CHO FED-BATCH STRATEGIES TO RAPIDLY INCREASE MAB TITER BY 100% WITHOUT SACRIFICING PRODUCT QUALITY

Amélie Mahé[1], Alexandra Martiné[1], Séverine Fagète [1*], Pierre-Alain Girod[1]
[1] Selexis SA, Route de la Galaise 36, 1228 Plan-Les-Ouates, Switzerland. www.selexis.com
*severine.fagete@selexis.com

14th Annual PEGS EUROPE
Protein & Antibody Engineering Summit
14 - 16 NOVEMBER 2022
InterContinental Barcelona | Barcelona, Spain

1

In the field of therapeutic antibody production, diversification of fed-batch strategies is flourishing in response to the market demand. All manufacturing approaches tend to follow the generally accepted dogma of increasing titer since it directly increases manufacturing output. While titer is influenced by the biomass (expressed as IVCD), the culture time and the cell specific productivity (q_p), we changed independently each of these parameters to tune our process strategy towards adapted solutions to individual manufacturing needs.
To do so, we worked separately on the increase of the IVCD as high seeding fed-batch capacity. Yet, as intensified fed-batch may not always be possible due to limited facility operational mode, we also separately increased the q_p with the addition of specific media additives. Both strategies improved titer by 100% in 14 days relative to the standard fed-batch process with moderate and acceptable changes in product quality attributes.
Since intensified fed-batch could rival the cell-specific productivity of a conventional fed-batch, we developed novel hybrid strategies to either allow for acceptable seeding densities without compromising productivity, or alternatively, to push the productivity the furthest in order to reduce timelines.

2 Process optimization

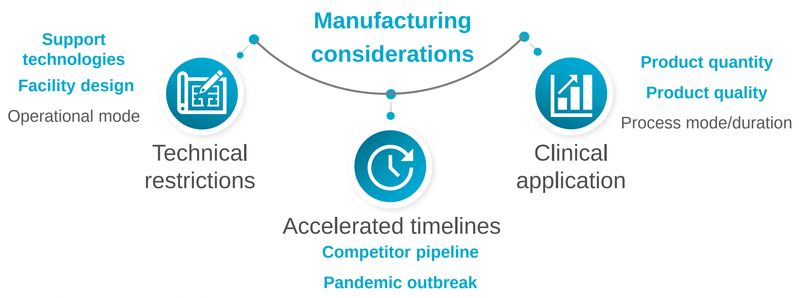


Fig. 1 Choosing appropriate early process solutions

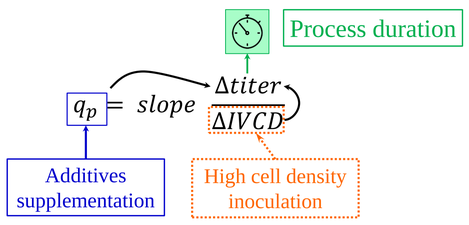


Fig. 2 Key determinants for increased productivity

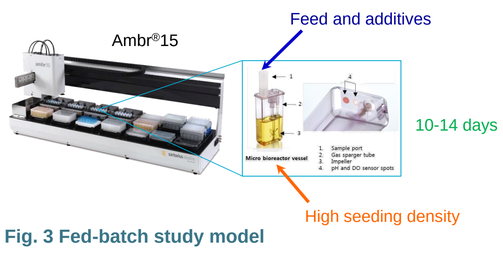


Fig. 3 Fed-batch study model

All manufacturing considerations taken in mind (Fig. 1), expression titer remains the most important process parameter. We aimed at exploring different paths to influence titer by playing either on biomass (expressed as IVCD), cell specific productivity (q_p) or process duration (Fig.2). We focused on Selexis CHO-M producing cell lines stably expressing IgG1 mAbs in a fed-batch mode using an Ambr®15 automated microscale bioreactor system (Fig. 3).

