ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Automated Dynamic Fed-Batch Process and Media Optimization for High Productivity Cell Culture Process Development

Franklin Lu,[1] Poh Choo Toh,[2] Iain Burnett,[1] Feng Li,[1] Terry Hudson,[1] Ashraf Amanullah,[1] Jincai Li[1]

[1]Oceanside Pharma Technical Development, Genentech, Inc., 1 Antibody Way, Oceanside, California 92056; telephone: +(86)21-50462814; fax: +(86)21-50461000; e-mail: jlisd2008@gmail.com

[2]Bioprocessing Technology Institute, Singapore 138668, Singapore

ABSTRACT: Current industry practices for large-scale mammalian cell cultures typically employ a standard platform fed-batch process with fixed volume bolus feeding. Although widely used, these processes are unable to respond to actual nutrient consumption demands from the culture, which can result in accumulation of by-products and depletion of certain nutrients. This work demonstrates the application of a fully automated cell culture control, monitoring, and data processing system to achieve significant productivity improvement via dynamic feeding and media optimization. Two distinct feeding algorithms were used to dynamically alter feed rates. The first method is based upon on-line capacitance measurements where cultures were fed based on growth and nutrient consumption rates estimated from integrated capacitance. The second method is based upon automated glucose measurements obtained from the Nova Bioprofile FLEX® autosampler where cultures were fed to maintain a target glucose level which in turn maintained other nutrients based on a stoichiometric ratio. All of the calculations were done automatically through in-house integration with a Delta V process control system. Through both media and feed strategy optimization, a titer increase from the original platform titer of 5 to 6.3 g/L was achieved for cell line A, and a substantial titer increase of 4 to over 9 g/L was achieved for cell line B with comparable product quality. Glucose was found to be the best feed indicator, but not all cell lines benefited from dynamic feeding and optimized feed media was critical to process improvement. Our work demonstrated that dynamic feeding has the ability to automatically adjust feed rates according to culture behavior, and that the advantage can be best realized during early and rapid process development stages where different cell lines or large changes in culture conditions might lead to dramatically different nutrient demands.

Biotechnol. Bioeng. 2013;110: 191–205.

© 2012 Wiley Periodicals, Inc.

Correspondence to: J. Li

Received 5 August 2011; Revision received 22 June 2012; Accepted 26 June 2012
Accepted manuscript online 5 July 2012;
Article first published online 1 September 2012 in Wiley Online Library (http://onlinelibrary.wiley.com/doi/10.1002/bit.24602/abstract)
DOI 10.1002/bit.24602

KEYWORDS: CHO cell culture; fed-batch; dynamic feeding; capacitance; automated sampling; Nova Flex

Introduction

Several different modes of bioreactor operations have traditionally been used to optimize production processes and maximize titer for monoclonal antibody production. Methods have varied from the simple batch and fed-batch process to the more complex continuous culture or perfusion culture (Bibila and Robinson, 1995). Among these approaches, fed-batch processes are the most widely employed due to their ease of operation, flexibility, and robustness. In these fed-batch processes, high monoclonal antibody yields of up to 10 g/L have been achieved through feeding enhancement and optimization, which increase cell productivity, maintain cell viability, and extend culture longevity (Huang et al., 2010; Yu et al., 2011). These strategies typically involve feeding cultures with a concentrated feed media either daily, continuously or at fixed intervals with fixed feed volumes. However, this strategy does not take into consideration the variation in growth and nutrient requirements that can occur in different cell lines or even with the same cell line due to biological variability which in turn can lead to nutrient depletion or accumulation of by-products in the culture.

Dynamic feeding on the other hand has been utilized as a method to adapt a process to the real time nutrient demand of cells through a variety of feeding algorithms (Wlaschin and Hu, 2006; Zhang et al., 2004). Early efforts in dynamic feeding largely focused on maintaining the cultures at a low glucose and/or glutamine level in order to reduce by-product accumulation, in particular lactate and ammonia, while also trying to balance overall nutrient demand (Gong et al., 2006; Kuwae et al., 2005; Sauer et al., 2000). This was achieved by growth and nutrient consumption modeling, coupled with more frequent manual sampling, calculation

© 2012 Wiley Periodicals, Inc.

and manual feeding (Xie and Wang, 1994a,b). Additionally, Zhou (1994) and Zhou et al. (1995) established automated oxygen uptake rate (OUR) measurement, and used that to estimate glucose consumption via the assumed stoichiometric ratio between glucose and oxygen, and then fed accordingly to maintain a low glucose level. However, periodic manual sampling for glucose measurement was required to adjust the glucose to oxygen ratio as it varied during the course of the culture. Other efforts included measuring and monitoring single specific nutrients, for example, glutamine, with an automated aseptic online sampling loop, and feeding glutamine accordingly to maintain a low level (Chee Furng Wong et al., 2005; Lee et al., 2003). More recently, dynamic feeding based on the manual calculation of integral viable cell concentration has been demonstrated to produce an optimized process with titers above 10 g/L (Huang et al., 2010). In all cases, better growth and productivity were demonstrated with successful control of the by-products lactate and ammonia. However, these feeding strategies are still very labor intensive involving frequent sampling, recalculation, and manual adjustments.

In spite of the various successful applications, dynamic feeding as a process development platform has not been explored extensively. Dynamic feeding has the benefit of adapting the feed strategy simultaneously with the evaluation of other process changes to reach an optimal process with significantly fewer experiments. For instance, a traditional problem with the use of a predefined platform during clone selection is that with a fixed feed strategy, the clone that best fits the platform will be chosen as opposed to the clone with the best potential. Alternatively, by allowing the process to adapt to the growth and consumption of each individual clone, the most efficient path to the optimal clone and process can be achieved simultaneously. In addition, due to further developments in the technology of on-line probes, bioreactor control systems, and automated sampling equipment it is of interest to evaluate such industrial platform processes with a completely automated dynamic fed-batch process. These advancements can supplement existing process measurements and adapt the cultures based on real-time nutrient demand.

In this work we demonstrate several distinct methods of on-line measurements in order to determine a dynamic feed rate for optimal cell culture protein productivity including capacitance and online measurements of viable cell density and glucose. Capacitance measurement has previously been proven to be a useful tool in providing real-time biomass monitoring in fed-batch fermentations (Arnoux et al., 2005) and cell culture (Opel et al., 2010). Capacitance measurements have also been successfully employed to obtain online cell concentration for feed rate calculations in the fed-batch fermentation of *Monascus* sp. for yellow pigment production (Krairak et al., 2000). In this work we incorporate the use of capacitance measurements as a surrogate for cell growth to dynamically determine feed rate through a completely automated, predictive method. Capacitance measurement is advantageous as an indicator for dynamic fed-batch process due to its continuous on-line signal and does not require any multivariate chemometric analysis (Junker and Wang, 2006; Kiviharju et al., 2008; Teixeira et al., 2009), thus simplifying the integration into feed algorithms. We also demonstrate the use of an automated on-line sampler to measure viable cell counts and glucose at pre-determined intervals which could then in turn be used by the bioreactor control system to modulate the feed rate of a complex stoichiometrically balanced feed. Previous methods of dynamic feeding have all required user intervention to manually adjust feed rates making these types of experiments labor intensive to implement into widespread development (Yu et al., 2011), but a fully automated system makes an adaptive platform feasible. We present two different models of control, a predictive method used to estimate future nutrient demand based on past consumption and growth, and a feedback mechanism used to maintain the setpoint of an indicator metabolite. Both of these methods use a feed that balances key components relative to one another through an iterative analysis of nutrient consumption. Taken together, we aim to demonstrate a rapid, systematic method of cell culture process development and optimization with the capability to rapidly develop high titer processes.

Materials and Methods

Cell Line and Culture Conditions

Two recombinant CHO cell lines, cell line A and cell line B, expressing two distinct IgG antibodies were maintained as continuous seed trains in a proprietary serum-free medium under selective pressure of either methotrexate or methionine sulfoximine. The cells were passaged every 3–4 days in shake flasks at a seeding density of 0.3E6 cells/mL. These seed train cultures were kept in incubators controlled at 37°C and 5% carbon dioxide, and were shaken at a speed of 150 rpm. The inoculum for the production cultures were cultivated in 3 L bioreactors (Applikon, Foster City, CA) without selective pressure with a starting densities of 0.8 to 1E6 cells/mL controlled at 37°C at pH 7 (Mettler Toledo, Bedford, MA) and 30% dissolved oxygen (DO; Broadley James, Irvine, CA) with an agitation of 275 rpm. Fed batch production cultures were seeded from inoculum cultures at 1.0 to 2.0E6 cells/mL in a proprietary chemically defined medium without selective pressure. These cultures were controlled at the same conditions as the inoculum cultures except for the temperature setpoint, which was shifted from 37 to 35°C on Day 2 or 3 of the production culture. For the initial baseline studies and improved feed testing, glucose was supplemented separately from the rest of the feed solution as a 500 g/L solution when the glucose concentration decreased to below 3 g/L. For later glucose feedback experiments, the glucose was incorporated into the feed solution. The culture duration for these fed-batch processes ranged between 14 and 20 days.

Cell Culture Media

All media for the fed-batch processes were proprietary and chemically defined. The feed medium was optimized as discussed in the Results Section. Basal media was pH and osmolality adjusted before use. Feed media was formulated without pH and osmolality adjustment.

