0.4	2.0	1.5	381	139
	C5/inoc	72.9	23.0	3.5	2.6	2.0	394	139
	C5/1	66.0	22.8	5.1	3.0	2.2	394	139
	C5/2	94.3	22.5	5.2	3.0	2.2	402	140
	C5/3	83.4	22.3	5.3	3.0	2.2	394	140
	C5/4	82.1	21.9	5.6	3.1	2.3	395	141
	C5/5	84.0	22.2	4.9	3.0	2.2	407	141
37 s	C5/6	82.7	21.7	5.5	3.2	2.4	398	140
	C5/7	82.3	22.6	5.5	3.2	2.4	409	139
	C5/8	80.8	22.5	5.6	3.2	2.4	392	139
35 t	C5/9	78.6	22.8	5.9	3.2	2.4	396	140
	C5/10	86.4	23.1	5.2	3.0	2.2	409	140
	C5/11	91.2	23.6	4.3	2.8	2.1	404	139
33 t	C5/12	88.2	23.4	4.3	2.9	2.2	395	139
	C5/13	89.0	23.5	4.3	3.0	2.2	395	139

Table A. 9 Data from Scale-down Transient Culture SD-C5. (continued)

Cond Set	Sample No	Total Xn (cells/mL)	Viability (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)	Calc'd Mu (1/h)	Dilution Rate (1/d)	Dilution Rate (1/h)
	C5/0	#DIV/0!	#DIV/0!	#DIV/0!				
	C5/inoc	1.28E+05	80.9	1.03E+05				
	C5/1	1.49E+05	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	C5/2	2.79E+05	96.4	2.69E+05	1.71E+05	0.042	0.6	0.025
	C5/3	2.56E+05	94.6	2.43E+05	1.61E+05	0.036	0.6	0.025
	C5/4	3.48E+05	99.0	3.45E+05	1.98E+05	0.054	0.7	0.029
	C5/5	2.37E+05	98.1	2.32E+05	1.57E+05	0.034	0.5	0.023
37 s	C5/6	2.13E+05	94.5	2.01E+05	1.45E+05	0.028	0.5	0.021
	C5/7	2.41E+05	95.4	2.30E+05	1.56E+05	0.036	0.6	0.023
	C5/8	2.04E+05	96.6	1.97E+05	1.43E+05	0.030	0.5	0.019
35 t	C5/9	2.05E+05	96.1	1.97E+05	1.43E+05	0.026	0.5	0.020
	C5/10	2.27E+05	95.6	2.17E+05	1.51E+05	0.032	0.6	0.024
	C5/11	1.74E+05	95.7	1.67E+05	1.31E+05	0.023	0.4	0.015
33 t	C5/12	1.57E+05	92.3	1.45E+05	1.21E+05	0.014	0.3	0.013
	C5/13	1.66E+05	90.7	1.51E+05	1.24E+05	0.018	0.4	0.015

Cond Set	Sample No	Glucose after media change (mM)	Lactate after media change (mM)	NH4 after media change (mM)	GUR (mmol/h)	LPR (mmol/h)	APR (mmol/h)	CSGUR (pmol/cell-d)
	C5/0							
	C5/inoc							
	C5/1	#DIV/0!	#DIV/0!	#DIV/0!	0.001	0.008	0.001	#DIV/0!
	C5/2	23.9	2.2	1.8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
	C5/3	23.8	2.4	1.8	0.007	0.013	0.002	9.93
	C5/4	24.0	1.9	1.8	0.008	0.014	0.002	9.82
	C5/5	23.7	2.3	1.8	0.007	0.012	0.002	10.86
37 s	C5/6	23.3	2.9	2.0	0.008	0.013	0.002	13.23
	C5/7	23.7	2.9	1.9	0.003	0.011	0.002	4.32
	C5/8	23.6	3.0	2.0	0.006	0.012	0.002	9.30
35 t	C5/9	23.8	3.2	2.0	0.003	0.011	0.002	5.44
	C5/10	24.0	2.6	1.9	0.003	0.008	0.001	4.45
	C5/11	24.1	2.7	1.9	0.002	0.007	0.001	3.39
33 t	C5/12	23.8	3.1	2.0	0.003	0.006	0.001	5.19
	C5/13	23.9	3.0	2.0	0.001	0.005	0.001	2.75

