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High-density mammalian cell cultures in stirred-tank bioreactor without external pH control

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ABSTRACT

Maintaining desired pH is a necessity for optimal cell growth and protein production. It is typically achieved through a two-sided pH control loop on the bioreactor controller. Here we investigated cell culture processes with minimum or no pH control and demonstrated that high-density mammalian cell cultures could be maintained for long-term protein production without pH control. The intrinsic interactions between pCO_2, lactate, and pH were leveraged to maintain culture pH. Fed-batch cultures at the same lower pH limit of 6.75 but different upper pH limits (7.05, 7.30, 7.45, 7.65) were evaluated in the 3 L bioreactors and comparable results were obtained. Neither CO_2 sparging nor base addition was required to control pH in the pH range of 6.75–7.65. The impact of sparger configurations (drilled hole sparger vs. frit sparger) and scales (3 L vs. 200 L) on CO_2 accumulation and culture pH was also demonstrated. The same principle was applied in two perfusion cultures with steady state cell densities at 42.5 ± 3.3 or $68.3 \pm 6.0 \times 10^6$ cells/mL with low cell specific perfusion rates (15 ± 2 to 23 ± 3 pL/cell/day), achieving up to 1.9 ± 0.1 g/L/day bioreactor productivity. Culture pH level in the 3 L perfusion bioreactors was steadily maintained by controlling the residual lactate and pCO_2 levels without the requirement of external pH control for up to 40 days with consistent productivity and product quality. Furthermore, culture pH could be potentially modulated via adjusting residual glucose levels and CO_2 stripping capability in perfusion cultures. To the best of our knowledge, this is the first time a systematic study was performed to evaluate the long-term cell cultivation and protein production in stirred-tank bioreactors without external pH control.

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1. Introduction

Importance of pH on mammalian cell growth, metabolism, and product formation has been extensively studied in different operational modes (Borys et al., 1993; Miller et al., 1988; Ozturk and Palsson, 1991; Yoon et al., 2005). In a typical bioreactor system, a feedback control loop is required to maintain pH at the setpoint that varies among cell lines and processes. When pH is lower than the target value, a base pump will be triggered to deliver base (e.g., NaOH, Na_2CO_3, or $NaHCO_3$) to bring pH up. When pH is higher than the target value, an acid pump or CO_2 valve will be turned on to deliver either acid (e.g., HCl or lactic acid) or CO_2 to lower pH. It is common to use CO_2 for pH control in combination with a bicarbonate-buffering system in mammalian cell cultures. When the base pump or CO_2 valve will be triggered is determined by the tuning parameters defined in the control loop. To avoid the negative impact from pH gradients created by base addition, optimization of pH control loop, tuning parameters, and location of base addition is critical (Langheinrich and Nienow, 1999; Nienow et al., 1996; Osman et al., 2002). However, this sometimes is a challenge in large-scale bioreactors since facility shutdown is often required for such activities. Minimum to no base addition is often viewed as an indicator for a good process, while a process that requires large amount of base addition could be problematic since base addition is typically related to high pCO_2 and/or high lactate accumulations (Charaniya et al., 2010; Nienow, 2006).

Controlling pH can be more challenging in high-density cell cultures and process scale-up. For fed-batch cultures at high densities (e.g., 20–30 $\times$ 10^6 cells/mL), high pCO_2 build-up in large-scale bioreactors could compromise process performance due to the additional base required and the accompanied osmolality increase (Nienow, 2006). Mixing difference between bioreactor configurations or scales could also cause issues during base addition, which could further impact product titer (Sieblist et al., 2015). For perfusion cultures, medium is continuously exchanged out and new balance has to be established continuously for pH control, which

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Table 1
Bioreactor geometry and engineering information.

Parameter	Equation/Symbol	3 L	200 L
Impeller type	–	Marine	Pitched-blade
Impeller power number	N_p	0.30	1.15
Impeller Number	–	1	1
Impeller speed (rpm)[a]	N	260–450	140
Impeller diameter (m)	D_i	0.070	0.203
Bioreactor diameter (m)	D_t	0.130	0.564
Ratio of impeller diameter to bioreactor diameter	D_i/D_t	0.54	0.36
Bioreactor height to diameter ratio	H/D_t	1.16	1.42
Sparger	–	• 0.5 mm drilled hole sparger (14 holes) for O_2 and CO_2 or • 20 μm frit sparger for O_2 and CO_2	• 1 mm drilled hole sparger (10 holes) for air, 20 μm frit sparger for O_2 and CO_2
Working volume (m^3)	V	0.002	0.2
Power input per volume (W/m^3)	$P/V = N_p \rho N^3 D_i^5/V$	20.5–106.4	25.2
Shear stress (N/m^2)	$\tau = (\varepsilon/\nu)^{1/2}\,\mu$	0.303–0.691	0.681
Tip speed (m/s)	$V_{tip} = \pi N D_i$	0.95–1.65	1.49
Impeller Reynolds number, *Re*	$Re = \rho N D_i^2/\mu$	23,751–41,107	107,555
Kolmogorov eddy size (μm)	$\eta = (\nu^3/\varepsilon)^{1/4}$	44–29	29