Fed-Batch Process

The standard fed-batch production process used a bolus feed addition at 10% (or 5% with concentrated feed medium) of culture volume, every 3 days starting on Day 3. Initial experiments continued the bolus feed until Day 9 but later fed batch experiments with optimized feed medium continued the feeds every 3 days until the end of the culture. Pumps were calibrated before each run through Delta V (Emerson, St. Louis, MO), and the feed source was placed on scale to monitor accuracy of additions. The dynamic fed batch algorithm employed in the experiments was set up through customized Delta V software. This algorithm enabled the culture to be fed based on one of two methods, a predictive method or a feedback-based algorithm. In the predictive method, the viable cell density was monitored using a BioProfile FLEX® (Nova Biomedical, Waltham, MA) automated cell counter or using the capacitance probe. At predetermined intervals, the current measure of the cell density (X_0), either from trypan blue counts or capacitance, and the previous measure of cell density (X_{-1}) were used to calculate a specific growth rate to predict the future cell density or capacitance (X_{+1}). The predicted future value could then be used to determine a future integrated capacitance or integrated viable cell count. A nutrient consumption rate (R_1) could then be calculated using the current nutrient concentration (Y_0), previous nutrient concentration (Y_{-1}), previous feed volume (F_{-1}), feed concentration (Y_c), current tank volume (V_0), and previous tank volume (V_{-1}) to determine the total amount of feed per cell per unit time required as shown by Equation (1)

$$\text{Nutrient consumption rate}\left(\frac{\text{mmol}}{\text{cell-d}}\right) = R_1 = \frac{\text{previous} - \text{current} + \text{fed}}{\text{integrated area}} = \frac{Y_{-1}V_{-1} - Y_0V_0 + F_{-1}Y_C}{\text{IVC}_0} \quad (1)$$

Alternatively, nutrient consumption rates from previous experiments could be used as estimates. Once the nutrient consumption rate was determined, the final feed rate could be calculated by Equation (2):

$$\text{Feed volume} = \frac{\text{predicted consumption (mmol)}}{\text{concentration in feed (mmol/mL)}} \text{ where}$$

$$\text{predicted consumption} = \text{consumption rate} \times \text{predicted IVC} - (\text{current nutrient concentration} - \text{desired nutrient concentration}) = (R_1\text{IVC}_{+1}) - (Y_0V_0 - (Y_SV_0)) \quad (2)$$

where R_1 is the nutrient consumption rate defined in Equation (1), IVC_{+1} is the predicted integral of viable cell concentration (or surrogate such as integrated capacitance), from the current time to the next timepoint, V_0 is the current volume of the culture, and Y_S is the desired setpoint of the nutrient concentration. The final term, $Y_0V_0 - (Y_SV_0)$, represents a correction factor to adjust feed volume in the case that the predicted nutrient consumption rate is inaccurate.

For glucose-based autosampler feedback, a separate algorithm was used in order to simplify the feed method. At each sample point, an amount of feed was added to the culture to bring the glucose concentration to a target level as described by Equation (3)

$$\text{mL to add} = \frac{[\text{target glucose (g/L)} - \text{measured glucose (g/L)}]V_0\text{(mL)}}{\text{glucose concentration in feed (g/L)}} \quad (3)$$

For viable cell concentration (VCC) based autosampler feedback cases, the calculation was based on a pre-determined per cell consumption rate and concentration of asparagine in the feed, as described by Equation (4).

$$\text{mL to add} = [\text{VCC}] \times V_0 \times \text{R/Freq} \times [\text{Asn}] \quad (4)$$

VCC: cell count (cells/mL)
V_0: culture volume (mL)
R: asparagine consumption rate(mmol asn/cell day)
Freq: sampling frequency (1/day)
Asn: asparagine feed concentration(mmol asn/mL)

In Process and Offline Measurements

Online capacitance (Fogale Biotech, Cambridge, MA) readings were taken continuously during the runs of the predictive model to facilitate the feeding algorithm. The capacitance reading was taken directly from the probe measurement with parameters set as described in Opel et al. (2010) and every 4 h these readings were used as described in the fed-batch process above. The offline VCC, viability, pH, ammonia, osmolality, pCO_2, pO_2, Ca++, K+, Na+, glutamine, glutamate, glucose and lactate were all monitored using a BioProfile FLEX® (Nova Biomedical). Autosamplers were setup and cleaned as described in Derfus et al. (2009). Amino acid analysis and vitamin analyses (by high performance liquid chromatography, HPLC), as well as trace element analysis (by inductively coupled plasma mass spectrometry, ICP-MS) were also performed after each experiment. Titer was measured through HPLC with Protein A columns.

Antibody Product Quality Analysis

The product quality attributes of antibodies, including level of aggregation, acidic and basic variants, and various glycoforms were measured and compared. The various product quality analysis methods were previously described (Li et al., 2012).

Errors, Uncertainties, and Reproducibility of Results

Most data presented here are from representative singlet conditions as the overall effort was an iterative process so each experiment varied slightly. Error bars presented are based on error of analytical measurements only and are described in each corresponding figure. We feel confident in the results and general applicability of the method, as several sets of iterative conditions were studied. We saw similar improvements in two cell lines (Fig. 9) with three different on-line inputs (capacitance, glucose, and OUR) and the performance trend was consistent throughout the optimization process (Table I).

Results

Baseline Data (Platform Feed Formulation, Various Timing)

In order to provide baseline data, cell lines A and B were evaluated in a standard fed-batch process as described in the Materials and Methods Section. These cell lines were chosen due to their high titer and high nutrient consumption rates in order to maximize the potential improvements of dynamic feeding. The results of cell line A are shown in Figure 1. Nutrient data was also collected in the initial baseline experiments in order to redesign the chemically defined feed media and to evaluate the impact of feed timing. The cultures were fed with the same feed medium, either by bolus feeding (10% of working volume on Day 2, 5, and 8), daily feeding (adjusted daily based on VCC), or continuous feeding (1.8 mL/L/h starting at Day 2). All cases were designed to deliver similar total feed volumes (Fig. 1F) and resulted in similar growth and titer profiles (Fig. 1A and B). This initial data suggested that when the total amount of nutrients is controlled, a variety of feed timings and volumes could still be used to produce comparable results. While growth and titer were not impacted by feed timing, amino acid time course profiles revealed that all three cultures were exhausted or limited in several key nutrients including cysteine, asparagine, and tyrosine during the late stage of the run (Fig. 1C–E) which may have led to slowed product accumulation during the late stage of the culture.

Feed Media Design and Optimization

The platform feed medium was redesigned and refined after several experiments according to the flow chart shown in Figure 2 to obtain a stoichiometrically balanced feed medium. For this iterative method, several experiments were required in order to assess the effectiveness of the feeding method for maintaining consistent nutrient consumption rates since adjustment of the feed could potentially change the relative consumption rates. Daily amino acid concentrations were used to calculate daily consumption rates that were then averaged to determine an overall consumption rate. The relative ratios for all components of the feed medium were then normalized to an indicator metabolite. When cultures were fed based on the indicator metabolite, other components were supplemented in stoichiometric amounts to avoid exhaustion or over accumulation. In these studies, asparagine was selected as one benchmark metabolite because of its role as one

Table I. Evolution of feed media.

	Platform feed	CDF1	CDF2	CDF3
Feed concentration of unlisted metabolites	1×	1×	2×	1.5×
Asparagine (g/L)	5.8	8.1	16.1	20.2
Tyrosine (g/L)	1.7	5.1	4.8	6.0
Cysteine (g/L)	2.0	2.0	5.2	6.6
Zinc sulfate (mg/L)	1×	15	30	23
Ferric citrate (mg/L)	1×	40	80	60
Other amino acids	1×	1.4× leucine, lysine, serine (others unchanged)	2× of CDF1	Glycine, threonine, valine, isoleucine, leucine, serine 1.8–2.5× of CDF1 (others 1.5× of CDF1)
Glucose (g/L)	50	50	50	80
Osmolality (mOsm/kg)	850–900	850–900	1,400–1,450	1,500
pH	6.5	6.5	7.5	7.5
Titer (cell line B)[a]				
Bolus feed	4.1 ± 0.2 (3 feeds to Day 9)	Not performed	6.0 ± 0.3 (3 feeds to Day 9)	7.0 ± 0.4 (5 feeds to Day 14)
Dynamic feed	Not performed	5.4 ± 0.3	7.6 ± 0.4	8.6 ± 0.4; 9.1 ± 0.5

[a]Titer ranges ($\pm t$) are based on analytical error.