Table A. 9 Data from Scale-down Transient Culture SD-C5. (continued)

Cond Set	Sample No	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)	tPA (U/mL)	[tPA] (mg/L)	tPA after media change (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	C5/0								
	C5/inoc								
	C5/1	#DIV/0!	#DIV/0!						
	C5/2	#DIV/0!	#DIV/0!						
	C5/3	18.81	2.60						
	C5/4	16.70	2.47	3234	5.58	1.62			
	C5/5	18.47	2.88	2988	5.15	2.22	0.014	3.42	21.82
37 s	C5/6	21.12	3.52	3607	6.22	3.10	0.016	3.86	26.71
	C5/7	16.85	2.72	4185	7.22	3.50	0.018	4.23	27.11
	C5/8	20.57	3.19	4431	7.64	3.88	0.019	4.47	31.23
35 t	C5/9	18.70	2.65	4925	8.49	4.31	0.018	4.31	30.15
	C5/10	13.05	1.74	4869	8.39	3.87	0.017	4.00	26.54
	C5/11	13.75	1.89	4510	7.78	4.67	0.017	4.16	31.84
33 t	C5/12	11.90	2.19	5013	8.64	5.97	0.015	3.67	30.33
	C5/13	9.98	2.15	5574	9.61	6.37	0.016	3.73	30.15

Table A. 10 Data from Scale-down Transient Culture SD-D5.

Cond Set	Sample No	Run Time (h)	Run Time (d)	Delta Time (h)	T (deg C)	pH	pCO2 (mm Hg)	pO2 (mm Hg)
	D5/0	-4.5	-0.2	0.0	37.0	7.347	45.9	98.6
	D5/inoc	0.0	0.0	4.5	36.9	7.177	59.9	88.9
	D5/1	21.0	0.9	21.0	36.9	7.134	68.6	81.6
	D5/2	44.3	1.8	23.3	36.9	7.029	95.1	125.0
	D5/3	69.0	2.9	24.7	37.0	7.321	41.6	118.3
	D5/4	92.1	3.8	23.1	36.9	7.288	42.4	112.7
	D5/5	116.9	4.9	24.8	37.0	7.305	41.0	112.8
37 s	D5/6	141.7	5.9	24.8	36.9	7.237	44.1	111.3
	D5/7	165.1	6.9	23.4	35.0	7.198	48.6	114.0
	D5/8	187.3	7.8	22.2	35.0	7.262	48.6	112.7
35 t	D5/9	213.0	8.9	25.7	35.0	7.241	49.6	109.5
	D5/10	237.5	9.9	24.5	33.0	7.231	55.9	119.8
	D5/11	260.0	10.8	22.5	33.1	7.219	55.3	130.3
33 t	D5/12	285.0	11.9	25.0	33.0	7.262	50.0	118.6
	D5/13	309.4	12.9	24.4	31.1	7.238	52.2	118.4

Table A. 10 Data from Scale-down Transient Culture SD-D5. (continued)

Cond Set	Sample No	DO (% air sat)	[Gluc] (mM)	[Lac] (mM)	[BUN] (mM)	[NH4] (mM)	Osmolality (mOsm/kg)	[Na+] (mM)
	D5/0	70.0	24.7	0.3	1.9	1.5	379	139
	D5/inoc	63.1	23.3	3.6	2.5	1.9	380	139
	D5/1	57.9	22.7	5.1	3.0	2.2	382	140
	D5/2	88.7	22.2	5.2	3.0	2.2	374	141
	D5/3	84.0	23.0	4.9	2.8	2.1	380	140
	D5/4	80.0	22.0	5.3	3.0	2.2	383	140
	D5/5	80.1	21.9	5.5	3.1	2.3	378	140
37 s	D5/6	79.0	21.1	6.1	3.2	2.4	381	139
	D5/7	80.9	22.4	6.5	3.3	2.4	380	139
	D5/8	80.0	23.0	5.5	3.0	2.2	386	140
35 t	D5/9	77.7	22.4	6.2	3.1	2.3	374	139
	D5/10	85.0	23.3	5.1	2.7	2.0	377	139
	D5/11	92.5	23.3	4.9	2.7	2.0	379	138
33 t	D5/12	84.2	22.6	5.2	2.8	2.1	373	138
	D5/13	84.0	22.3	6.0	3.2	2.4	375	139

