



Successful Transfer and 200-Fold Scale-Up of a r-CHO Cell Fed-Batch Process Using a New cGMP Compliant Basal and Feed Medium Combination

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Abstract

A research process developed in shake flasks and 0.6L bioreactors was transferred from an independent development laboratory and rapidly scaled up to a 100L production bioreactor. A newly developed, commercially available, chemically defined basal and feed medium combination designed specifically for cGMP fed-batch production was a key element in this success. A single 5L bioreactor run using 10%(v/v) feeds on Days 3, 5, and 7 with glucose supplementation (≥ 1 g/L) indicated a need for a more aggressive feed schedule in order to replicate the productivity and culture properties obtained at the research scale. Without further experimentation, the feed schedule was changed to 10%(v/v) feeds on Days 2, 4, 6, and 8 without glucose supplementation. In a 5L bioreactor the modified feed schedule replicated the 900 mg/L productivity of the research bioreactors and exhibited similar culture and metabolic properties. The modified process was incorporated easily into formal production records for a 100L bioreactor by the GMP manufacturing group. The feed schedule and bioreactor operating parameters transferred successfully to the 100L bioreactor without further modification. The productivity was again >900 mg/L and the culture properties replicated those seen during process development.

The new basal medium GIBCO® CD OptiCHO™ and the feed media CHO CD Efficient Feed™ A and B were developed by Invitrogen as cGMP compliant production media. These chemically defined media were designed specifically for fed-batch bioreactor production with a broad variety of cells types, to contain a minimal number of components in the formulation, and to favor the establishment of a metabolic state which maximizes recombinant protein production and which minimizes wasteful energy source catabolism and inhibitory waste product accumulation. The utility of these new media in supporting the successful transfer of a fed-batch production process between development laboratories and scale up into a GMP production facility is demonstrated.

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Introduction

Invitrogen PD-Direct® partnered with Cytovance Biologics to demonstrate rapid process transfer and scale up using new media developed specifically for fed batch processes. The base medium, GIBCO® CD OptiCHO™, and the feed media, GIBCO® CHO CD Efficient Feed™ A and B, were developed by Invitrogen as cGMP compliant, chemically defined fed batch production media for a broad variety of cells. They were designed to simplify and accelerate fed batch process development by reducing the initial development step to a feed schedule matrix experiment in shake flasks, with productivity and cell density as the performance factors. Optimization for bioreactor operations and scale up then consists of minor adjustments to the feed schedule to maximize product yield and cell density and to minimize nutrient depletion between feeds. Accumulation of waste products and feed nutrients, and elevation of osmolality to inhibitory levels are sufficiently well controlled that they are secondary issues.

Invitrogen PD-Direct® developed a fed-batch bioreactor process for a recombinant CHO cell line producing an IgG antibody at the shake flask and 0.6L bioreactor scale. The process description and cell line were transferred to Cytovance Biologics for scale up to 5L Process Development bioreactors and to the 100L production bioreactor in the GMP Manufacturing Facility. The feed medium, GIBCO® CHO CD Efficient Feed™ A, was provided as a prototype of the medium being prepared for commercial launch. The procedures for process transfer and scale up were standard Cytovance practices for customer interaction and preparation of a formal manufacturing record suitable for GMP production.

Materials & Methods

Media

Liquid GIBCO® CD OptiCHO™ medium, Invitrogen part numbers 12681 (1L bottle) and 07-0063 (20L bag), supplemented with 8 mM GlutaMAX™ (Invitrogen part number 35050-061) was both the growth medium for inoculum cultures and the base medium for fed batch cultures in bioreactors and shake flasks. Liquid GIBCO® CHO CD Efficient Feed™ A prototype, Invitrogen part numbers A10234 (1L bottle) and 07-0063 (5L bag), was the feed medium for all fed batch cultures. A sterile 7.5% (w/v) Sodium Bicarbonate solution was used for pH control in bioreactors. A sterile 0.5 M Glucose solution was used for glucose additions to only the first 5L bioreactor run.

Cell Culture Practices

Standard culture practices and conditions were used for shake flasks and the Wave bioreactor without optimization for the cell line. Cell density and viability were determined by Trypan Blue exclusion with manual hemacytometer cell counts. Metabolic patterns were monitored with a Nova BioProfile 400 Analyzer to measure pH, pO₂, pCO₂, glutamine, glutamate, glucose, lactate, ammonia, and bicarbonate. A freezing point osmometer was used to confirm the calculated osmolality value provided in the Nova assay panel for bioreactor 1 (Fig. 2h). Spent medium analysis and IgG quantitation were done by Invitrogen. The cell, provided by Invitrogen PD-Direct®, was a recombinant CHO cell line producing an IgG antibody.