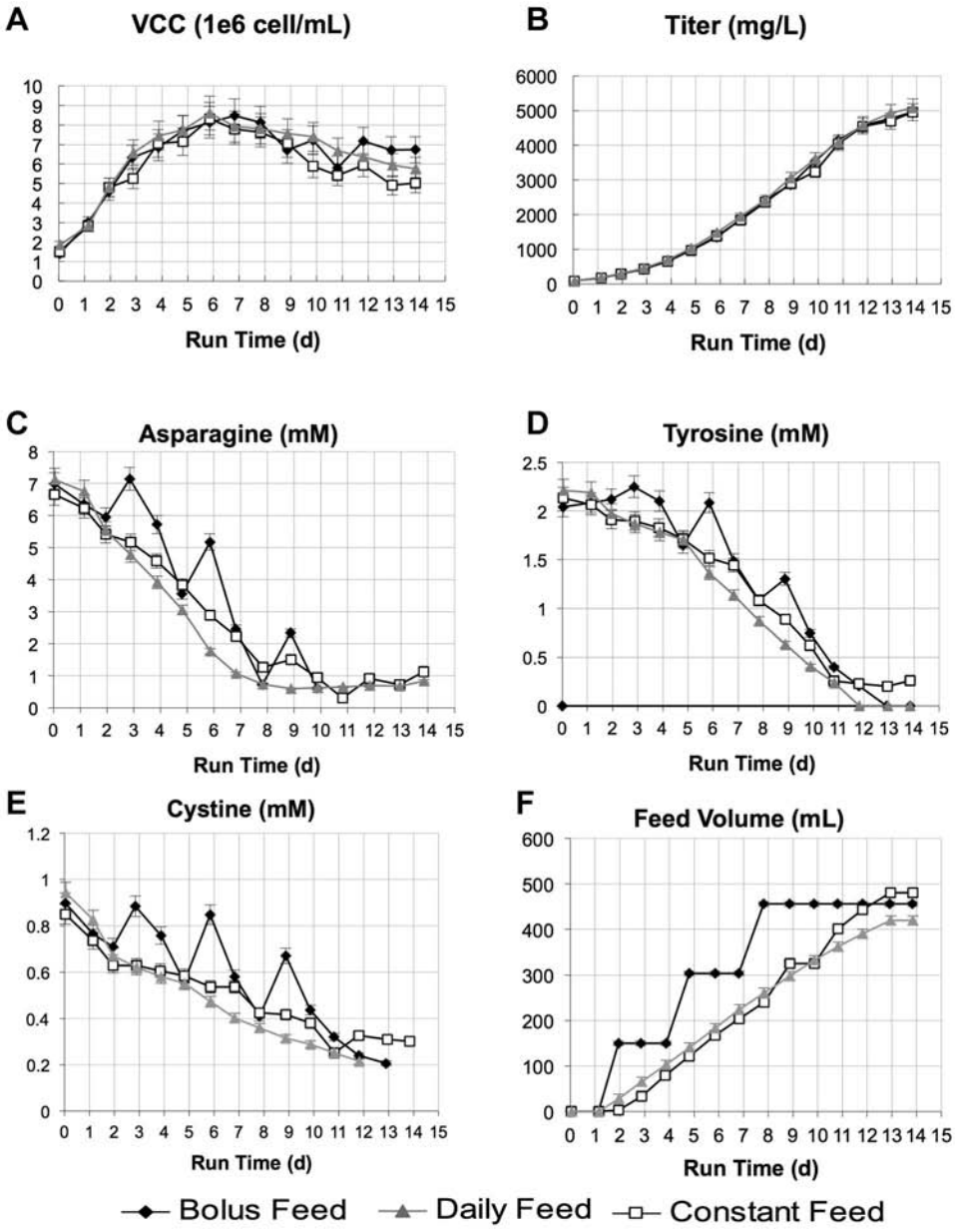

Figure 1. Three cultures of cell line A, designed to receive about the same volume of a chemically defined feed formulation (CDF1) were evaluated with either bolus feeding (10% WV on Day 2, 5, 8), daily feeding (adjusted daily based on VCC), or continuous feeding (1.8 mL/L/h starting at Day 2). **A**: VCC; (**B**) titer profiles of baseline fed-batch runs were similar independent of feeding method; (**C**) asparagine; (**D**) tyrosine; (**E**) cystine amino acid profiles indicating nutrient limitation in later part of culture; and (**F**) total feed volume profile. Error bars are based on analytical accuracy of measurements: VCC ± 5%, titer ± 5%, amino acids ± 5%, and feed volume (by weight) ± 5 g.

of the key energy sources in the tricarboxylic acid (TCA) cycle and its relatively consistent consumption rate throughout our baseline study cultures. In addition, asparagine is regarded as a key amino acid for product synthesis, as depletion of asparagine had been shown to lead to decreases in cell specific productivity (unpublished observations). Other metabolites such as glucose were also used as an indicator due to its key role as a primary energy source.

The evolution of the feed media is described in Table I. As shown in Figure 1, cultures with the initial platform feed showed depletion of several amino acids. Therefore, the original platform feed was adjusted with higher levels of asparagine, tyrosine, leucine, lysine, and serine to create chemically defined feed #1 (CDF1). In addition, zinc and iron levels were also increased in CDF1. With CDF1, the culture was able to maintain appropriate levels of most of the amino acids, for example, asparagine, tyrosine, serine,

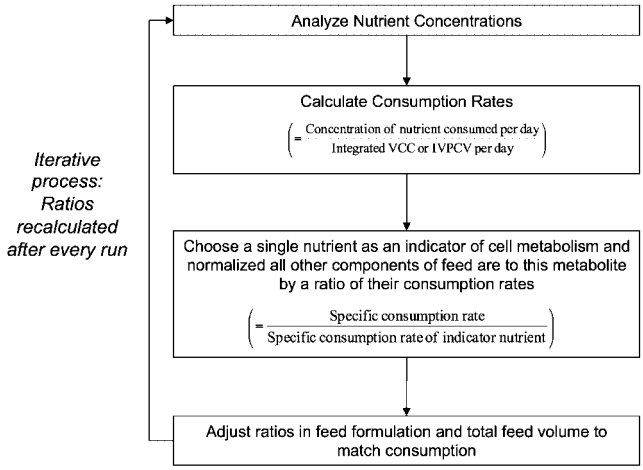

Figure 2. Iterative method of a complex stoichiometrically balanced feed.

and isoleucine. However, the total feed volume increased about 40% compared to the control culture (data not shown), resulting in significant dilution and thus limiting increase of volumetric productivity. The majority of the feed components were then concentrated by 2× in order to minimize dilution effects, and relative ratios were further adjusted (chemically defined feed #2, CDF2). CDF2 was tested for cell line B with asparagine as indicator metabolite, and average asparagine consumption rate from platform bolus feed culture was used as pre-determined nutrient consumption rate. However, significant accumulation of asparagine as well as serine and isoleucine was observed, as actual asparagine consumption rate on cell line B culture was lower than predicted. The overall concentration of the feed medium was then lowered slightly to 1.5× of CDF1 and ratios of several components were further refined for the final version, chemical defined feed #3 (CDF3), to accommodate the changes in consumption rates. This resulted in balanced nutrient profiles, as shown in Figure 3 of asparagine, tyrosine, serine, and isoleucine profiles. In addition, vitamin and trace metals profiles were also monitored and adjustments were made throughout the feed medium optimization (data not shown). The titer results in

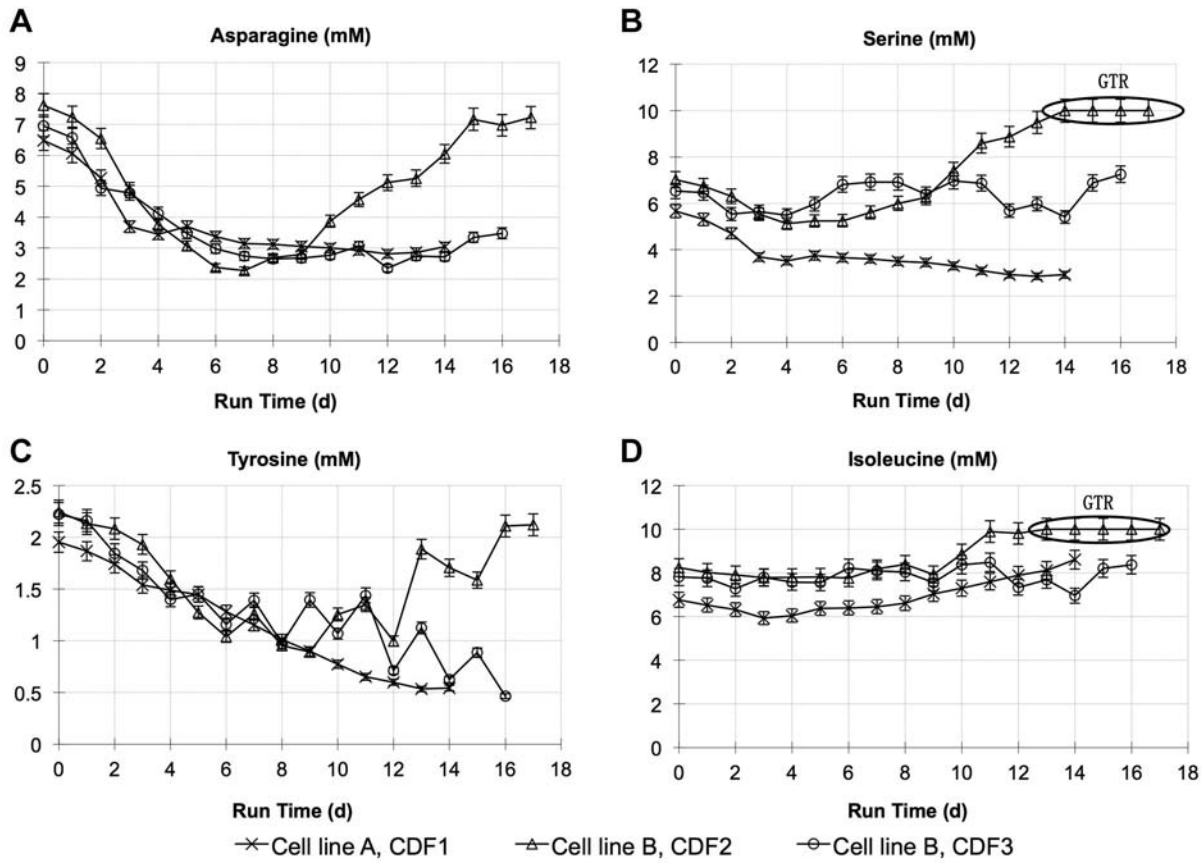

Figure 3. Amino acid profiles of (**A**) asparagine, (**B**) serine, (**C**) tyrosine, and (**D**) isoleucine during dynamic feeding with evolved feed medias CDF1, CDF2, and CDF3 with both cell line A and B. Error bars are based on analytical accuracy of measurements: amino acids ± 5%. GTR (greater than measurement range of 10 mM).