Cond Set	Sample No	Total Xn (cells/mL)	Viability (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)	Calc'd Mu (1/h)	Dilution Rate (1/d)	Dilution Rate (1/h)
	D5/0	#DIV/0!	#DIV/0!	#DIV/0!				
	D5/inoc	9.06E+04	85.5	7.75E+04				
	D5/1	2.04E+05	89.7	1.83E+05	1.23E+05	0.041	0.5	0.019
	D5/2	3.70E+05	96.4	3.57E+05	2.02E+05	0.054	0.7	0.029
	D5/3	2.42E+05	94.5	2.29E+05	1.56E+05	0.034	0.6	0.024
	D5/4	2.81E+05	96.4	2.71E+05	1.72E+05	0.043	0.6	0.025
	D5/5	2.32E+05	97.6	2.27E+05	1.55E+05	0.033	0.5	0.022
37 s	D5/6	2.13E+05	89.5	1.91E+05	1.41E+05	0.026	0.5	0.020
	D5/7	3.85E+05	93.5	3.60E+05	2.03E+05	0.055	0.7	0.030
	D5/8	2.17E+05	94.9	2.06E+05	1.47E+05	0.033	0.5	0.020
35 t	D5/9	2.86E+05	95.5	2.73E+05	1.72E+05	0.039	0.6	0.026
	D5/10	1.95E+05	96.3	1.88E+05	1.39E+05	0.026	0.5	0.021
	D5/11	2.29E+05	91.7	2.10E+05	1.48E+05	0.033	0.5	0.021
33 t	D5/12	1.46E+05	91.7	1.34E+05	1.16E+05	0.012	0.3	0.010
	D5/13	1.78E+05	93.0	1.66E+05	1.30E+05	0.021	0.4	0.017

Table A. 10 Data from Scale-down Transient Culture SD-D5. (continued)

Cond Set	Sample No	Glucose after media change (mM)	Lactate after media change (mM)	NH4 after media change (mM)	GUR (mmol/h)	LPR (mmol/h)	APR (mmol/h)	CSGUR (pmol/cell-d)
	D5/0							
	D5/inoc							
	D5/1	23.6	2.9	1.9	0.003	0.007	0.002	5.59
	D5/2	24.0	1.7	1.7	0.006	0.010	0.001	7.17
	D5/3	24.0	2.3	1.7	0.004	0.013	0.002	6.24
	D5/4	23.7	2.1	1.8	0.008	0.013	0.002	11.85
	D5/5	23.5	2.6	1.8	0.007	0.014	0.002	11.29
37 s	D5/6	22.8	3.3	1.9	0.010	0.014	0.002	16.25
	D5/7	23.9	2.3	1.8	0.002	0.014	0.002	2.09
	D5/8	23.9	2.8	1.8	0.004	0.014	0.002	6.96
35 t	D5/9	23.9	2.5	1.8	0.006	0.013	0.002	8.01
	D5/10	24.0	2.9	1.8	0.002	0.011	0.001	3.92
	D5/11	24.0	2.5	1.7	0.003	0.009	0.001	4.71
33 t	D5/12	23.1	3.9	1.9	0.006	0.011	0.001	11.83
	D5/13	23.3	3.7	2.0	0.003	0.008	0.002	6.32