Abbreviations: *P*: power; *V*: bioreactor working volume; N_p: impeller power number; *N*: impeller speed; D_i: impeller diameter; D_t: bioreactor diameter; *H*: bioreactor height; V_{tip}: tip speed; ρ: culture density; μ: culture viscosity; τ: shear stress; ν: kinetic viscosity (μ/ρ); ε: rate of energy dissipation per unit of mass ($\varepsilon = N_p N^3 D_i^2$).
[a] In fed-batch cultures, impeller speed of 260 rpm was used; in perfusion cultures, impeller speed was increased in the range of 260–450 rpm to improve oxygen k_La at high cell densities.

calls for more base for pH control. A few studies have evaluated the effect of different bases (NaOH, Na_2CO_3, and $NaHCO_3$) on CO_2 accumulation and cell viability in perfusion cultures (Goudar et al., 2007; Matanguihan et al., 2001; Ozturk, 1996). Maintaining constant glucose and lactate levels in perfusion cultures through online glucose/lactate monitoring has been shown to be effective in reducing or eliminating base addition (Konstantinov et al., 1996; Ozturk et al., 1997). To achieve such goals, additional real-time glucose/lactate monitoring equipment will have to be used, and this could add complexity in process development. Recently, a few studies have shown the possibility of maintaining good cell growth without pH and DO controls through optimizing engineering parameters of either Wave bioreactors or orbitally shaken bioreactors (OSR) for 7–14 days (Tissot et al., 2011; Yuk et al., 2011). However, none of them demonstrated desired pH control for long-term protein production.

In this study, we explored the possibility of cultivating Chinese hamster ovary (CHO) cells with minimum or no pH control, in both high-density fed-batch (14 days at both 3 L and 200 L scales) and perfusion cultures (up to 40 days at 3 L scale). Typically, pH is determined by the lactate and pCO_2 levels when no base is added in a pre-defined mammalian culture process. Thus, lactate and pCO_2 could be potentially leveraged to maintain a desired culture pH without the need of external pH control loops. In such case, studies on the impact of different bases on cell viability and CO_2 accumulation at different scales could be avoided since base addition will not be required. For fed-batch cultures, process performances at different controlling pH ranges were evaluated. High-density cultures ($21.7 \pm 1.8 \times 10^6$ cells/mL) in the 3 L and 200 L bioreactors were evaluated to demonstrate the pCO_2 difference from different bioreactor scales and sparger configurations, and the potential impact of that on culture pH. For perfusion cultures, the effectiveness of maintaining bioreactor pH in two distinguishably different processes with steady state cell densities at 42.5 ± 3.3 or $68.3 \pm 6.0 \times 10^6$ cells/mL without CO_2 sparging and base addition was assessed. The impact of antifoam addition on CO_2 stripping and the impact of pH fluctuations on product quality attributes were also discussed.

2. Materials and methods

2.1. Cell line and inoculum expansion

A recombinant CHO cell line developed in-house was used in the study. It was a glutamine synthetase (GS) cell line designed to produce a monoclonal antibody. The inoculum train started from vial thaw and expanded in shake flasks and Wave bioreactors (GE Healthcare, Uppsala, Sweden). Both shake flasks and Wave bioreactors were maintained at 5% CO_2 and 36.5 °C.

2.2. Batch cultures (15 mL)

Microbioreactors (ambr15™, Sartorius Stedim, Germany) were used in the initial pH evaluation. The microbioreactors were inoculated at a target cell density of 0.5×10^6 cells/mL with a working volume of 15 mL. Merck proprietary basal medium was used (contains 2 g/L $NaHCO_3$). Bioreactor temperature was controlled at 36.5 °C. DO was controlled at 30% of air saturation using pure O_2. A pH range of 6.60–7.50 was evaluated, in which CO_2 sparging and 0.5 N NaOH were used to control pH at the setpoints with a dead-band of 0.10. Viable cell density (VCD) and viability were measured using the trypan blue dye exclusion method on an in-line Cedex Hi-Res cell counter (Roche Diagnostics GmbH, Mannheim, Germany). Glucose, lactate, glutamine, glutamate, and ammonium were analyzed on a RX Imola analyzer (Randox Laboratories, Ltd., Crumlin, UK). Final culture osmolality and sodium concentration were measured using a Nova FLEX bioanalyzer (Nova Biomedical, Waltham, MA). Additional 2.5 M D-Glucose solution (Sigma-Aldrich, St. Louis, MO) was bolus fed on day 4 and day 6 as shown in Supplementary materials Fig. S1.

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