Bioreactor Engineering and Operating Parameters

To the extent that scale up considerations and design differences among the bioreactors would allow, the original bioreactor operating conditions used with Invitrogen's DASGIP 0.6L bioreactors were used in the Cytovance Sartorius/B. Braun 5L and 100L bioreactors. No specialized engineering or operating procedures were required to implement the fed batch process. Information about the design and operation of the bioreactors used in this work is provided in Table 1. The Fed Batch Process Description for each fed batch bioreactor run is provided in Table 2.

5L Bioreactor Operation

The Cytovance Cell Culture Process Development group uses Sartorius/B. Braun A-Plus bioreactors with 5L glass vessels. The A-Plus controller uses individual solenoid valves to control flow of each gas. The instantaneous flow rate through an open valve is controlled by individual rotameters for each gas. Instantaneous flow rates for air, oxygen, and carbon dioxide were 100 mL/min, 600 mL/min, and 20 mL/min, respectively. The sparger was an open tube to minimize the foam generation. A constant flow of air to the sparger at 100 mL/min was used to aerate the culture, and to smooth DO and pH control by rapidly carrying the short pulses of oxygen and carbon dioxide to the culture. No overlay

gas flow was used. The vessels are operated at atmospheric pressure. Agitation is provided by two Braun large area, variable pitch impellers with three blades per impeller. Blade pitch was set to 45° to pump down. Bioreactor operation is monitored and data collected with the Sartorius/B. Braun MFCS SCADA program.

For each bioreactor run, one vial of the recombinant CHO cell line was recovered from the cell bank to generate the inoculum. The inoculum culture was grown in suspension in shake flasks at 37°C in 8%CO₂ at 120rpm. Shake flask cultures were maintained between 0.3 – 1.5x10⁶ cells/mL by dilution with growth medium. Cells were passed at least three times in CD OptiCHO™ medium before being used to seed the bioreactor. Bioreactor and shake flask experiments were seeded at cell densities of 0.3-0.4x10⁶ cells/mL in initial culture volumes of 3L and 200 mL, respectively.

A volume of Efficient Feed A equal to 10%(v/v) of the initial culture volume on Day 0 was added on each of the days indicated in the feed schedule for each experiment. No adjustments were made to the feed volume to compensate for changes in culture volume due to sampling and feed additions. Efficient Feed A was added at a rate of 2 mL/minute to the bioreactor to avoid oxygen depletion. Glucose was added to bioreactor 1 (5L Brx1) to maintain glucose at 2-4 g/L before addition of the feed medium. Analytical samples were taken daily from the bioreactors and parallel shake flask culture, centrifuged and stored at -20°C until shipment to Invitrogen for IgG and spent medium analyses.

100L Bioreactor Operation

The Cytovance Contract Manufacturing Facility uses a Sartorius/B. Braun 100L stirred tank bioreactor system designed for GMP production. The bioreactor is designed for automated CIP and SIP and has been fully qualified and validated. The controller uses individual mass flow controllers for air, oxygen, and carbon dioxide. A constant air flow to the sparger at 0.4 L/min and to the headspace at 10 L/min was used. Oxygen and carbon dioxide flows were to the sparger only and were actively controlled. A sparge bar with six 1.6 mm diameter holes was used. The vessel was operated at 1.5 psig. Agitation is provided by two Braun large area, fixed angle pitched blade impellers with three blades per impeller. Blade pitch is fixed at 45° to pump down. Bioreactor operation is monitored and data collected with the Sartorius/B. Braun MFCS SCADA program.

The 5L fed batch process was transferred from Cytovance Process Development Laboratories with parameters scaled as necessary for the production bioreactor. The scaled-up process was incorporated into the Cytovance platform for GMP manufacturing, including development of approved master batch records, and formal quarantine and release of raw materials against approved specifications by Cytovance Quality Assurance.

One vial of IgG-expressing CHO cells was recovered from the cell bank and expanded in shake flask culture to multiple 2L shake flasks using CD OptiCHO™ medium with 8 mM GlutaMAX as the growth medium. Shake flasks were incubated at 37°C and 5% CO₂ on orbital shaker platforms at 97-120 rpm. Cell counting by trypan blue exclusion and hemacytometer was generally performed daily. Splits (1:5) were done at a target viable cell density of 1.5x10⁶ cells/mL.

Cells were transferred from 5x2L shake flasks into one 25L Wave Bioreactor. The working volume in the Wave bioreactor was limited to 19L to maintain the split ratio of 5. The Wave bioreactor was rocked at an angle of 6 degrees and a rate of 12 rpm. Temperature was maintained at 37°C with a constant flow of 7.5% CO₂ in air at 0.5 L/min.

