Repurposing fed-batch media and feeds for highly productive CHO cell perfusion processes

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Repurposing fed-batch media and feeds for highly productive CHO cell perfusion processes

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Introduction

We have developed a method for identifying first-generation perfusion culture media based on existing fed-batch media and feeds. Here, we show that we can obtain culture media that successfully support perfusion cultures in bioreactors at cell-specific perfusion rates (CSPR) below 25 pL/cell/day. High productivities and favorable product quality were also achieved.

Materials and methods

Batch cultures

We used a design of experiments (DoE) approach to test a variety of medium and feed combinations on two cell lines in eight day batch shake flask experiments. Cell line 1 is a CHO DG44 cell line producing a recombinant IgG mAb binding TNF-α. Cell line 2 is a proprietary MedImmune CHO cell clone producing a mAb/peptide fusion protein. Table 1 shows the range of experimental conditions tested in these batch shake flask cultures.

Perfusion cultures

The medium and feed combinations selected from the batch shake flask experiments were used in a steady state perfusion culture in ReadyToProcess WAVE™ 25 (Fig 1) or stirred tank bioreactor (STR)/ATF2 perfusion bioreactor systems. Under all conditions, a perfusion rate of < 1.5 volume per volume per day (VVD) was used. Experimental conditions are listed in Table 2.

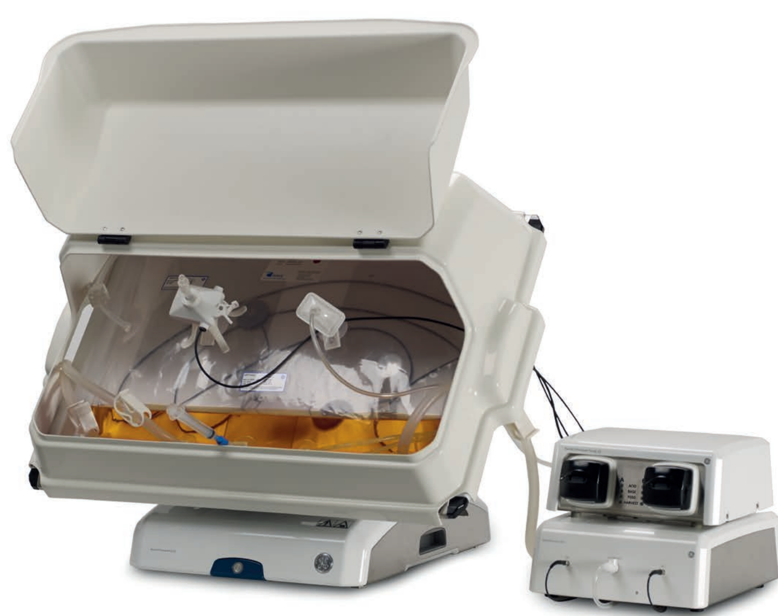

Fig 1. ReadyToProcess WAVE 25 perfusion bioreactor system.

Conclusions

- We have developed a rapid batch shake flask method, requiring limited resource to derive first-generation perfusion media.
- The method predicted both successful and unsuccessful medium and feed combinations.
- Successful perfusion media allowed good productivity and product quality in perfusion bioreactors at CSPR < 25 pL/cell/day, allowing operation at < 1.5 VVD.
- Further refinement of media can be carried out in scale-down models of perfusion such as pseudo perfusion in ambr™ 15 (The Automation Partnership) or TubeSpin™ (TPP Techno Plastic Products AG) bioreactors.

Results

Batch cultures

Table 3 shows the quality of the model fit for the shake flask experiments for the output parameters studied, the conditions selected for perfusion verification in bioreactors, as well as the predicted shake flask levels for maximum viable cell density (VCD), titre, and specific productivity (qP), also indicating the levels for these parameters relative to the maximum achievable output in the design space. These results show that it is not possible to identify conditions that maximise all output parameters. For Cell line 1, combinations were chosen favoring titer as this cell line does not grow to the very high cell densities that Cell line 2 is capable of, and therefore does not represent any bioreactor operational constraint. Because Cell line 2 is capable of reaching a very high cell density in perfusion mode (VCD > 150 × 10^6 cells/mL), which is difficult to control, conditions favoring qP (and hence suboptimal for cell growth) were chosen.

