OPTIMIZATION OF CHO-S CELL CULTURE MEDIUM BY SUPPLEMENTATION WITH NON-ANIMAL DERIVED COMPONENTS USING DESIGN OF EXPERIMENTS (DOE)

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OUTLINE

The optimization of CHO-S cell culture is studied for two different cell lines. The first part focuses on the improvement of CHO-S cell growth by addition of non-animal derived components to serum-free and protein-free media through design of experiments (DoE). Eight different supplements are tested and the best results are obtained for a chemically-defined medium (Medium 1) supplemented with Insulin, Transferrin and Polysorbate 80. In the second part of this work, the effect of insulin as an additive to three additional chemically-defined media is studied for a CHO-S mab producing cell-line. Insulin has a positive effect on both cell growth and protein production for the three media tested: Medium 2, Medium 3 and Medium 4.

CHO-S CELL LINE

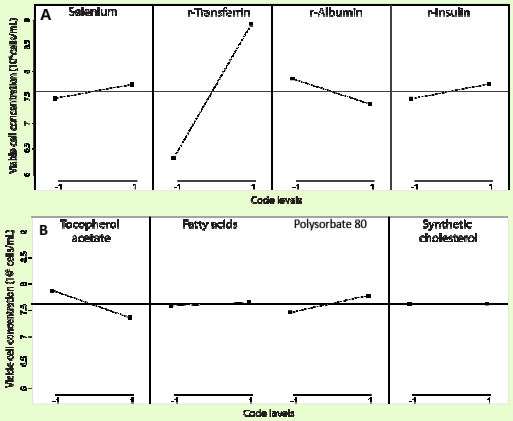
SCREENING SUPPLEMENTS WITH SIGNIFICANT EFFECT ON CHO-S CELL GROWTH

Medium 1 was selected as the cell culture medium to be optimized in a first round of experiments.

Plackett-Burman design

Independent variables	Code levels		Independent variables	Code levels	
	Low	High		Low	High
r-Albumin (g/L)	0	1	Tocopherol acetate (X)	0	3
r-Insulin (mg/L)	0	30	Synthetic cholesterol (X)	0	3
r-Transferrin (mg/L)	0	30	Fatty acids (X)	0	3
Selenium (µg/L)	0	10	Polysorbate 80 (X)	0	1

Plackett-Burman results



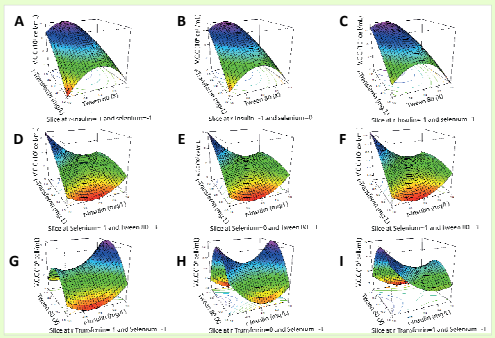
DEFINING OPTIMAL SUPPLEMENT LEVELS FOR CHO-S CELL GROWTH

r-Insulin, r-transferrin, selenium and polysorbate 80 are the supplements selected for the Box-Behnken optimization step.

Box-Behnken design

Independent variables	Code levels		
	-1	0	1
r-Insulin (mg/L)	0	1	2
r-Transferrin (mg/L)	0	30	60
Selenium (µg/L) T	0	10	20
Polysorbate 80 (X)	0	2	4

Box-Behnken results



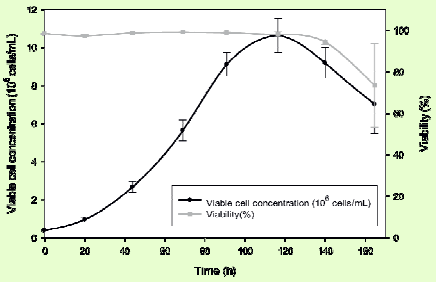
Data was fitted to a second order model:

A $Yi = \beta o + \Sigma \beta i\, Xi + \Sigma \beta ii\, Xi^2 + \Sigma \beta ij\, XiXj$

$Yi = 7.98 + 0.30 \times r\text{-Trans} + 0.01 \times r\text{-Ins} - 0.19 \times Sel - 0.39 \times Polys. - 0.59 \times r\text{-Trans} \times r\text{-Ins} - 0.01 \times r\text{-Trans} \times Sel - 0.46 \times rTrans \times Polys. - 0.04 \times r\text{-Ins} \times Sel + 0.36 \times r\text{-Ins} \times Polys. - 0.27 \times Sel \times Polys. - 0.27 \times r\text{-Trans}^2 + 0.77 \times r\text{-Ins}^2 + 0.26 \times Sel^2 - 1.35 \times Polys.^2$

B	Optimum levels of additives
r-Insulin (mg/L)	2
r-Transferrin (mg/L)	15.3
Selenium (µg/L)	0
Polysorbate 80 (X)	2.3