The cell suspension from the Wave Bioreactor cell suspension was used to seed the 100L bioreactor at a working volume of 70L and a cell density of 0.62x10⁶ cells/mL. The seeding density was higher than the 5L reference bioreactor run but was believed to be within the range of acceptable variation for the process. On Days 2, 4, 6, and 8 after inoculation, Efficient Feed A at 10%(v/v) of the initial culture volume was added. The feed medium was added over a period of 3-4 hours to avoid oxygen depletion. The culture was terminated when viability decreased below 50%.

Table 1. Comparison of Bioreactor Design & Operation

Culture System	DASGIP	5L Bioreactor	100L Bioreactor
Total Vessel Vol.	0.6 L	5L	100L
Agitation	1 Impeller	2 Impellers	2 Impellers
Impeller	Pitched Blade	3 Large Blades 45° Pitch Down	3 Large Blades 45° Pitch Down
pH Control	Active: CO ₂ /HCO ₃ ⁻	Active: CO ₂ /HCO ₃ ⁻	Active: CO ₂ /HCO ₃ ⁻
DO Control	Active: O ₂ Enrichment	Active: O ₂ Enrichment	Active: O ₂ Enrichment
Gas Flow Control	Mass Flow Controller	Solenoid Valve & Rotameter	Mass Flow Controller
Overlay Gassing	na	na	Air
Sparge Gassing	DO & pH Control	DO & pH Control	DO & pH Control
Sparger Design	1 x 2 mm id	1 x 6 mm id	6 x 1.6 mm id
O ₂ Enrichment	Continuous Blend	Pulsed	Continuous
CO ₂ Enrichment	Continuous Blend	Pulsed	Continuous
Operating Conditions			
Working Volume Range	0.4-0.6 L	3-3.8 L	70-98 L
Agitation	110 rpm	80 & 100 rpm	50 rpm
Overlay Gassing	na	na	Air 10 L/min
Sparge Air	Blend	Fixed 1.2 vvh	Fixed 0.2 vvh
O ₂	Blend	Pulsed 7.2 vvh	0 - 3.7 vvh
CO ₂	Blend	Pulsed 0.4 vvh	0.4 vvh
Max O ₂ Duty Cycle	na	25%	na
Gas Blend (21-90%O ₂)	1 - 4 vvh	na	na
Min Total Flow	1 vvh	1.2 vvh	0.2 vvh
Max Instantaneous Flow	na	8.4 vvh	na
Max Average Total Flow	1 - 4 vvh	2.9 vvh	3.9 vvh

* vvh – volume gas/volume vessel/hour

Table 2. Fed Batch Process Descriptions

Bioreactor	DASGIP	5L Brx1	5L Brx2	100L
Feed Schedule	Day 3, 6, 8	Day 3, 5, 7	Day 2, 4, 6, 8	Day 2, 4, 6, 8
Feed Volume	CHO CD Efficient Feed™ A 10% (v/v) of Day 0 volume	CHO CD Efficient Feed™ A 10% (v/v) of Day 0 volume	CHO CD Efficient Feed™ A 10% (v/v) of Day 0 volume	CHO CD Efficient Feed™ A 10% (v/v) of Day 0 volume
Glucose Feed	No	Yes	No	No
Seed Density	0.3e ⁶ cells/mL	0.4e ⁶ cells/mL	0.4e ⁶ cells/mL	0.6e ⁶ cells/mL
Initial Volume	0.5L	3L	3L	70L
Temperature	37°C	37°C	37°C	37°C
pH	7.05±0.02	7.05±0.05	7.05±0.05	7.05±0.05
DO SP	50% air sat'n	50% air sat'n	50% air sat'n	50% air sat'n

Process Transfer and Optimization

At the time the process was transferred, the optimization of the feed schedule was not complete. The reference 0.6L run in DASGIP bioreactors (IVG 0.6L Brx) used a feed schedule of 10% (vol feed/initial culture vol) CHO CD Efficient Feed™ A feeds on Days 3, 6, and 8 (Figure 1a, 1b). However, glucose was exhausted by Day 5 and glucose, lactate and glutamine were exhausted by Day 9 (Figure 2c, 2d, 2e).

Consequently, the Cytovance 5L bioreactor process transfer run (5L Brx1) used a feed schedule of 10% (vol feed/initial culture vol) CHO CD Efficient Feed™ A on Days 3, 5, and 7 (Figure 1, 1b; Figure 2a-2h). In addition, glucose supplementation was used as required to prevent the glucose concentration from falling below 1 g/L (Fig. 1a, Fig. 2c).

This schedule increased the culture life to 14 days, prevented nutrient exhaustion and limited waste metabolite accumulation (Figure 2c, 2d, 2e, 2g). However, the maximum cell density and product yield were low (Fig. 1a, 1b). The product concentration increased less than 7% after Day 10 (Fig. 1b).

The conclusions drawn from run 5L Brx1 were 1) there is no benefit from glucose supplementation; 2) cell density may have been limited because the Day 5 feed came too late; and 3) the Day 7 feed came too late to prevent nutrient depletion which severely reduced productivity.