Table I showed the performance improvement trends for cell line B throughout the optimization process.

Design of Dynamic Feeding algorithm

Two different types of dynamic feeding algorithms were employed in this work. The predictive method utilized an online indicator of cell growth and calculated the expected growth rate and cell density in order to estimate future nutrient demand. The alternative approach was a feedback-based approach in which the historical nutrient consumption is used to determine amount of feed to maintain an indicator metabolite at a target. With the automated sampling method, the sampling interval is set frequent enough (~6 h) to avoid dramatic changes in cell mass and the target concentration is set high enough to avoid exhaustion between feeding intervals. In either method, dynamic feeding is not being used in a nutrient limited mode to alter metabolism, but solely as a means to determine how much and how often to feed, which may avoid potential changes in product quality due to altered metabolism (Chee Furng Wong et al., 2005). In addition, the dynamic feed was restricted to small bolus feeds that occurred several times a day. Consequently, nutrient composition was never fully static, but was controlled within desired ranges to optimize productivity.

One of the key advancements of this work was integration of our feeding algorithms into our control system. Delta V is a process control platform that can be easily scaled for large bioreactor networks. In this work, we were able to build custom modules within the control system that linked to novel inputs to build an additional layer of control beyond that of the typical PID control. Two primary advancements were key to these developments: the integration of on-line process monitoring which was able to provide real time analysis of key variables (i.e., capacitance) and the automation of at-line sampling that is typically done by hand by researchers using Nova Bioprofile Automated Sampler system. In collaboration with Nova Biomedical, we were able to build a custom OPC module to read additional parameters that could be measured on an adaptable schedule.

Figure 4 depicts the set up of the automated fed-batch process. Initially, a signal (capacitance, OD, OUR, cell count, or glucose) was collected in order to assess the current state of the bioreactor. Once the current state of the bioreactor had been assessed, previous data could also be used to predict future nutrient demand. In the case of capacitance, the specific growth rate was coupled with a predetermined nutrient consumption rate to account for changes in cell mass before the next feed point. Finally, after each pre-determined interval, the feed pump was activated to supply the required volume of feed.

In order to account for noise and aberrant readings, correction factors were incorporated into both algorithms. For the predictive methods an additional term were built in to the algorithms to handle over or underfeeding (the second term of Equation 2).For the feedback method, the response to fluctuation is built into the feedback control so that if at any one data point, an excess of feed is added, the feed volume added will be reduced or eliminated upon the next measurement. Neither of these algorithms are able to immediately discard an errant data point, which is a weakness of the system, as an incorrect measurement could lead to a large spike of unneeded feed. While not

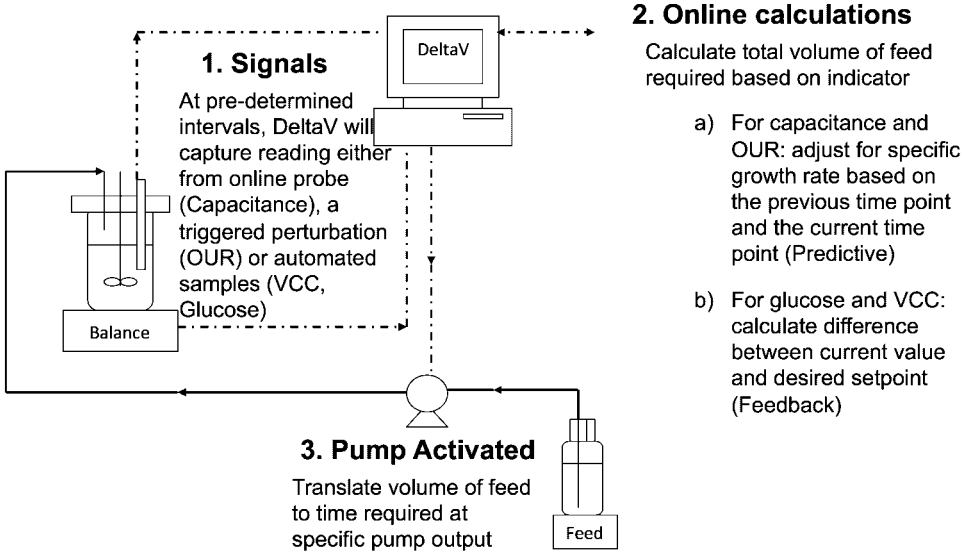

Figure 4. Design of automated dynamic feeding system.

implemented for these experiments, a criteria could be implemented that would trigger a re-calibration and recalculation of feed addition if, for instance, the calculated consumption rate changed more drastically than expected. These sort of error checks would be simple for an automated method to incorporate once bi-directional communication with analytical instruments is enabled. Currently, this is possible with the Nova Flex and was in development at the time of this work.

Capacitance Based Predictive Method Using Optimized Feed Medium: Cell Line A

The automated system described above was first attempted using the predictive model with online capacitance measurement. In addition, the feed medium formulation was optimized from the original platform feed medium after several iterative experiments. Figure 5 shows the results using the optimized feed medium CDF3 for cell line A. The dynamic feed was based on capacitance measurement and was added every 4 h. A bolus feed culture was also performed for comparison, where a feed volume of 5% initial culture volume was delivered every 3 days until Day 15. Overall, the dynamic feeding case and the manual bolus feeding case had very similar performance, as shown by the growth and productivity profiles (Fig. 5A and B). Ammonium increased more rapidly in late phase of the dynamic feeding culture (Fig. 5C), potentially due to higher levels of asparagine available, which may have resulted in higher rate of deamidation to aspartatic acid. Asparagine concentration was well maintained throughout the cultures, although there were more day-to-day changes for the bolus feeding case compared to the dynamic feeding case (Fig. 5D). Other nutrient and metabolism profiles were also similar between the two cultures (data not shown). Titer reached 6.1 and 6.3 g/L for the bolus and dynamic feeding cases, respectively, which represents about 25% increase when compared to the platform bolus feeding cultures shown in Figure 1. The improvement in titer appeared to be primarily due to optimization of feed formulation and cell line A was not sensitive to the feeding frequency using the optimized feed medium.

Capacitance Based Predictive Method Using Optimized Feed Medium: Cell Line B

The capacitance-based dynamic feeding method was also applied to cell line B with the same optimized feed medium CDF3. Cell line B had marked difference on growth and nutrient consumption rates compared to cell line A (data

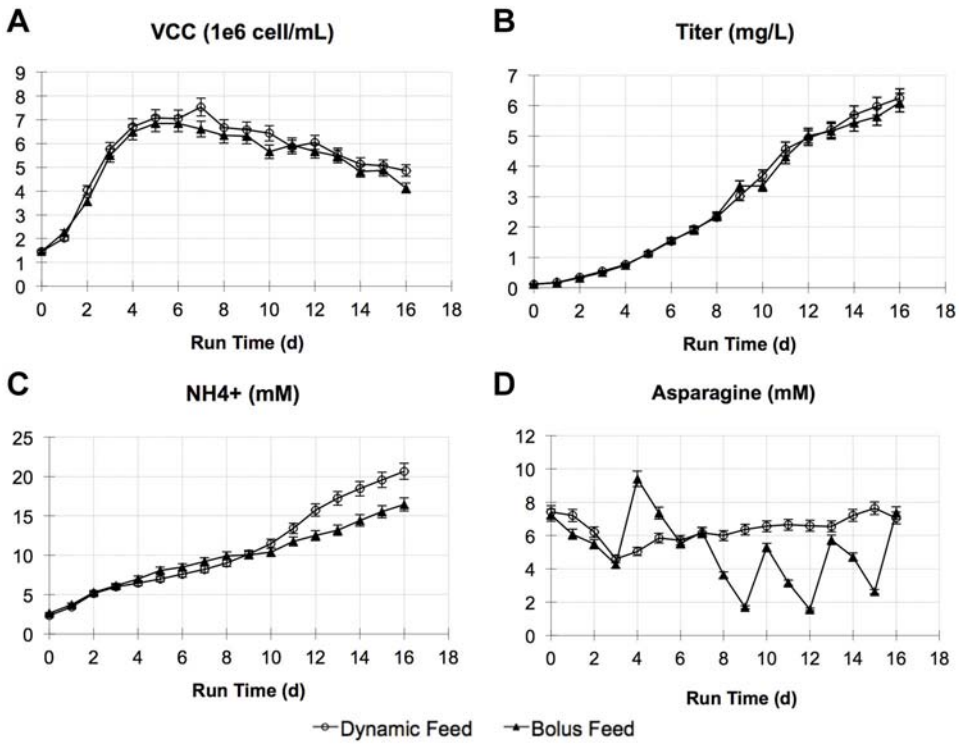

Figure 5. Two cultures of cell line A were tested with optimized feed medium (CDF3) using either the standard bolus feeding method (5% every 72 h) or using the dynamic feeding method with capacitance as on-line feedback signal (fed every 4 h). Comparison of (**A**) VCC, (**B**) titer, (**C**) ammonium, and (**D**) asparagine profiles. Error bars are based on analytical accuracy of measurements: VCC ± 5%, titer ± 5%, ammonium ± 5%, and amino acids ± 5%.

not shown), and thus the pre-determined feeding rate had to be adjusted for each cell line. Specifically, the asparagine feed rate was reduced by about 30% to account for lower specific asparagine consumption rate. For the control case, manual bolus feeding was done every 3 days at 5% initial culture volume using the same CDF3 feed medium. The results are shown in Figure 6. In addition, results from the original platform bolus feed culture are also depicted in Figure 6 for comparison.