3 Playing independently with process parameters

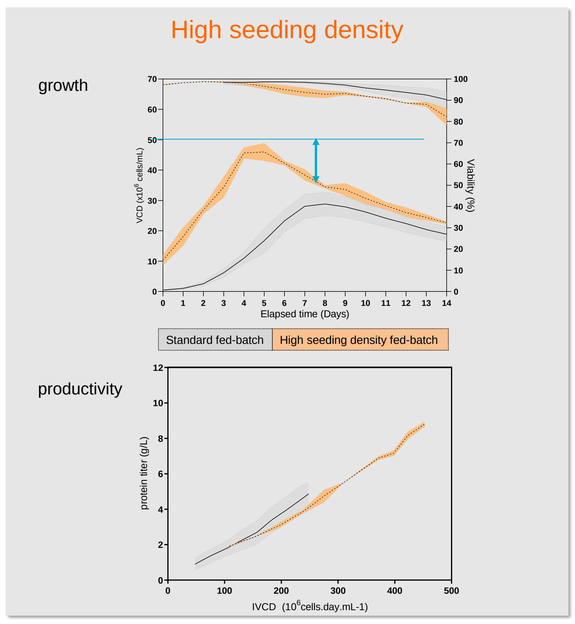


Fig. 4 Influencing expression titer during fed-batch : biomass

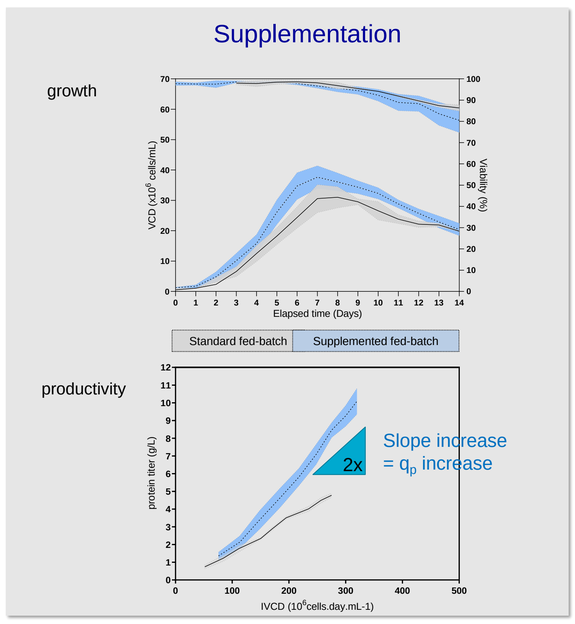


Fig. 5 Influencing expression titer during fedbatch : additives

Seeding density
- from 0.45 x 10^6 c/mL to 10 x 10^6 c/mL

By increasing seeding density and adjusting the feed regimen solely, we were able to sustain productivity and hence increase final titer, while keeping very good cell growth during a 14 days production time.

Supplementation of the production media:
- early phase : copper acetate
- late phase : ferric citrate

Combination of additives with medium inoculation density (1 x 10^6 c/mL) had an additive effect on improving titer and cell- specific productivity (q_p).

4 Process impact

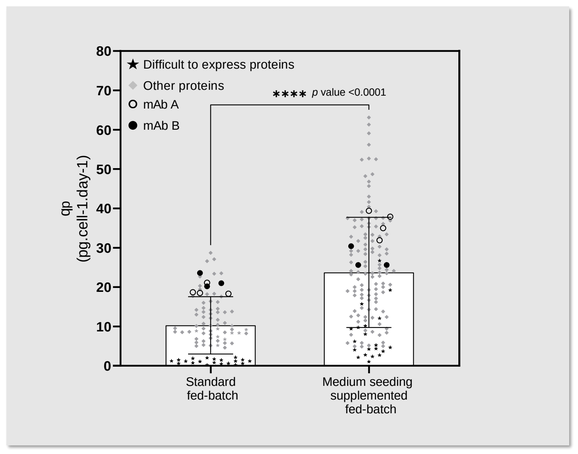


Fig. 6 Intensified process impact on specific productivity of different CHO-M expressing cell lines.

- 2 fold q_p increase for mAbs and other proteins
- Up to 7 fold q_p increase with difficult-to-express proteins

Process intensification is not restricted to a unique CHO-M producing cell line but rather appears to be appropriate to any CHO-M producing cell line whatever the nature of the expressed protein.

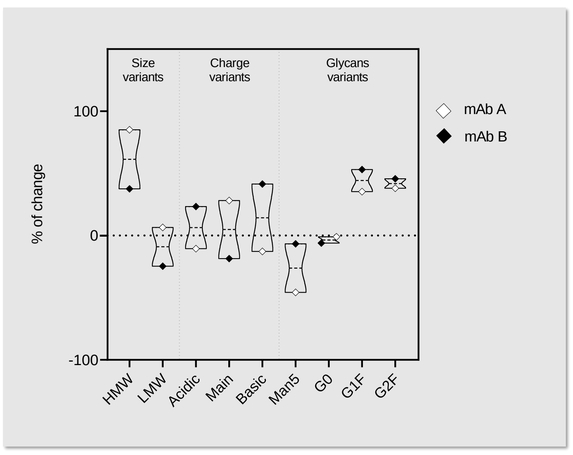


Fig. 7 Minor impact on quality attributes

- Increase in HMW still acceptable <5%
- Man5 reduction due to copper
- Increase in galactosylation due to copper and iron acting as galactosyltransferase cofactors

Specific productivity (q_p) was not improved at the expense of product quality as only moderate changes in product quality attributes were observed.

5 On demand process

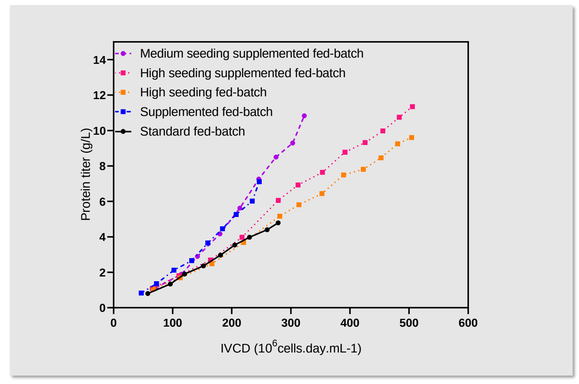


Fig. 8 Productivity performance of different intensified processes.