Cond Set	Sample No	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)	tPA (U/mL)	[tPA] (mg/L)	tPA after media change (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	D5/0								
	D5/inoc								
	D5/1	13.98	3.19						
	D5/2	11.60	1.74						
	D5/3	20.15	2.53						
	D5/4	18.11	2.93	3181	5.48	2.02			
	D5/5	20.99	3.40	3033	5.23	2.31	0.013	3.11	20.07
37 s	D5/6	24.09	3.63	3434	5.92	3.10	0.015	3.49	24.83
	D5/7	15.99	2.49	4878	8.41	2.76	0.023	5.45	26.86
	D5/8	23.31	3.23	4170	7.19	3.49	0.020	4.79	32.63
35 t	D5/9	18.33	2.48	4877	8.41	3.08	0.019	4.60	26.71
	D5/10	18.54	1.73	4299	7.41	3.94	0.018	4.24	30.43
	D5/11	14.71	1.84	4553	7.85	3.74	0.017	4.16	28.08
33 t	D5/12	22.37	2.93	5425	9.35	6.97	0.022	5.39	46.35
	D5/13	15.49	3.26	6515	11.23	6.77	0.017	4.19	32.23

Table A. 11 Data from Scale-down Transient Culture SD-A6.

Cond Set	Sample No	Run Time (h)	Run Time (d)	Delta Time (h)	T (deg C)	pH	pCO2 (mm Hg)	pO2 (mm Hg)
	A6/0	-1.2	-0.1	0.0	37.0	7.716	20.3	103.7
	A6/Inoc	0.0	0.0	1.2	37.0	7.250	28.6	87.7
	A6/1	21.7	0.9	21.7	37.0	7.395	39.2	104
	A6/2	44.2	1.8	22.5	37.0	7.378	34.8	92.3
	A6/3	69.9	2.9	25.7	37.0	7.311	45.4	97.9
	A6/4	92.2	3.8	22.3	37.0	7.320	48.1	103.2
	A6/5	116.4	4.9	24.2	37.0	7.362	42.1	102.1
37ss	A6/6	140.0	5.8	23.5	37.0	7.326	40.5	99.2
35-1t	A6/7	163.9	6.8	24.0	35.0	7.326	42.0	116.3
33-1t	A6/8	189.5	7.9	25.6	33.0	7.405	33.6	99.1
32-1t	A6/9	212.5	8.9	23.0	32.0	7.458	35.0	103.0
31-1t	A6/10	236.0	9.8	23.5	31.1	7.447	31.0	98.9
	A6/11	259.1	10.8	23.1	31.1	7.376	40.7	111.5
	A6/12	284.9	11.9	25.8	31.2	7.415	34.5	108.5
	A6/13	309.0	12.9	24.1	31.0	7.392	35.0	106.9
	A6/14	332.5	13.9	23.5	31.6	7.426	33.0	96.6
31s	A6/15	355.9	14.8	23.3	32.2	7.468	32.3	100.9
	A6/16	380.7	15.9	24.8	33.0	7.443	30.0	107.4
	A6/17	403.8	16.8	23.0	33.0	7.459	30.2	110.9
33t	A6/18	430.9	18.0	27.1	33.0	7.449	28.4	94.7
	A6/19	454.0	18.9	23.1	35.1	7.435	27.5	uncal
	A6/20	478.1	19.9	24.2	35.0	7.497	28.0	103.7
35t	A6/21	500.6	20.9	22.5	35.1	7.473	26.6	97.5
	A6/22	524.6	21.9	24.1	37.0	7.357	38.3	122.2
	A6/23	548.6	22.9	24.0	37.0	7.338	39.5	109.1
37t	A6/24	573.1	23.9	24.5	37.0	7.321	41.0	93.7

Table A. 11 Data from Scale-down Transient Culture SD-A6. (continued)