Cell growth was significantly higher in the dynamic feeding case than both the improved bolus feed case and the initial platform bolus feed case (Fig. 6A). Peak density was reached around Day 8 in both improved bolus feed and dynamic feeding cases. Osmolality was maintained below 400 mOsm/kg at the end of the culture, indicating absence of significant metabolite accumulation (Fig. 6B). Capacitance profiles tracked closely with offline VCC values until peak VCC was reached. Capacitance then continued to increase for several days before beginning to decrease around Day 11. The divergence between capacitance and VCC profiles had been reported previously, as capacitance depends on cell size and physiological state in addition to biomass (Opel et al., 2010). Titer was also dramatically improved with the optimized feed formulation. Combined with dynamic feeding, cultures were able to reach titers up to 8.6 g/L, a two-fold improvement over the baseline process (Fig. 6C). Cell specific productivity was similar during the early stage of culture, and the capacitance based predictive method maintained productivity at higher levels and for a longer period than bolus feeds (Fig. 6D). The bolus feed case with the same optimized feed was also improved from the baseline, reaching 7 g/L titer, indicating that the rebalancing of the feed as well as higher inoculation density were also significant contributions to the increase in titer. However, even in the optimized bolus feed case shown here, rapid changes of over 50 mOsm/kg still occurred after each bolus feed, potentially negatively impacting growth and productivity relative to the dynamic feeding case. In addition, the total feed volume was not controlled as in the baseline studies. The dynamic feed culture actually used approximately 50% more total feed volume (Fig. 6E) over the entire culture duration compared to the bolus feed

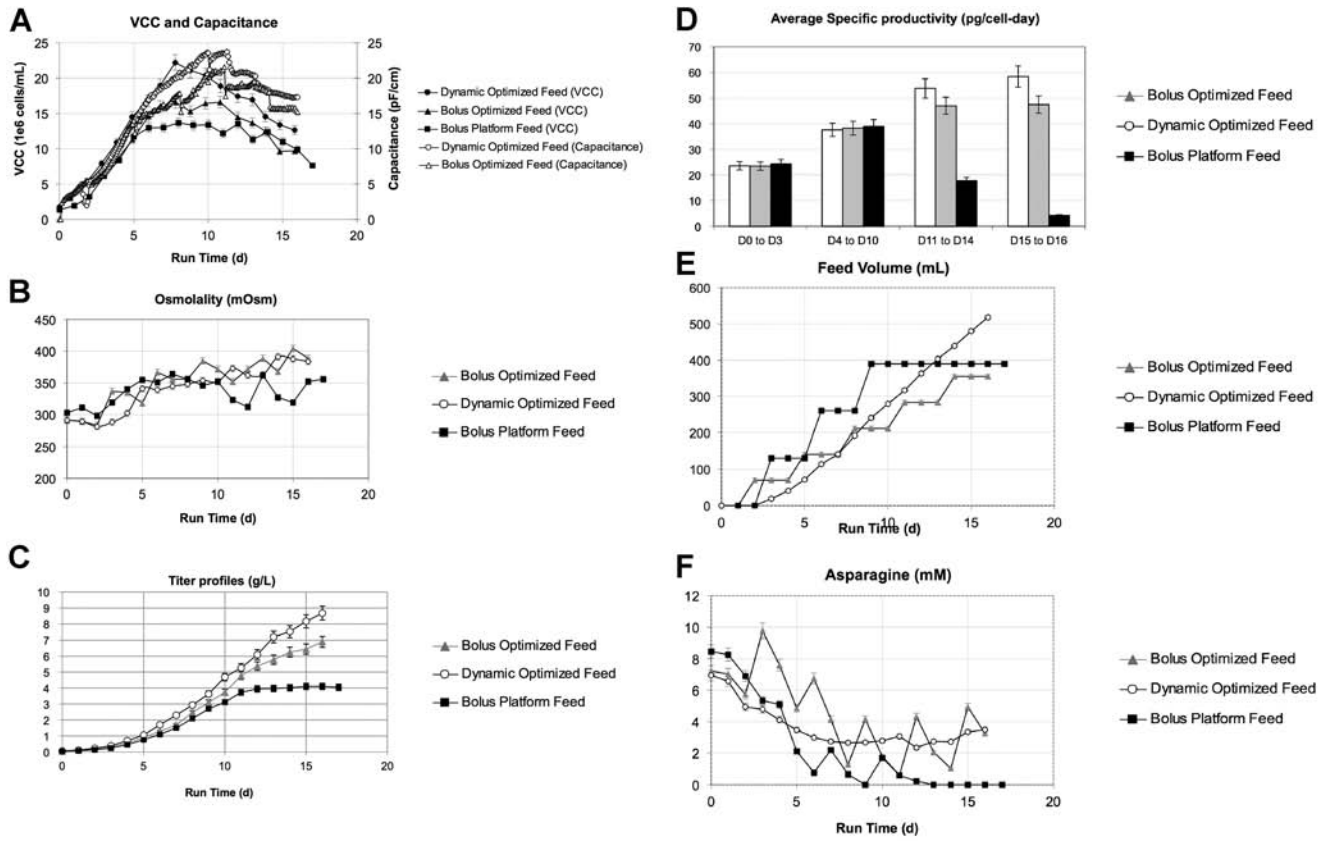

Figure 6. Two cultures of cell line B were tested with CDF3 using either the standard bolus feeding method (5% every 72 h) or using the dynamic feeding method with capacitance as on-line feedback signal (fed every 4 h). Both cultures also incorporated a higher inoculation density of 2e−6 cells/mL to increase build up of cell mass. The platform bolus feed process (with initial platform feed medium and lower inoculation density) is shown for reference: (**A**) VCC (solid symbols) and capacitance (open symbols) profiles, steep drops in capacitance profile in the optimized bolus case were due to volume changes due to feeds; (**B**) osmolality profiles, (**C**) titer, (**D**) average specific productivity, (**E**) total feed volumes, and (**F**) asparagine concentration profiles. Error bars are based on analytical accuracy of measurements: VCC ± 5%, osmolality ± 5 mOsm, titer ± 5%, specific productivity ± 7% (based on propagation of error), feed volume (by weight) ± 5 g, and amino acids ± 5%.

culture, although no amino acids dropped below detectable levels (0.2 mM) in either case. While the lack of nutrient exhaustion demonstrated the successful redesign of the feed formulation using the iterative method (Fig. 6F), it also indicated that feeding the additional volume in the dynamic feeding case led to greater nutrient consumption and increased antibody production. These differences underscore the possibilities that different feed algorithms can result in alternative feed volumes that could result in even higher titers. Consequently, simply searching for nutrient exhaustion might not be sufficient to determine when a process has reached an optimal feed regime.

A key aspect of process improvement is product quality. Differences in product quality have been observed in previous applications of dynamic feeding that was focused on altering cellular metabolism to improve process productivity (Chee Furng Wong et al., 2005). However, in our case our feeding algorithms are not designed to operate in nutrient limited mode, but to more efficiently predict the demand of the culture and adjust feed according to shifts in cell density and cell physiology. Consequently, in our cases only minor differences in key product quality attributes were observed that were all within acceptable ranges for typical molecules (Fig. 7).

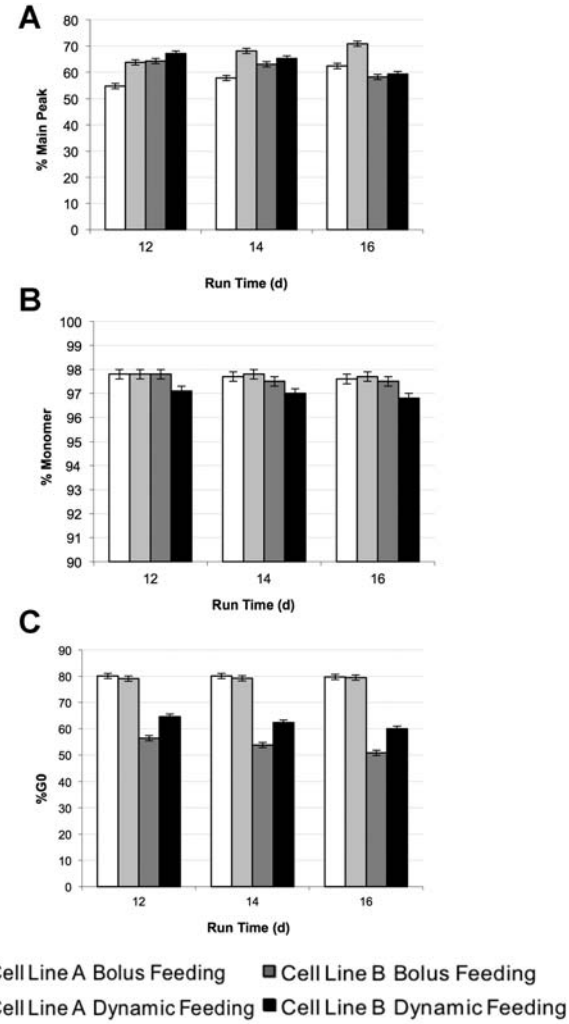

Figure 7. Product quality comparison between dynamic feeding and bolus feeding cases. **A**: %Main peak of charged variants as measured by iCIEF at Day 12, 14, and 16 using the capacitance based predictive method for the two test cell lines. **B**: %Monomer analysis of product by size exclusion chromatography, (**C**) %G0 glycan analysis by capillary electrophoresis. Error bars are based on analytical accuracy of measurements: monomer ± 0.2%, charge variants ± 1%, and glycoforms ± 1%.