Perfusion cultures

The selected medium and feed combinations were used in a steady state perfusion culture in ReadyToProcess WAVE 25 or STR/ATF2 perfusion bioreactor systems. In all conditions, a CSPR of < 25 pL/cell/day was achieved. Figure 2 shows the perfusion bioreactor performance of the supplemented ActiCHO™ P medium with Cell line 1 (Fig 2A) and for Cell line 2 perfused with supplemented M1 medium (until day 27), supplemented M2 medium (from day 27) (Fig 2B), as well as the latter medium in a perfused STR (Fig 2D). All three fortified media supported growth of the respective cell lines in the expected ranges. Cell viabilities remain high throughout for Cell line 1 (> 95%). Cell line 2 initially grew slowly and showed viability as low as 88%, although it recovered to an average of 97% for the period at which the culture was perfused (day 8 and onwards). We believe this was due to the culture being inoculated in the fortified medium, which negatively affects cell growth and viability as compared with the STR run inoculated in basal M1 medium. Cell line 2 did not perform well in supplemented ActiCHO P medium in the batch experiments (Table 3) and the ActiCHO basal medium as well as the supplemented perfusion medium failed to support growth in the perfused STR (Fig 2C).

For Cell line 1, the titer ranged between 0.41 and 0.99 g/L (Fig 2A) whereas for Cell line 2, the titer ranged between 0.18 and 0.66 g/L when perfused with fortified M1 (days 8–27), with the lower titers occurring during the initial days of perfusion. After switching to fortified M2, the titer increased to between 0.56 and 1.00 g/L (day 27 and onwards) (Fig 2B). In the STR/ATF2 setup, the supplemented M2 perfusion medium resulted in a titer ranging from 0.90–1.16 g/L at steady state (Fig 2D). The STR/ATF2 bioreactor, started with OptiCHO™ medium, failed to reach the cell density perfusion trigger. Nevertheless, perfusion with the supplemented OptiCHO medium was started on day 8, however, failed to have a positive impact as viability continued to decline and the culture was terminated.

Regarding product quality of ReadyToProcess WAVE 25 harvest material purified on Protein A, Table 4 shows that material analyzed by size exclusion chromatography (SEC) from both Cell lines 1 and 2 had lower levels of aggregated material when derived from perfusion as compared with that derived from fedbatch. Material from Cell line 1 was further analyzed by cation exchange chromatography (CIEX) and TNF-α binding kinetics, and results were compared between an average of ten fed-batch cultures using the same medium and feeds. Significantly lower levels of acidic charge variants were observed for perfusion culture samples as compared with those derived from fed-batch cultures.