Process Scale Up

1 Based on the conclusions drawn from run 5L Brx1, the feed schedule was changed to 10%(v/v) feeds of CHO CD Efficient Feed™ A on Days 2, 4, 6, and 8 without glucose supplementation for the 5L Reference Bioreactor run (5L Brx2). The new feed schedule achieved the desired result of increasing production and cell density, and of preventing exhaustion, or excessive accumulation, of nutrients and metabolites (Fig. 3, Fig. 4).

Consequently, the conditions for 5L Brx2 were used to design operating parameters for the 100L bioreactor run (100L Brx). The bioreactor operating parameters are provided in Table. The data from these bioreactor runs are presented in Figures 3 and 4. Although there are differences in the growth profile, by the measures of final product yield, specific productivity, and the general metabolic properties of the cultures, the results in the 5L and 100L bioreactors are very similar. In addition, Invitrogen PD-Direct® obtained very similar results with this feed schedule in their 0.6L DASGIP and 5L Sartorius/B. Braun bioreactors (production data for the Invitrogen bioreactors is included in Figure 6).

Process Transfer & Optimization

Invitrogen 0.6L Bioreactor & Cytovance 5L Bioreactor 1

Figure 1. Growth & Productivity

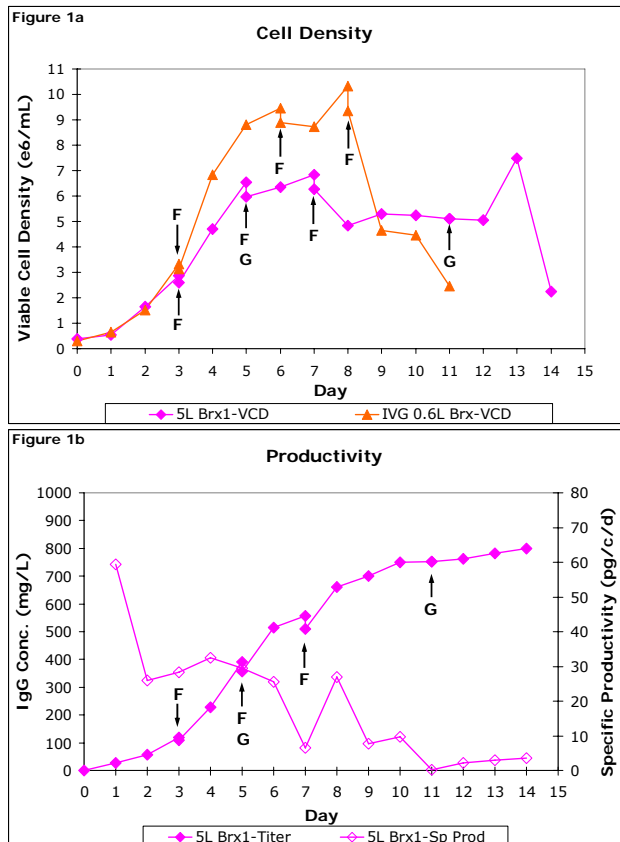


Figure 2. Metabolic Panel

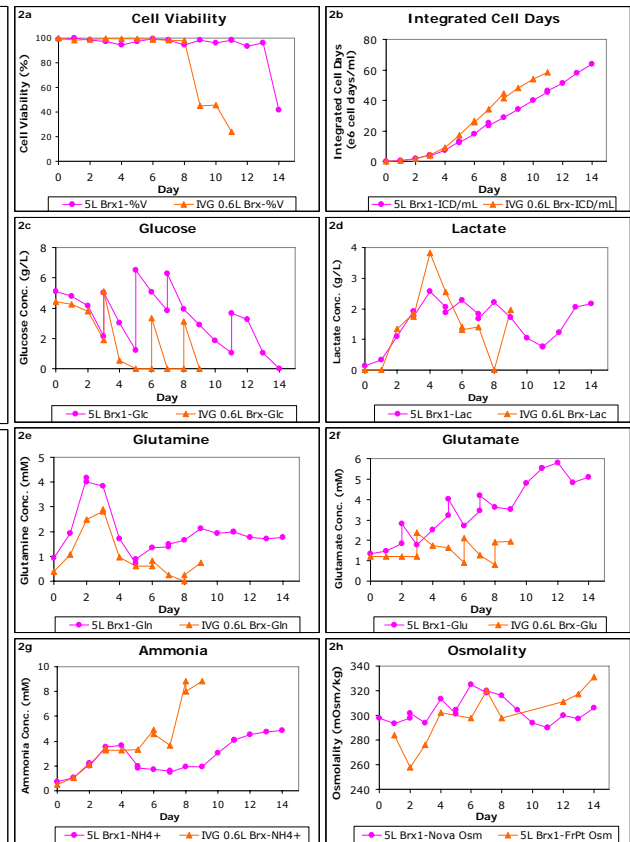


Figure 1. Process Transfer: Growth and Production Profiles

The feed schedule of 10%(v/v) feeds on Days 3, 5, and 7 done in a Cytovance 5L bioreactor (5L Brx1) is compared to a feed schedule of 10%(v/v) feeds on Days 3, 6, and 8 done in an Invitrogen 0.6L bioreactor (IVG 0.6L Brx). Addition of Feed Medium is indicated by F and addition of Glucose is indicated by G.