Autosampler Based Feedback Control Method Using Optimized Feed Medium: Cell Line B

As an alternative to a predictive model of nutrient demand used in previous studies, we explored a direct feedback mechanism where feed rates were altered dynamically to maintain a preset target of an indicator metabolite. From the limited number of metabolites directly measured by the BioProfile FLEX instrument, glucose was chosen as a key indicator of metabolism. Glucose has the advantage of being a direct indicator of nutrient consumption that inherently incorporates changes in cell growth and volumetric consumption rate. From the capacitance based predictive dynamic feeding study, it was observed that there was a relatively constant ratio between glucose consumption rate and the asparagine consumption rate throughout the culture. This suggested that glucose could be a surrogate indicator for the previously asparagine balanced feed formulation (CDF3) to dynamically feed the cultures. Feed rate in glucose feedback control is determined by Equation (3) described in the Materials and Methods and by in-process glucose measurements. The glucose feedback method benefits from incorporating multiple sources of variability such as changes in cell mass and metabolism into a single direct input that could be used to determine the feed rate.

The feed medium used in this study had one change from CDF3, which was used in the experiment described in Figure 6. Glucose, which had previously been added separately as a second feed, was added to the feed medium and balanced stoichiometrically with other metabolites. The rest of the CDF3 composition remained unchanged. With this method, additional components could be modified or added easily to a chemically defined feed as long as they are balanced relative to other nutrients based on their consumption rates.

Figure 8 shows the results of the autosampler based dynamic feed back control study. In addition to feeding based on the measured glucose concentration, VCC was also used as a feeding indicator in this same study. For the glucose-based feeding, the target glucose level was set at 5 g/L, which was chosen based on previous capacitance based feeding study. For the VCC-based feeding, feed rate was calculated automatically according to Equation (4) in the Materials and Methods Section. In addition, a fourth case from a separate experiment, labeled as ''Manual adjusted feed, every 6 h'' was also included for comparison, where the

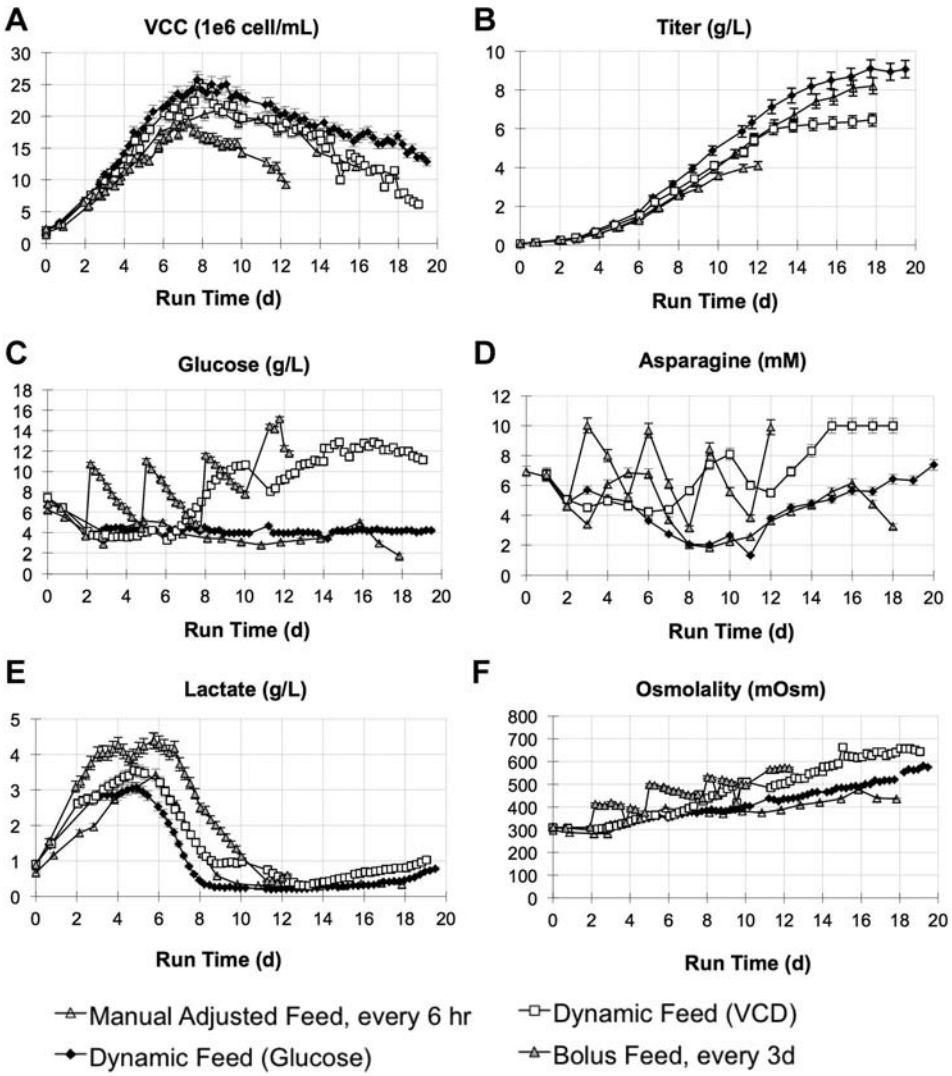

Figure 8. Auto sampler based dynamic feeding results of cell line B. Cultures were inoculated at initial VCC of 2.0e−6/mL and feeding with CDF3 was initiated on Day 2. For the glucose and VCC-based feeding, cultures were sampled every 6 h via the autosampler, and fed automatically based on Equations (3) and (4) after each sampling. A fourth case from a separate experiment, labeled as "manual adjusted feed, every 6 h" was also included for comparison, where the feed medium was also delivered every 6 h, but no auto sampler was used and feed volume was preset once a day based on off-line glucose measurement. The daily adjustment on feed volume was done to target culture glucose level of between 4 and 6 g/L. The bolus feed case was fed every 3 days at 6.7% initial culture volume. **A**: VCC and (**B**) titer profiles indicating cell mass and productivity improved with glucose feedback. **C**: Glucose and (**D**) asparagine profiles illustrating direct and indirect metabolite control through dynamic feeding. **E**: lactate and (**F**) osmolality profiles. Error bars are based on analytical accuracy of measurements: VCC ± 5%, titer ± 5%, glucose ± 0.2 g/L, asparagine ± 5%, lactate ± 5%, and osmolality ± 5 mOsm.

feed medium was delivered in the same frequency as with the glucose based feeding, that is, every 6 h. But no auto sampler was used and feed volume was adjusted only once a day based on off-line glucose measurement so that the same feed volume would be used four times before the next adjustment. The daily adjustment on feed volume was done to target culture glucose level of between 4 and 6 g/L. The design can be interpreted as manual dynamic feeding.

The highest titer of 9.1 g/L was achieved using the glucose feedback method (Fig. 8B). In the auto sampler based VCC feedback case, glucose, osmolality, and asparagine levels were increasing late in the culture, indicating potential nutrient accumulation (Fig. 8C, D, and F). This may be due to the fact that VCC is an indirect indicator of nutrient consumption and the total cell mass may not be an accurate indicator of changing metabolic activity. As a result, the continual overfeeding and resulting osmolality increases may have led to more rapid reduction in VCC (Fig. 8A) and reduced productivity. The bolus feeding case (fed every 3 days at 6.7% initial culture volume) did not perform as well in this study, with culture viability dropping to <60% by Day 12 and titer reaching 4.1 g/L. Compared to the bolus feeding case in Figure 5 where titer reached 7 g/L, a key difference between the two cultures was the increased feed