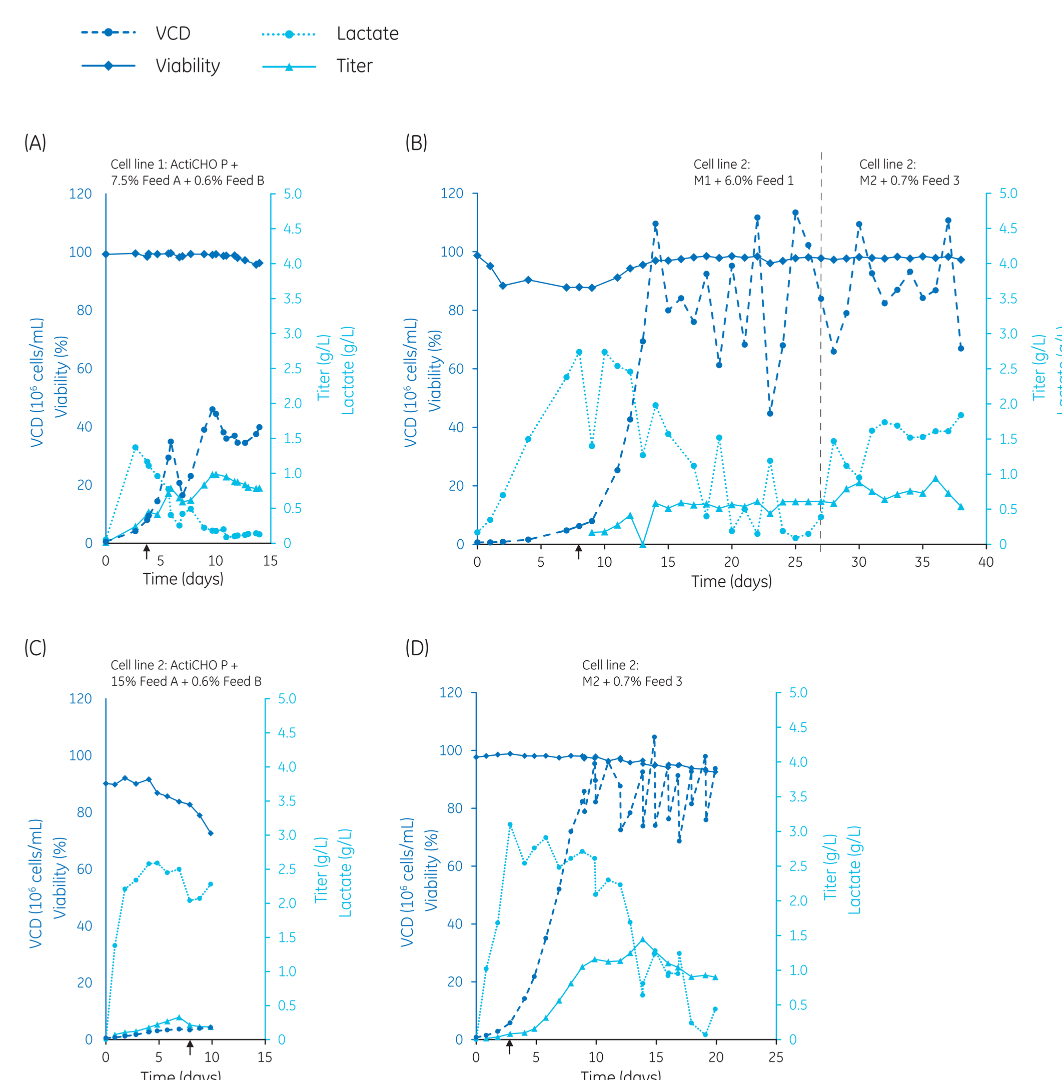

Fig 2. Verification bioreactor runs. (A) Cell line 1 perfused at 1 RV/d with 0.71× ActiCHO P medium fortified with 7.5% Feed A and 0.9% Feed B. (B) Cell line 2 initially perfused at 1.2 VVD with M1 supplemented with 6.0% Feed 1 followed from day 27 by 1.2 RV/d of M2 fortified with 0.7% Feed 3. (C) Cell line 2 perfused at 1.4 RV/d with ActiCHO P medium supplemented with 15% Feed A and 0.6% Feed B. (D) Cell line 2 perfused at 1.4 RV/d with M2 medium supplemented with 0.7% Feed 3. (A) and (B) were carried out in ReadyToProcess WAVE 25 perfusion bioreactor systems, whereas (C) and (D) were performed in STR/ATF2 perfusion setups. Start of perfusion is indicated with an upward arrow on the x-axis.