Cond Set	Sample No	DO (% air sat)	[Gluc] (mM)	[Lac] (mM)	[BUN] (mM)	[NH4] (mM)	Osmolality (mOsm/kg)	[Na+] (mM)
	A6/0	72.3	24.1	0.3	2.2	1.7	361	137
	A6/Inoc	61.1	22.2	2.4	2.4	1.8	413	139
	A6/1	74.1	19.9	6	3	2.2	386	138
	A6/2	65.7	18.2	8.9	3.3	2.4	391	139
	A6/3	71.1	18.9	8.4	3.4	2.5	381	140
	A6/4	74.6	19.7	6.8	3.1	2.3	398	140
	A6/5	73.4	21.6	6.5	3.2	2.4	398	140
37ss	A6/6	71.6	19.4	8.7	3.4	2.5	402	140
35-1t	A6/7	81.4	20.5	7.4	2.8	2.1	395	141
33-1t	A6/8	69.9	20.4	7.5	2.8	2.1	396	140
32-1t	A6/9	72.7	21.5	5.1	2.7	2.0	404	141
31-1t	A6/10	69.8	21.1	6.7	3.0	2.2	384	140
	A6/11	78.9	21.3	6.3	2.8	2.1	378	141
	A6/12	77.1	21.3	6.1	2.9	2.2	392	141
	A6/13	76.2	20.5	8.2	3.4	2.5	393	142
	A6/14	68.5	20.3	7.5	3.3	2.4	380	141
31s	A6/15	71.0	20.9	6.5	3.1	2.3	392	142
	A6/16	76.1	20.4	7.3	3.5	2.6	395	142
	A6/17	77.8	20.4	6.9	3.3	2.4	392	141
33t	A6/18	66.9	20.4	7.7	3.6	2.6	386	142
	A6/19		19.4	8.3	3.6	2.6	370	140
	A6/20	74.1	20.1	7.5	3.2	2.4	388	139
35t	A6/21	69.7	20.0	8.2	3.4	2.5	392	141
	A6/22	88.1	19.1	7.7	3.4	2.5	380	141
	A6/23	80.0	19.7	7.5	3.4	2.5	378	141
37t	A6/24	68.2	19.6	8.4	3.3	2.4	385	142

Table A. 11 Data from Scale-down Transient Culture SD-A6. (continued)

Cond Set	Sample No	Total Xn (cells/mL)	Viability (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)	Calc'd Mu (1/h)	Dilution Rate (1/d)	Dilution Rate (1/h)
	A6/0	#DIV/0!	#DIV/0!	#DIV/0!				
	A6/Inoc	1.45E+05	93.1	1.35E+05				
	A6/1	1.5E+05	94.0	1.5E+05	1.4E+05	0.003	0.3	0.0
	A6/2	3.1E+05	93.1	2.9E+05	1.8E+05	0.047	0.7	0.0
	A6/3	3.9E+05	90.4	3.6E+05	2.0E+05	0.049	0.7	0.0
	A6/4	3.78E+05	94.2	3.56E+05	2.02E+05	0.057	0.7	0.0
	A6/5	2.27E+05	92.6	2.10E+05	1.48E+05	0.031	0.5	0.0
37ss	A6/6	3.30E+05	92.9	3.07E+05	1.84E+05	0.048	0.7	0.028
35-1t	A6/7	2.83E+05	91.9	2.60E+05	1.67E+05	0.040	0.6	0.024
33-1t	A6/8	3.06E+05	97.7	2.99E+05	1.82E+05	0.043	0.7	0.028
32-1t	A6/9	1.41E+05	92.9	1.31E+05	1.15E+05	0.012	0.2	0.010
31-1t	A6/10	1.67E+05	90.5	1.51E+05	1.24E+05	0.017	0.3	0.014
	A6/11	1.68E+05	91.3	1.54E+05	1.25E+05	0.019	0.4	0.015
	A6/12	1.24E+05	82.4	1.03E+05	1.01E+05	0.001	0.0	0.001
	A6/13	1.47E+05	92.7	1.36E+05	1.17E+05	0.012	0.3	0.011
	A6/14	1.67E+05	91.5	1.53E+05	1.24E+05	0.018	0.3	0.014
31s	A6/15	1.29E+05	90.3	1.16E+05	1.08E+05	0.006	0.1	0.006
	A6/16	1.52E+05	91.1	1.39E+05	1.18E+05	0.013	0.3	0.012
	A6/17	1.33E+05	90.0	1.20E+05	1.10E+05	0.008	0.2	0.007
33t	A6/18	1.36E+05	92.2	1.25E+05	1.12E+05	0.008	0.2	0.008
	A6/19	1.95E+05	92.5	1.80E+05	1.36E+05	0.025	0.4	0.019
	A6/20	1.78E+05	93.9	1.67E+05	1.31E+05	0.021	0.4	0.017
35t	A6/21	2.80E+05	98.0	2.74E+05	1.73E+05	0.045	0.6	0.026
	A6/22	3.06E+05	93.9	2.87E+05	1.77E+05	0.044	0.7	0.027
	A6/23	3.01E+05	98.1	2.96E+05	1.81E+05	0.045	0.7	0.028
37t	A6/24	2.47E+05	98.6	2.43E+05	1.61E+05	0.036	0.6	0.025