Depending on manufacturing constraints, initial seeding density can be adjusted in combination with supplementation to maintain high production performance.

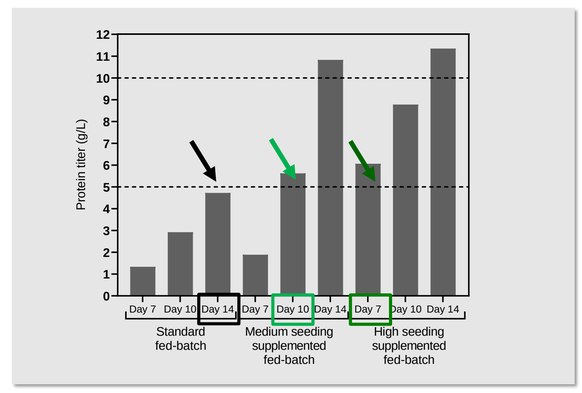


Fig. 9 Accelerating timelines on demand

- Titer target 5 g/L

30% reduction production time (10 days) with medium seeding supplemented fed-batch

50% reduction production time (7 days) with high seeding supplemented fed-batch

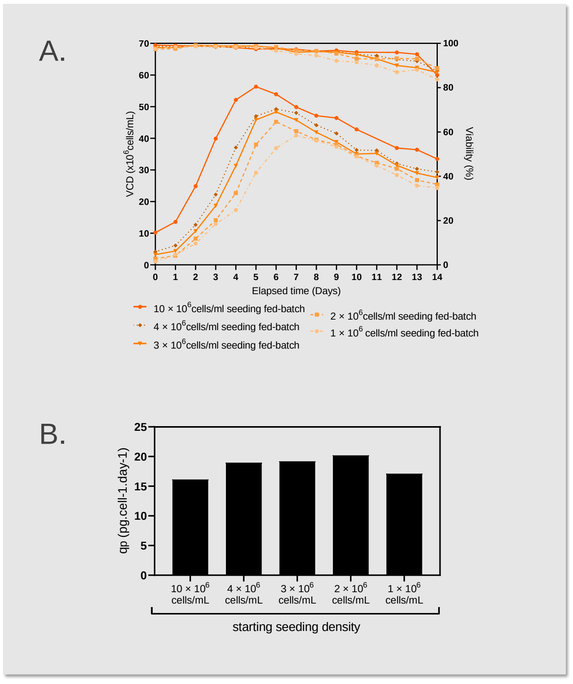


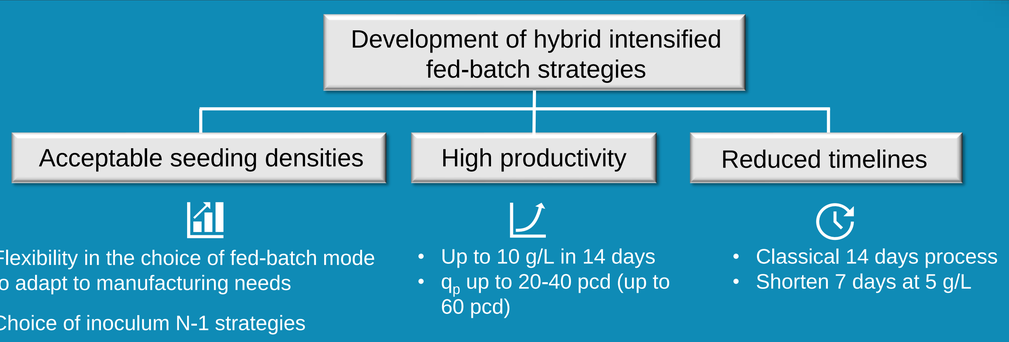
Fig. 10 Supplemented fed-batch performance with various starting densities: VCD/viability (A), q_p (B).

- Playing on different seeding densities
1, 2, 3, 4 and 10 x 10^6 c/mL

To gain greater flexibility in supplemented fed-batch culture process, we developed processes working accross a range of seeding densities to minimize the seeding density impact.

The five fed-batches showed that the optimized feeding conditions maintained the cells in the production state. Peak values were reached in both time and value in accordance with seeding density (A) and a similar specific productivity was observed (B).

Conclusion



Take home message

We successfully influenced the expression titer of mAbs above 10 g/L in 14 days, solely by acting on either an increased biomass reaching 50 x 10^6 c/mL peak density or by ameliorating q_p (40 pg.cell-1.day-1) using a chemical engineering approach.
Our study demonstrated the undeniable plasticity of the platform's process parameters and highlights that understanding of consecutive stages of mAb development is key determinant to provide new adapted and on-demand CHO fed-batch early process solutions.

Acknowledgements
Analytical Team, Selexis SA