Figure 2. Process Transfer: Metabolic Profile

The 5L Process Confirmation bioreactor (5L Brx1) with a feed schedule of 10%(v/v) feeds on Days 2, 5, and 7 was characterized with the Nova panel of assays, cell viability, and cell yield (ICD/mL). Panel 2h compares the absolute osmolality of the medium determined by Freezing Point osmometry and the calculated value in the Nova panel.

Process Scale Up

Cytovance 5L Bioreactor 2 & 100L Bioreactor

Figure 3. Growth and Productivity

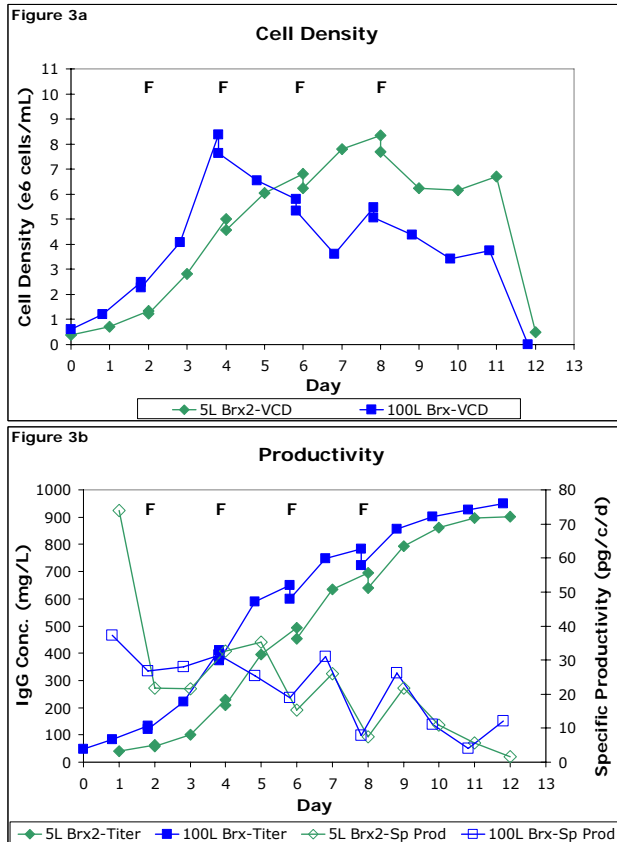


Figure 4. Metabolic Panel

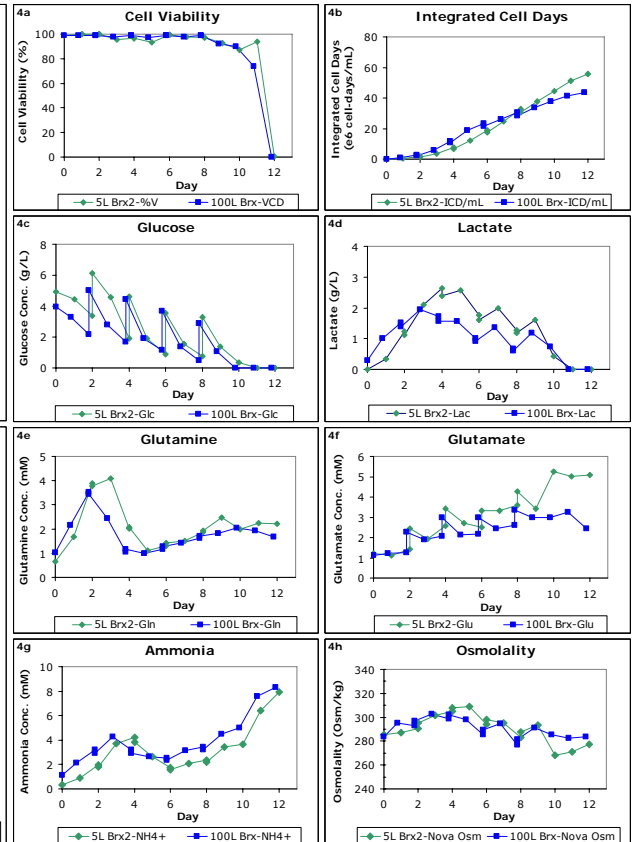


Figure 3. Process Scale Up: Growth and Production Profiles

The feed schedule of 10%(v/v) feeds on Days 2, 4, 6, and 8 done in a Cytovance 5L bioreactor (5L Brx2) is compared to the same feed schedule done in the Cytovance 100L bioreactor (100L Brx).