Table A. 11 Data from Scale-down Transient Culture SD-A6. (continued)

Cond Set	Sample No	Glucose after media change (mM)	Lactate after media change (mM)	NH4 after media change (mM)	GUR (mmol/h)	LPR (mmol/h)	APR (mmol/h)	CSGUR (pmol/cell-d)
	A6/0							
	A6/Inoc							
	A6/1	21.2	4.2	2.1	0.011	0.017	0.002	18.14
	A6/2	22.1	3.3	1.9	0.013	0.021	0.002	18.04
	A6/3	22.6	2.6	1.9	0.012	0.020	0.002	14.64
	A6/4	22.9	2.1	1.9	0.013	0.019	0.002	15.69
	A6/5	22.9	3.3	2.0	0.005	0.018	0.002	8.44
37ss	A6/6	22.6	3.0	1.9	0.015	0.023	0.002	19.41
35-1t	A6/7	22.5	3.4	1.9	0.009	0.018	0.001	12.36
33-1t	A6/8	22.9	2.7	1.8	0.008	0.016	0.001	11.03
32-1t	A6/9	22.1	4.0	1.9	0.006	0.010	0.001	12.37
31-1t	A6/10	22.1	4.5	2.0	0.004	0.012	0.001	8.35
	A6/11	22.3	4.2	1.9	0.004	0.008	0.000	6.75
	A6/12	21.4	6.0	2.1	0.004	0.007	0.001	9.00
	A6/13	21.5	6.1	2.3	0.004	0.009	0.001	7.37
	A6/14	21.6	5.0	2.2	0.005	0.006	0.001	9.52
31s	A6/15	21.3	5.6	2.2	0.003	0.006	0.001	6.74
	A6/16	21.4	5.4	2.3	0.004	0.007	0.001	7.74
	A6/17	21.0	5.8	2.3	0.004	0.007	0.000	9.78
33t	A6/18	21.1	6.2	2.4	0.002	0.007	0.001	4.86
	A6/19	21.5	4.7	2.2	0.008	0.009	0.001	13.36
	A6/20	21.7	4.6	2.1	0.006	0.011	0.001	10.55
35t	A6/21	22.6	3.2	2.0	0.008	0.016	0.002	10.58
	A6/22	22.4	2.9	2.0	0.015	0.019	0.002	19.70
	A6/23	22.6	2.7	2.0	0.011	0.019	0.002	14.75
37t	A6/24	22.3	3.6	2.0	0.012	0.023	0.002	18.33

Table A. 11 Data from Scale-down Transient Culture SD-A6. (continued)

Cond Set	Sample No	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)	tPA (U/mL)	[tPA] (mg/L)	tPA after media change (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	A6/0								
	A6/Inoc								
	A6/1	28.39	3.24						
	A6/2	28.03	2.26						
	A6/3	23.75	2.60						
	A6/4	22.55	2.06	3276	5.65	1.59			
	A6/5	29.22	3.42	2321	4.00	1.91	0.010	2.39	16.13
37ss	A6/6	30.12	2.74	4073	7.02	2.29	0.022	5.22	28.29
35-1t	A6/7	26.08	0.85	3475	5.99	2.60	0.015	3.71	22.14
33-1t	A6/8	21.26	1.20	4697	8.10	2.71	0.021	5.15	28.39
32-1t	A6/9	21.72	1.86	3814	6.58	5.03	0.017	4.03	35.13
31-1t	A6/10	22.53	2.36	5474	9.44	6.26	0.019	4.50	36.39
	A6/11	14.62	0.40						
	A6/12	17.45	1.95						
	A6/13	19.03	3.01	7871	13.57	9.95			
	A6/14	11.55	1.24	7807	13.46	8.83	0.015	3.58	28.79
31s	A6/15	14.08	1.17	7085	12.22	10.51	0.015	3.48	32.28
	A6/16	13.62	2.94	7907	13.63	9.84	0.013	3.02	25.54
	A6/17	14.70	1.05	7537	13.00	10.83	0.014	3.29	29.97
33t	A6/18	14.99	2.61	8957	15.44	12.32	0.017	4.08	36.40
	A6/19	16.02	1.48	8718	15.03	8.35	0.012	2.82	20.72
	A6/20	20.93	1.16	6341	10.93	6.54	0.011	2.56	19.61
35t	A6/21	22.25	2.55	5959	10.27	3.75	0.017	4.00	23.13
	A6/22	25.40	2.94	5123	8.83	3.08	0.021	5.07	28.59
	A6/23	25.65	2.97	4389	7.57	2.56	0.019	4.50	24.91
37t	A6/24	34.48	2.89	3572	6.16	2.53	0.015	3.53	21.90