Table 1. Experimental details for the shake flask experiments using two cell lines, three media, and five feeds

Cell line	Basal medium	Medium range tested	Feeds	Feed range tested (%)	Number of combinations tested
1	ActiCHO P	0.5–1×	Feed A	0–15	25
			Feed B	0–1.5	
2	M1	1×	F1	0–7.5	26
			F2	0–0.8	
			F3	0–0.9	
	M2	1×	F1	0–7.5	22
			F2	0–0.8	
			F3	0–0.9	
	ActiCHO P	1×	Feed A	0–15	24
			Feed B	0–1.5	

Table 2. Experimental details for the bioreactor experiments

Cell line	Perfusion medium	Perfusion rate (RV/d)	VCD trigger for perfusion start (× 10^6 cells/mL)	Target VCD (× 10^6 cells/mL)	Temperature set point (°C)	pH setpoint (observed range)	DO set point (%)	Rock/ stirrer rate (rpm)
ReadyToProcess WAVE 25								
1	0.71× ActiCHO P 7.5% Feed A 0.9% Feed B	1.0	≥ 4	40–60	36.5	7.10 (6.99–7.13)	40	25–30
2	M1a 6.0% Feed 1	1.2	≥ 6	60–100	35.5	7.00 (6.85–7.12)	50	15–32
	M2 0.7% Feed 3		NA					
Stirred tank bioreactors								
2	ActiCHO P 15% Feed A 0.6% Feed B	1.4	≥ 6	80–100	35.5	7.00 (6.62–7.11)	50	300
	M2 0.7% Feed 3					7.10 (6.78–7.20)		300–500

Table 3. Summary model data generated from the batch cultures detailed in Table 1

Cell line	Basal medium	Model fit		Selected conditions	Predicted (% of maximum predicted)		
		R^2	R^2 adjusted		Max VCD (10^6 cells/mL)	Ab titer (mg/L)	qP ($pg.cell^{-1}.day^{-1}$)
1	ActiCHO P	Max VCD: 0.95 Titer: 0.98 qP: 0.88	Max VCD: 0.91 Titer: 0.97 qP: 0.83	0.71×ActiCHO P 7.5% Feed A 0.9% Feed B	15.4 (94.4)	1419 (97.7)	44.1 (54.3)
2	M1	Max VCD: 0.53 Titer: 0.58 qP: 0.69	Max VCD: 0.43 Titer: 0.48 qP: 0.62	6.0% Feed 1	8.9 (50.9)	400 (37.4)	26.1 (87.3)
	M2	Max VCD: 0.88 Titer: 0.84 qP: 0.89	Max VCD: 0.84 Titer: 0.79 qP: 0.83	0.7% Feed 3	5.4 (37.0)	610 (74.5)	29.9 (87.2)
	ActiCHO P	Max VCD: 0.95 Titer: 0.78 qP: 0.97	Max VCD: 0.92 Titer: 0.61 qP: 0.96	15% Feed A 0.6% Feed B	1.6 (26.2)	285 (72.9)	50.1 (94.3)

Included are the model fit parameters for maximum VCD, titer, and qP; chosen conditions for the verification runs; as well as the percentage of the maximum achievable levels for maximum VCD, titer, and qP predicted by the model for these chosen conditions.

Table 4. Quality attributes for samples taken from the perfusion runs shown in Figures 2A and 2B, and compared with historical fed-batch harvest sample data for the same cell line

Cell line	Analytical technology	Analyte	Results	
			Fed-batch	Perfusion
1	CIEX	Acidic charge variants	> 60%	25%
		Alkaline charge variants	3%	2%
	SEC	Aggregates	1%	0.4%
	TNF-α binding kinetics	On rate, k_a	5.2 × 10^5 Ms^{-1}	3.82±0.07 × 10^5 Ms^{-1}
		Off rate, k_d	14.9 × 10^{-5} s^{-1}	8.64±0.72 × 10^{-5} s^{-1}
		Affinity, K_D	287 pM	226 pM
2	SEC	Aggregates	3.45%	2.5%
		Fragments	0%	0%

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