Figure 4. Process Scale Up: Metabolic Profile

The 5L Process Confirmation bioreactor run (5L Brx2) with a feed schedule of 10%(v/v) feeds on Days 2, 4, 6, and 8 was characterized with the Nova panel of assays, cell viability, and cell yield (ICD/mL).

Scale Comparisons

A critical issue in process transfer and scale up is that any significant differences in results among groups and scale of operation can be used to better understand the process. This proved to be true in this project as shown when Invitrogen PD-Direct™ used the final feed schedule in their 0.6L DASGIP and 5L Sartorius/B. Braun bioreactors and obtained productivity very similar to the Cytovance bioreactors (Fig. 6).

Culture Properties

In spite of differences in the cell density profiles and the integrated cell days of the 5L Reference Bioreactor (5L Brx2) and the 100 L bioreactor (Fig. 3a, Fig. 4b) the impact on nutrient and metabolite concentrations is minimal (Fig. 4c-4g). The extent to which these differences are a consequence of the high seeding density in the 100L bioreactor is not clear. Although the feed schedule should be linked to the time course of cell density, this process is sufficiently robust that the terminal product yield was stable in spite of the variations between runs.

Metabolic Properties

In both the 5L and the 100L bioreactors, the metabolic properties of the cells made maintenance of a stable culture environment an easily managed process. Control at the acid side of the pH control dead band required only limited additions of base and was, for the most part, controlled by the CO₂ stripping action of the fixed air sparge. Control at the basic side of the pH dead band was managed by low CO₂ flow rates and the lactate generated after each feed. In both reactors, there was no significant change in osmolality (Fig. 4h). Oxygen demand was satisfied without difficulty.

The feed schedule maintained the major nutrients and metabolites in acceptable ranges until exhaustion of the Day 8 feed (Fig. 4c -4g). Lactate was generated primarily early in the run and immediately after each feed, but was consumed as the cultures age (Fig. 4d). Ammonia concentrations did not exceed 5 mM until Day 11 following glucose exhaustion (Fig. 4g).

Spent medium analysis was done by Invitrogen on the Day 10 sample from the 100L bioreactor. Even though this sample was taken very late in the run, two days after the last feed, only asparagine, cystine, and tyrosine were less than 10% of the fresh CD OptiCHO™ concentration and only alanine and glycine accumulated significantly. The other amino acids had residual concentrations of 30-85% (Table 3). No investigation was done to determine whether the depleted amino acids were responsible for the cycling in oxygen demand discussed below.

Table 3. Amino Acid Depletion: 100L Bioreactor Day 10

Amino Acid	% Residual	Amino Acid	% Residual	Amino Acid	% Residual
Alanine	873.8	Glycine	2148.0	Phenylalanine	57.0
Arginine	67.3	Histidine	73.8	Proline	84.5
Asparagine	4.0	Hydroxy-Proline	112.3	Serine	31.2
Aspartic Acid	51.4	Isoleucine	39.0	Threonine	63.9
Cystine	2.9	Lysine	60.6	Tyrosine	0.5
Glutamine	10.0	Methionine	65.2	Valine	42.9
Glutamic Acid	34.7				

Productivity

Although there are significant differences in the cell density profiles between the bioreactors, the final product concentration is remarkably consistent at 900 mg/L and 950 mg/L. Similar yields were obtained in the Invitrogen bioreactors (Fig. 6). Thus consistent yields were obtained across an approximately 200-fold range of bioreactor volumes (0.6-100L), in two unrelated companies, and in three different facilities.

The specific productivity for both bioreactors for Days 2-9 is in the range of 20-30 pg/c/d (peak productivity following feeds) with no significant, consistent difference between them (Fig. 3b). However, there is very consistent oscillation in the specific productivity in both bioreactors starting after the feed on Day 4.

When the specific productivity, nutrient, and metabolite data are combined with the bioreactor parameters, several interesting correlations are observed. Data from the 100 L Bioreactor is shown in Figure 5. The oscillations of specific productivity, oxygen flow, and glucose concentration are all approximately in phase, and the oscillations in lactate concentration are approximately 24 hours out of

phase. The continuously recorded pH and oxygen flow data provide the highest resolution indicators of the timing of these metabolic changes. Within 10-30 minutes after starting addition of feed medium, the oxygen flow increases.

At high cell densities, nutrients required for high metabolic activity appear to be depleted in about 24 hours. As the oxygen consumption falls, lactate consumption begins and the pH rises to the high side of the pH control band. The decreased metabolic activity does not appear to be caused by exhaustion of the energy sources (Fig. 4c-4f); levels of waste metabolites or osmolality (Fig. 4d-4h); nor to oscillations in concentrations of glutamine, glutamate, or ammonia (Fig. 4e-4g).