Table A. 12 Data from Scale-down Transient Culture SD-B6.

Cond Set	Sample No	Run Time (h)	Run Time (d)	Delta Time (h)	T (deg C)	pH	pCO2 (mm Hg)	pO2 (mm Hg)
	B6/0	-1.2	0.0	0.0	37.0	7.641	24.8	119.7
	B6/inoc	0.0	0.0	1.2	37.0	7.480	34.4	110.7
	B6/1	21.7	0.9	21.7	37.0	7.377	42.0	92.9
	B6/2	44.2	1.8	22.5	37.0	7.361	39.4	85.4
	B6/3	69.9	2.9	25.7	37.0	7.358	40.7	96.2
	B6/4	92.2	3.8	22.3	37.0	7.301	46.5	90.6
	B6/5	116.4	4.9	24.2	37.0	7.384	41.3	94.6
37ss	B6/6	140.0	5.8	23.5	37.0	7.402	39.4	100.8
35t	B6/7	164.0	6.8	24.0	35.0	7.395	28.4	120.8
33t	B6/8	189.5	7.9	25.6	33.0	7.439	32.6	98.2
32t	B6/9	212.6	8.9	23.0	31.7	7.507	30.4	90.4
31t	B6/10	236.1	9.8	23.5	30.6	7.517	27.3	94.9
	B6/11	259.1	10.8	23.1	31.0	7.418	38.8	108.3
	B6/12	284.9	11.9	25.8	31.1	7.431	35.6	102.5

Table A. 12 Data from Scale-down Transient Culture SD-B6. (continued)

Cond Set	Sample No	DO (% air sat)	[Gluc] (mM)	[Lac] (mM)	[BUN] (mM)	[NH4] (mM)	Osmolality (mOsm/kg)	[Na+] (mM)
	B6/0	83.4	24.5	0.3	2.1	1.6	387	139
	B6/inoc	77.2	22.1	2.3	2.3	1.7	378	139
	B6/1	66.2	20.9	5.2	3.0	2.2	402	140
	B6/2	60.8	19.7	7.2	3.1	2.3	373	139
	B6/3	69.8	21.3	7.7	3.3	2.4	381	139
	B6/4	65.5	20.6	7.6	3.2	2.4	368	139
	B6/5	68.0	21.9	5.7	3.1	2.3	374	139
37ss	B6/6	72.7	22.3	4.9	2.9	2.2	372	139
35t	B6/7	84.5	22.3	5.3	2.7	2.0	369	140
33t	B6/8	69.3	21.6	5.9	2.9	2.2	367	140
32t	B6/9	63.8	22.2	4.8	2.8	2.1	369	139
31t	B6/10	67.0	22.4	5.1	3.0	2.2	369	141
	B6/11	76.7	23.4	4.5	2.8	2.1	370	141
	B6/12	72.8	22.8	5.0	3.2	2.4	366	141