VALIDATION OF THE MODEL



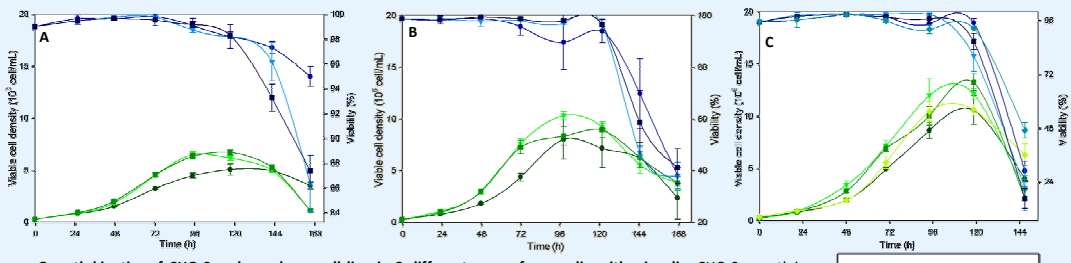
Growth kinetics of CHO-S cells in optimized cell culture conditions Cell density and viability are shown. Values presented are the mean ± SD (n=3). The maximum cell concentration attained was 10.6 x 10^6± 0.89 x 10^6 cells/mL, closer to the value predicted by the model (9.33 x 10.6x10^6 ± 1.23 x 10^6 cells/mL), and significantly higher than the negative control (6.48 x 10.6x10^6 ± 0.13 x 10^6 cells/mL).

CONCLUSIONS

- An improved cell growth of CHO-S cells in Medium 1 was achieved by adding three supplements: r-insulin (2 mg/L), r-transferrin (15.3 mg/L) and Polysorbate 80 (2.3 X).
- The maximum cell concentration was 60% higher than the control.
- The optimization of Medium 1 was successfully achieved by means of Design of Experiments.

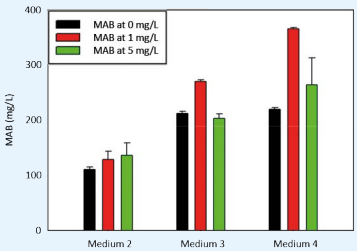
CHO-S MAB PRODUCER CELL LINE

r-INSULIN SUPPLEMENTATION TO 3 COMMERCIAL MEDIA



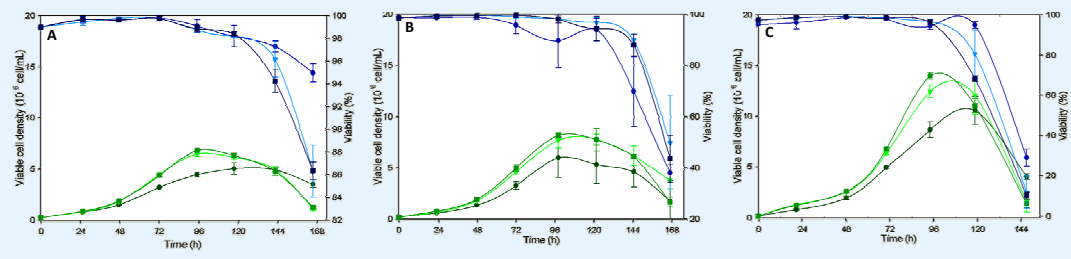
Growth kinetics of CHO-S mab producer cell line in 3 different serum-free media with r-insulin. CHO-S growth in Medium 1, Medium 2 and Medium 3 serum-free formulations with different concentration of r-insulin as indicated in the legend. Values presented are the mean ± SD (n=3). Cell density was significantly improved in all the media, especially with 1 mg/L.

MAB PRODUCTION



MAB Production : Comparison of mab production at 120h with different concentrations of r-insulin: 0 mg/L (black), 1 mg/L (red) and 5 mg/L (green).

BOLUS ADDITION OF r-INSULIN



Growth kinetics of cell line producewith bolus addition of r-insulin. CHO-S growth in chemically defined Medium 2, Medium 3 and Medium 4 , without r-insulin, with 1 mg/L of r-insulin and with bolus additions of 1 mg/L insulin at 48 and 96h. Values presented are the mean ± SD (n=3).

Medium	Conditions	Growth improvement	MAB improvement
Medium 2	Initial	45%	16%
	Bolus	53%	-
Medium 3	Initial	28%	27%
	Bolus	37%	-
Medium 4	Initial	31%	66%
	Bolus	53%	-

Media supplementation with r-insulin. Percentage of improvement comparing viable cell density at 96h and mab production at 120h. Two conditions are shown: initial addition of 1 mg/L of r-insulin at 0h (initial) and 1 mg/L initial concentracion and bolus addition of r-insulin at 48 and 96h (bolus).

FUTURE WORK

- Analysis of the main compounds in the media
- Fed-batch studies with commercial feeds + r-insulin.

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