Although these correlations were not investigated further, the correlations of specific productivity with the bioreactor pH control state, and with the oxygen delivery rate, offer intriguing opportunities for feed schedule optimization and, perhaps, automation of feed medium addition.

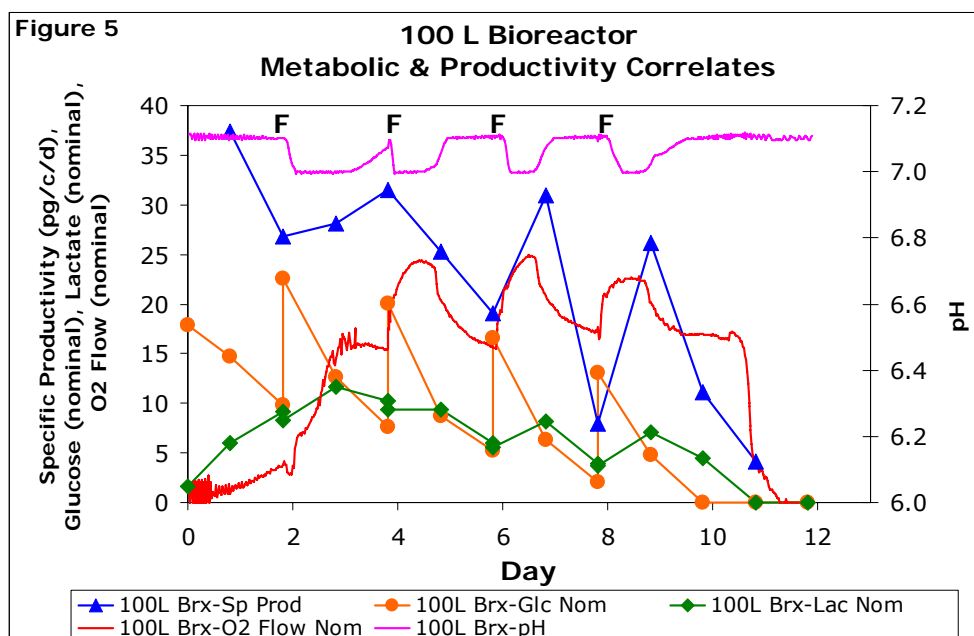


Figure 5. Correlation of Bioreactor Parameters with Productivity and Metabolism
Continuously recorded values for pH and oxygen flow from the 100L Bioreactor run are plotted with daily measurements for specific productivity and glucose and lactate concentrations. Glucose, lactate, and oxygen flow are plotted as nominal values with independent scaling factors.

Tips for Use

Control the Rate of Feed Medium Addition

The rate of oxygen consumption increases very rapidly as feed medium is added. If the bioreactor control system is tuned to provide a slow response, anoxic conditions which may develop and persist for several hours. The feed medium should be added at a slow rate to provide time for the bioreactor control system to respond.

Use CHO CD Efficient Feed™ A as the Glucose Source

For the cell line used in this work, unidentified nutrients required for high productivity and aerobic metabolism are depleted more rapidly than the glucose. Supplementation with glucose alone supports cell growth and viability but not IgG production. Adjusting the feed schedule to maintain glucose concentration will also maintain the balance of all the nutrients required for high productivity.

Use Bioreactor Parameters to Recognize Nutrient Depletion

When the interval between feed medium additions is sufficiently long to allow cells to deplete the medium, a large drop in oxygen consumption provides an easily evaluated signal for nutrient

depletion. The associated shift in pH from the lower edge of the control dead band to the upper edge is also easily evaluated and is a signal for lactate consumption. With the current cell line these changes occur even though glucose and glutamine are not depleted. Although the use of these signals was not investigated, their potential for optimizing the feed schedule or automating feed medium addition is intriguing.