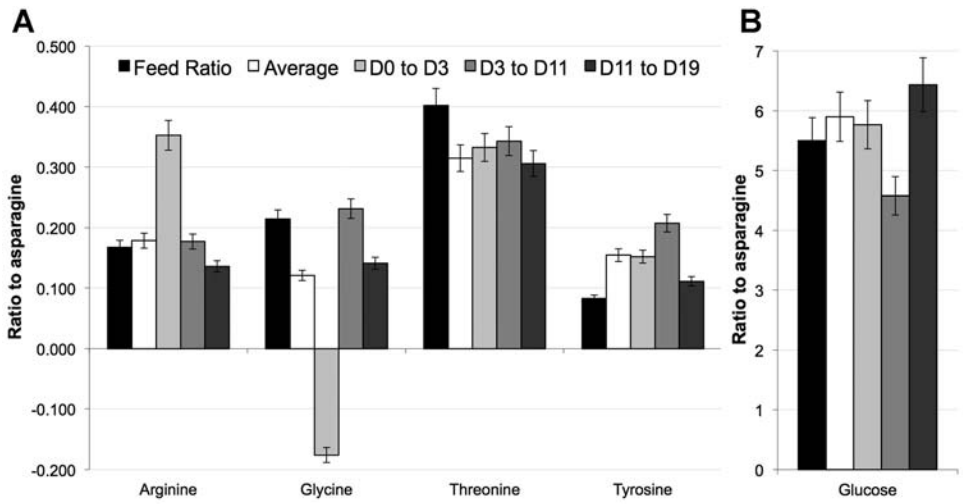

Figure 9. **A**: Ratio of feed concentrations and consumption rates of arginine, glycine, threonine, and tyrosine relative to feed concentration and consumption rates of asparagine in feed versus consumption rates in various stages of culture for auto sampler based glucose feedback culture: (black) indicates ratio in feed medium, (white) indicates consumption rate across entire run, (light gray) consumption rate from Day 0 to Day 3, (gray) consumption rate from Day 3 to Day 11 and (dark gray) consumption rates from Day 12 to Day 19 based on amino acid analysis of cell culture supernatants by HPLC. **B**: Ratio of glucose feed concentrations and consumption rates to asparagine feed concentrations and consumption rates for auto sampler based glucose feedback case. Error bars are based on analytical accuracy of measurements and propogation of error: amino acids ratio $\pm$7%, glucose ratio $\pm$6.7% (based on glucose error of 0.2 g/L and average glucose measurement of 4.5 g/L).

volume from 5% to 6.7% initial culture volume per feed, with the objective of increasing total feed volume to match the dynamic feeding case. However, the bolus feed case might have suffered from apparent high osmolality and high nutrient concentration shock accompanied by each feed, as each bolus feed added about 100 mOsmo to the culture (Fig. 8F). The fourth case with the daily manual adjusted feed rate performed quite well, reaching titer of 8.2 g/L, which was only slightly lower than the 9.1 g/L reached by the glucose-based dynamic feeding case and comparable with the 8.6 g/L reached by the capacitance-based dynamic feeding case in Figure 6.

When looking at the metabolite and nutrient profiles, the glucose feedback case and the daily manual adjusted case tracked very closely in glucose, lactate, asparagine, and osmolality (Fig. 8C–F). In contrast, the VCC-based dynamic feeding case and the bolus feed case both had higher glucose, asparagine, and osmolality levels.

Even with glucose as a direct indicator of metabolism, the relative consumption rates of other components varied during the culture, and in some cases the overall consumption rates also differed slightly than predicted from previous studies (Fig. 9A and B). For instance, the average consumptions rate (white bar) of arginine and glucose matched well with the ratios predicted in feed formulation (black bars), but other components such as tyrosine and glycine showed slight differences from expected relative consumption. In addition, as expected, the relative consumption rates also varied throughout the length of the culture from relatively minor changes of glucose to more dramatic shifts of components such as glycine.

Discussion

Most industrial mammalian cell culture processes still use manual bolus feeding methods for nutrient feeding (Kelley, 2009). This is in contrast to an industrial microbial fermentation process where automatic, dynamic feeding often is necessary to avoid nutrient over-accumulation and waste product formation. Examples include widely used dissolved oxygen (DO-stat) or pH (pH-stat) based feeding strategies for *E. coli* fermentation (Chen et al., 1997; Kim et al., 2004). The difference in practice is largely due to the fast metabolism of microbial cells in comparison to mammalian cells and hence the perception that mammalian cultures do not need to be fed so frequently. In this work, part of our objective was to understand whether given the same feed medium, the feeding strategy and frequency difference would lead to different culture performance. Little has been reported on such comparison studies. In their work, Pörtner et al. (2004) did compare the various control and feed strategies for a hybridoma cell line expressing a Mab. However, the studies with the various feed strategies were not conducted in parallel; rather they were done over several years, when specific Mab productivity varied significantly. The feed medium used for the studies also varied slightly. Thus strict comparison on productivity across the different methods was not possible. Many other reports compared performance between batch culture and optimized fed-batch culture, but again not among fed-batch culture modes with the same feed medium (Xie and Wang, 1994a,b, 1996; Xie et al., 1997; Yu et al., 2011; Zhou et al., 1995, 1997). To our knowledge, our work is the first that

Table II. Summary of comparisons between preset bolus feed and automatic dynamic feed cultures.

Data source	Cell line	Feed medium	Feeding strategy	Feeding frequency	Feed volume (mL)[a]	Feed volume ratio between the cases	Culture duration (day)	Titer (g/L)[b]
Figure 1[c]	A	Platform feed	Bolus	3 days	420	1	14	5 ± 0.3
			Bolus (daily adjusted)	6 h	456	1.09	14	5.1 ± 0.3
			Continuous	—	480	1.14	14	5 ± 0.3
Figure 5	A	CDF3	Bolus	3 days	388	1	16	6.1 ± 0.3
			Dynamic	4 h	362	0.94	16	6.3 ± 0.3
Figure 6	B	CDF3	Bolus	3 day	373	1	16	7 ± 0.4
			Dynamic	4 h	544	1.46	16	8.6 ± 0.4
Figure 8[d]	B	CDF3[e]	Bolus	3 day	390	1	12[d]	4.1 ± 0.2
			Dynamic, VCC	6 h	746	1.91 (1.18 on Day 12)	18	6.5 (5.4 on Day 12)
			Dynamic, glucose	6 h	640 (395 on Day 12)	1.64 (1.01 on Day 12)	18	9.1 ± 0.5 (6.3 ± 0.3 Day 12)
			Bolus (daily adjusted)	6 h	530 (335 on Day 12)	1.36 (0.86 on Day 12)	18	8.2 ± 0.4 (5.4 ± 0.3 Day 12)

[a]Adjusted based on 1.45 L initial culture volume, ±5 g.
[b]Titer ranges (±5%) are based on analytical error.
[c]Nutrient depletion seen on all three cultures.
[d]Bolus feed rate changed from 5% to 6.7%. High nutrient accumulation, high osmolality seen on the culture with bolus, every 3-day feeding, which resulted in early termination of bolus culture.
[e]The glucose concentration was adjusted to 133 g/L (739 mM) in CDF3 for this experiment to combine a previously separate glucose and nutrient feed into one solution.

offers direct comparison between manual bolus feeding and automatic dynamic feeding with industrially relevant and high producing cell lines. We offered four sets of data (Figs. 1, 5, 6, and 8) across two cell lines for the direct comparison. Table II summarized the studies by highlighting the feeding strategy, frequency and total feed volume. Each set of the studies used exactly the same feed medium, with the only difference being feeding strategy and frequency. The studies shown in Figures 1 and 5 show basically identical performance between manual bolus feeding and dynamic feeding for cell line A, whereas studies on Figures 6 and 8 show higher titer with the dynamic feeding case than the bolus feeding case for cell line B. Consequently, improvements from dynamic feeding will vary based on relative sensitivity of cell line, which may be a combination of sensitivity to large shifts in osmolality from bolus feeds and sensitivity to variations in metabolites that may alter nutrient uptake and metabolism. For some cell lines, such as cell line A, this difference may be negligible.

An interesting observation from this work was that the relative performance among the cases appear to be correlated with total feed volume, with the total feed volume approximately the same between the feeding methods for Figure 1 studies and Figure 5 studies, respectively, whereas for Figure 6 studies, feed volume was about 1.5× higher with the dynamic feeding case. This suggests that it is possible to achieve similar culture performance when similar volumes of feed are delivered via different feeding methods. Consequently, with further adjustments a manual bolus feed could potentially achieve the same titer as a dynamic feed with cell line B. However, two key points need to be considered a priori. The total bolus feed volume would need to be predetermined, and the frequency of bolus feeding needs to be optimized for different feed medium and different processes. It should be noted, however, that for manual bolus feeding to work optimally, appropriate feed dose, and feeding frequency still need to be established for different cell lines and processes. This is especially true for highly concentrated feed medium, for example, CDF3, where feeding in every 3 days at 5% initial volume seemed not enough for cell line B, and feeding in every 3 days at 6.7% initial volume resulted in large osmolality and nutrient shock.

In essence, the key advantage of dynamic feeding, when coupled with feedback control, is the ability to automatically adjust feed rates according to culture behavior, aka, feed by demand, thus avoiding either under-feeding or over-feeding of the cultures. The advantage can be best realized during early and rapid process development stages where different cell lines or large changes in culture conditions might lead to dramatically different nutrient demands, making it impossible to determine feed rates beforehand. Conversely, once a process is well established, a fixed rate, predefined bolus feeding method could very well deliver similar performance. The actual dynamic feed profiles (Figs. 1F and 6E) of this work appear to be relatively constant, where a continuous feed could also potentially replicate results while simplifying implementation. However, these simplifications require first performing dynamic feeding experiment to determine the appropriate feed rate. In addition, even in well established manufacturing processes, the bolus strategy does not take into consideration the variation in growth and nutrient requirements that can occur due to variations in parameters like inoculation density, raw materials or cell age, which in turn can lead to nutrient depletion, or accumulation of by-products in the culture.