Cond Set	Sample No	Total Xn (cells/mL)	Viability (%)	Xn viable (cells/mL)	Xv log mean (cells/mL)	Calc'd Mu (1/h)	Dilution Rate (1/d)	Dilution Rate (1/h)
	B6/0	#DIV/0!	#DIV/0!	#DIV/0!				
	B6/inoc	1.26E+05	97.0	1.22E+05				
	B6/1	1.62E+05	97.1	1.57E+05	1.39E+05	0.012	0.4	0.015
	B6/2	2.99E+05	97.1	2.90E+05	1.78E+05	0.047	0.7	0.027
	B6/3	3.05E+05	92.9	2.83E+05	1.76E+05	0.040	0.6	0.027
	B6/4	4.50E+05	95.1	4.28E+05	2.26E+05	0.065	0.8	0.032
	B6/5	4.46E+05	91.5	4.08E+05	2.19E+05	0.058	0.8	0.031
37ss	B6/6	2.13E+05	96.9	2.07E+05	1.47E+05	0.031	0.5	0.022
35t	B6/7	1.80E+05	97.5	1.75E+05	1.34E+05	0.023	0.4	0.016
33t	B6/8	1.89E+05	96.2	1.82E+05	1.37E+05	0.023	0.5	0.019
32t	B6/9	1.34E+05	94.0	1.26E+05	1.12E+05	0.010	0.2	0.009
31t	B6/10	1.51E+05	94.4	1.42E+05	1.20E+05	0.015	0.3	0.012
	B6/11	1.02E+05	92.6	9.44E+04	9.72E+04	-0.003	-0.1	-0.002
	B6/12	1.34E+05	95.5	1.28E+05	1.13E+05	0.012	0.2	0.009

Table A. 12 Data from Scale-down Transient Culture SD-B6. (continued)

Cond Set	Sample No	Glucose after media change (mM)	Lactate after media change (mM)	NH4 after media change (mM)	GUR (mmol/h)	LPR (mmol/h)	APR (mmol/h)	CSGUR (pmol/cell-d)
	B6/0							
	B6/inoc							
	B6/1	22.2	3.4	2.0	0.006	0.013	0.002	9.56
	B6/2	22.8	2.7	1.8	0.011	0.017	0.001	15.00
	B6/3	23.4	2.9	1.9	0.006	0.020	0.002	8.19
	B6/4	23.6	2.0	1.8	0.012	0.021	0.002	13.22
	B6/5	23.9	1.6	1.8	0.007	0.015	0.002	7.65
37ss	B6/6	23.4	2.5	1.9	0.007	0.014	0.002	10.84
35t	B6/7	23.1	3.4	1.9	0.005	0.012	0.001	8.47
33t	B6/8	22.9	3.4	1.9	0.006	0.010	0.001	10.53
32t	B6/9	22.7	3.9	2.0	0.003	0.006	0.001	6.53
31t	B6/10	23.0	3.7	2.0	0.001	0.005	0.001	2.34
	B6/11	23.3	4.8	2.1	-0.002	0.004	0.000	-4.02
	B6/12	23.2	4.0	2.2	0.002	0.001	0.001	4.38

Cond Set	Sample No	CSLPR (pmol/cell-d)	CSAPR (pmol/cell-d)	tPA (U/mL)	[tPA] (mg/L)	tPA after media change (mg/L)	tPAPR (mg/h)	VtPAPR (mg/L-d)	CStPAPR (pg/cell-d)
	B6/0								
	B6/inoc								
	B6/1	23.09	3.82						
	B6/2	22.61	1.75						
	B6/3	26.60	3.10						
	B6/4	22.37	2.21	4277	7.37	1.72			
	B6/5	16.73	2.30	3649	6.29	1.54	0.019	4.53	20.68
37ss	B6/6	22.73	2.64	2959	5.10	2.47	0.015	3.63	24.69
35t	B6/7	20.70	1.09	2389	4.12	2.55	0.007	1.65	12.32
33t	B6/8	17.14	2.01	3948	6.81	3.74	0.017	3.99	29.16
32t	B6/9	13.16	1.65	4407	7.60	6.03	0.017	4.02	35.72
31t	B6/10	10.46	2.01	4735	8.16	5.74	0.009	2.18	18.18
	B6/11	8.86	0.50	4510	7.78	8.24	0.009	2.12	21.83
	B6/12	2.05	2.01	6816	11.75	9.18	0.014	3.27	28.81