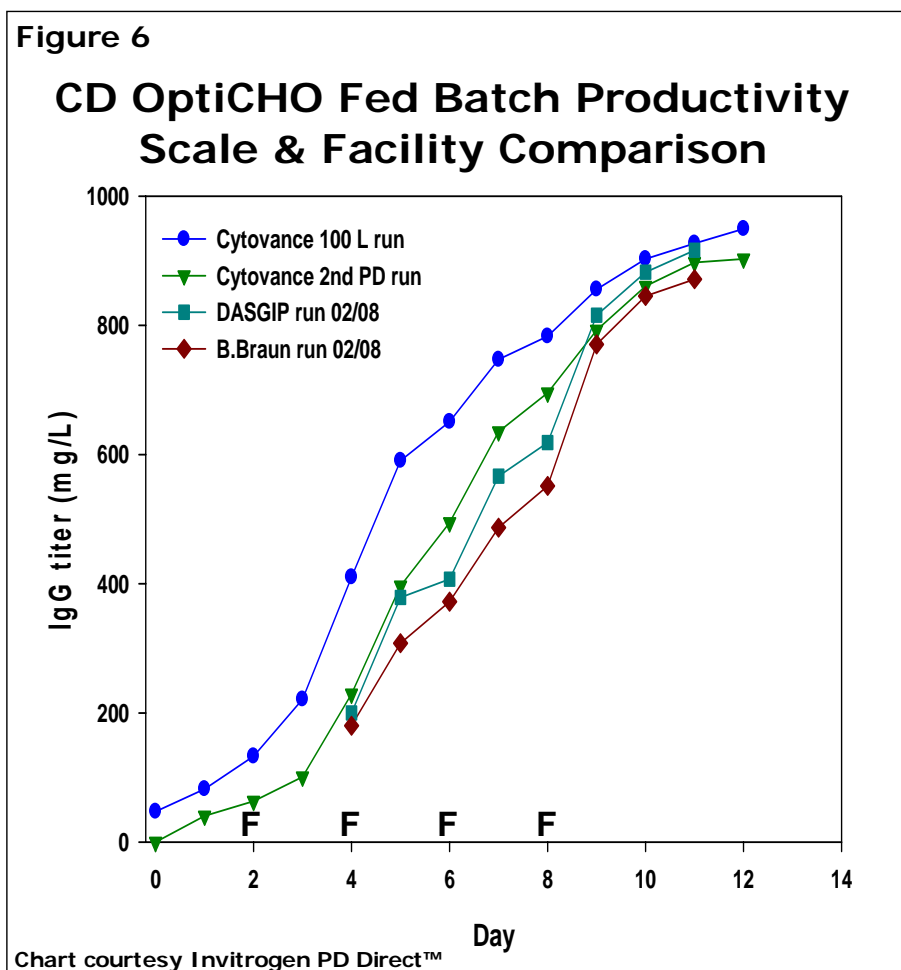


Figure 6. Scalability and Transferability of Productivity

The final fed batch process of 10% (v/v) CD CHO Efficient Feed™ A on Days 2, 4, 6, and 8 was run by Cytovance in 5L and 100L bioreactors and by Invitrogen in 0.6L and 5L bioreactors. The time course of product concentration from each run is shown.

User Perspective

Simplicity of Use

CHO CD Efficient Feed™ A and CD OptiCHO™ provide a well balanced, complete medium pair for fed batch processes. For the cell line used in this work, CHO CD Efficient Feed™ A contains glucose at levels that appear to be well matched to the other nutrients in the medium. Changing the frequency of the feed corrected the depletion of glucose and glutamine and maintained the overall nutrient balance of the culture. Addition of glucose or glutamine as separate supplements was not necessary.

Culture Robustness

The cells used in this work were thoroughly adapted to CD OptiCHO™ by Invitrogen PD-Direct® and were remarkable for their consistent rapid growth, very high viability, and "healthy" appearance. In shake flask cultures, the pH of high density fed batch cultures was generally stable and in the 6.8-7.0 range. This pH appears to be low enough to slow glucose consumption but did not limit cell growth or productivity. In the controlled environment of the bioreactor, viability was very high and cell appearance good until two days after the final feed, when the culture died rapidly.

Metabolic Parameters

The metabolic patterns of the bioreactor cultures are well suited to the production environment. With the feed schedules used in this work, lactate and ammonia accumulation is well controlled. Very little base addition is required and osmolality is extremely well controlled. The feed schedule and the formulation of the media maintained the amino acid balance over an extended period.

Metabolic States

Although there are changes in the metabolic profile and productivity with culture age, and in response to the addition of feed medium, they appear to be due to temporary nutrient depletion rather than to induction or loss of a unique, high productivity metabolic state.

Conclusions

- A fed-batch bioreactor process developed in 0.6L bioreactors by Invitrogen PD-Direct® for a recombinant CHO cell line was transferred to Cytovance Biologics and scaled 200 fold with only two process development 5L bioreactor runs.
- The fed batch process transferred from the 0.6L scale to the 5L and 100L scales with a simple adjustment to the feed schedule.
- Product yields of 900 mg/L were obtained at the 0.6L, 5L, and 100L bioreactor scales.
- Metabolic profiles were similar at the three bioreactor scales.
- Adjustments to the feed schedule were guided by commonly measured culture parameters (Glucose, Glutamine, Lactate, Product Quantitation, & Cell Density).
- Distinctive changes in bioreactor parameters (Oxygen Flow, pH, CO2 Flow) correlated with nutrient depletion and specific productivity and may prove useful for feed schedule optimization.
- GIBCO® CD OptiCHO™ and CHO CD Efficient Feed™ A, a commercially available base and feed medium combination, simplified and accelerated the process transfer and scale up of a fed batch process from bench to production scale.

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