In our efforts, we evaluated both the capacitance-based predictive method and the auto sampler-based feedback control method. Similar performance was achieved between the two methods. The predictive method requires accurate

modeling of culture growth rates or nutrient consumption rates (Dowd et al., 2001a,b; Kurokawa et al., 1994; Xie and Wang, 1994a,b). However, since growth rates and nutrient consumption rates vary during the course of any given culture, which have impact on specific substrate uptake rates, an on-line or off-line measurement is needed to either automatically or manually adjust feed rates. In our work, the capacitance signal was successfully used as a surrogate of culture growth, and constant re-calculation of growth rates was done automatically to allow for change of feed rates. The asparagine consumption rate, used as an indicator metabolite for feed rate calculation, was initially derived from historical data and then adjusted throughout evolution of the feed medium formulations. On the other hand, the feedback-based method does not rely on growth or nutrient consumption rate models. Instead, it relies on more frequent measurement of the indicator metabolite, for example, glucose, and the subsequent feeding that is designed to bring the indicator metabolite to a preset control level (Chee Furng Wong et al., 2005; Lee et al., 2003; Zhou et al., 1995, 1997). This strategy has been successfully used to control low levels of glucose and glutamine, but for this work glucose was maintained at around 4–5 g/L in order to keep the culture from either over-feeding or under-feeding. Glucose as a feed indicator is simple, requires the least prior knowledge and is adaptable to both changes in cell density and metabolism.

It is also important to note that the overall improvements on productivity, for example, from 4 g/L of the baseline platform process to 9.1 g/L for the dynamic feeding process, is only possible with the optimized feed medium. The automatic dynamic feeding method, however, made it possible to evaluate the various feed medium formulations without the need to establish a priori how these different feed media should be administered in terms of dose and frequency. Therefore, the combined approach of dynamic feeding and iterated feed medium optimization has the clear synergistic benefit. Automation is also a main feature in our studies, as many earlier efforts on dynamic feeding had to rely on manual adjustments based on off-line measurements (Xie and Wang, 1994a,b, 1996; Xie et al., 1997; Yu et al., 2011; Zhou et al., 1995, 1997a,b). Advances in automation have enabled more advanced feedback control mechanisms without a concomitant increases in labor. A fully integrated sampling, data acquisition, and control system has the capability of responding to a wide variety of inputs. Alternative methods could also be developed for subgroups of metabolites that could be broken up into separate feeds that vary in predetermined ratios to one another. An expansion in the number of available inputs could further improve feeding algorithms to develop a control system that is dependent on inherent cell metabolism. An evolution of this feeding algorithm could define a platform process that adapts to natural variations in cell lines in order to ensure optimal productivity and quality is reached in the shortest time possible, translating technological advancements into high throughput process development.

The authors are grateful for the valuable contributions and technical discussions by Srikanth Chary, Gayle Derfus, Veronica Carvalhal, Jason Goodrick, and Robert Kiss.

References

Arnoux AS, Preziosi-Belloy L, Esteban G, Teissier P, Ghommidh C. 2005. Lactic acid bacteria biomass monitoring in highly conductive media by permittivity measurements. Biotechnol Lett 27(20):1551–1557.

Bibila TA, Robinson DK. 1995. In pursuit of the optimal fed-batch process for monoclonal antibody production. Biotechnol Prog 11(1):1–13.

Chee Furng Wong D, Tin Kam Wong K, Tang Goh L, Kiat Heng C, Gek Sim Yap M. 2005. Impact of dynamic online fed-batch strategies on metabolism, productivity and N-glycosylation quality in CHO cell cultures. Biotechnol Bioeng 89(2):164–177.

Chen W, Graham C, Ciccarelli RB. 1997. Automated fed-batch fermentation with feed-back controls based on dissolved oxygen (DO) and pH for production of DNA vaccines. J Ind Microbiol Biotechnol 18: 43–48.

Derfus GE, Abramzon D, Tung M, Chang D, Kiss R, Amanullah A. 2009. Cell culture monitoring via an auto-sampler and an integrated multi-functional off-line analyzer. Biotechnol Prog 26(1):284–292.

Dowd JE, Kwok KE, Piret JM. 2001a. Glucose-based optimization of CHO-cell perfusion cultures. Biotechnol Bioeng 75:252–256.

Dowd JE, Kwok KE, Piret JM. 2001b. Predictive modeling and loose-loop control for perfusion bioreactors. Biochem Eng J 9:1–9.

Gong X, Li D, Li X, Fang Q, Han X, Wu Y, Yang S, Shen BQ. 2006. Fed-batch culture optimization of a growth-associated hybridoma cell line in chemically defined protein-free media. Cytotechnology 52(1): 25–38.

Huang YM, Hu W, Rustandi E, Chang K, Yusuf-Makagiansar H, Ryll T. 2010. Maximizing productivity of CHO cell-based fed-batch culture using chemically defined media conditions and typical manufacturing equipment. Biotechnol Prog 26(5):1400–1410.

Junker BH, Wang HY. 2006. Bioprocess monitoring and computer control: key roots of the current PAT initiative. Biotechnol Bioeng 95(2):226–261.

Kelley B. 2009. Industrialization of mAb production technology: The bioprocessing industry at a crossroads. MAbs 1(5):443–452.

Kim BS, Lee SC, Lee SY, Chang YK, Chang HN. 2004. High cell density fed-batch cultivation of *Escherichia coli* using exponential feeding combined with pH-stat. Bioprocess Biosyst Eng 26:147–150.

Kiviharju K, Salonen K, Moilanen U, Eerikainen T. 2008. Biomass measurement online: The performance of in situ measurements and software sensors. J Ind Microbiol Biotechnol 35(7):657–665.

Krairak S, Yamamura K, Irie R, Nakajima M, Shimizu H, Chim-Anage P, Yongsmith B, Shioya S. 2000. Maximizing yellow pigment production in fed-batch culture of *Monascus* sp. J Biosci Bioeng 90(4):363–367.

Kurokawa H, Park YS, Iijima S, Kobayashi T. 1994. Growth characteristics in fed-batch culture of hybridoma cells with control of glucose and glutamine concentrations. Biotechnol Bioeng 44:95–103.

Kuwae S, Ohda T, Tamashima H, Miki H, Kobayashi K. 2005. Development of a fed-batch culture process for enhanced production of recombinant human antithrombin by Chinese hamster ovary cells. J Biosci Bioeng 100(5):502–510.

Lee YY, Yap MG, Hu WS, Wong KT. 2003. Low-glutamine fed-batch cultures of 293-HEK serum-free suspension cells for adenovirus production. Biotechnol Prog 19(2):501–509.

Li J, Wong CL, Vijayasankaran N, Hudson T, Amanullah A. 2012. Feeding lactate for CHO cell culture processes: Impact on culture metabolism and performance. Biotechnol Bioeng 109:1173–1186.

Opel CF, Li J, Amanullah A. 2010. Quantitative modeling of viable cell density, cell size, intracellular conductivity, and membrane capacitance in batch and fed-batch CHO processes using dielectric spectroscopy. Biotechnol Prog 26(4):1187–1199.

Pörtner R, Schwabe JO, Frahm B. 2004. Evaluation of selected control strategies for fed-batch cultures of a hybridoma cell line. Biotechnol Appl Biochem 40:47–55.

Sauer PW, Burky JE, Wesson MC, Sternard HD, Qu L. 2000. A high-yielding, generic fed-batch cell culture process for production of recombinant antibodies. Biotechnol Bioeng 67(5):585–597.

Teixeira AP, Oliveira R, Alves PM, Carrondo MJ. 2009. Advances in on-line monitoring and control of mammalian cell cultures: Supporting the PAT initiative. Biotechnol Adv 27(6):726–732.

Wlaschin KF, Hu WS. 2006. Fedbatch culture and dynamic nutrient feeding. Adv Biochem Eng Biotechnol 101:43–74.

Xie L, Wang DI. 1994a. Fed-batch cultivation of animal cells using different medium design concepts and feeding strategies. Biotechnol Bioeng 43(11):1175–1189.

Xie L, Wang DI. 1994b. Applications of improved stoichiometric model in medium design and fed-batch cultivation of animal cells in bioreactor. Cytotechnology 15:17–29.

Xie L, Wang DI. 1996. High cell density and high monoclonal antibody production through medium design and rational control in a bioreactor. Biotechnol Bioeng 51:725–729.

Xie L, Nyberg G, Gu X, Li H, Möllborn F, Wang DIC. 1997. Gamma-interferon production and quality in stoichiometric fed-batch cultures of Chinese hamster ovary (CHO) cells under serum-free conditions. Biotechnol Bioeng 56:577–582.

Yu M, Hu Z, Pacis E, Vijayasankaran N, Shen A, Li F. 2011. Understanding the intracellular effect of enhanced nutrient feeding toward high titer antibody production process. Biotechnol Bioeng 108(5):1078–1088.

Zhang L, Shen H, Zhang Y. 2004. Fed-batch culture of hybridoma cells in serum-free medium using an optimized feeding strategy. J Chemi Tech Biotech 79:171–181.

Zhou W, Hu W-S. 1994. On-line characterization of a hybridoma cell culture process. Biotechnol Bioeng 44:170–177.

Zhou W, Rehm J, Hu WS. 1995. High viable cell concentration fed-batch cultures of hybridoma cells through on-line nutrient feeding. Biotechnol Bioeng 46(6):579–587.

Zhou W, Chen CC, Buckland B, Aunins J. 1997. Fed-batch culture of recombinant NS0 myeloma cells with high monoclonal antibody production. Biotechnol Bioeng 55(5):783–792.