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 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 Freestyle CHO supplemented with Insulin, Transferrin and Twin 80. In the second part of this work, the effect of insulin as an additive to chemically defined media is studied for a CHO-S cell producing an antibody (Herceptin). Insulin has a positive effect on both cell growth and protein production for the three media tested: ActiCHO, CDCCHO and FortiCHO

CHO-S CELL LINE

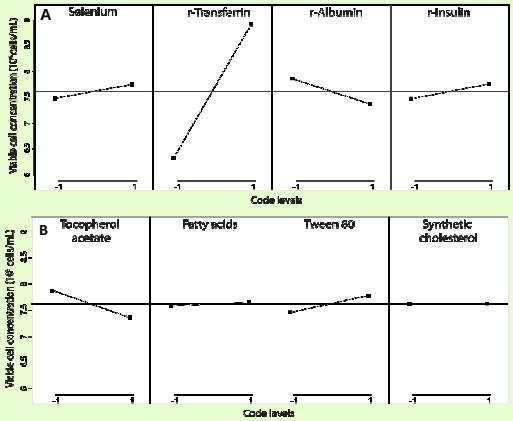
SCREENING SUPPLEMENTS WITH SIGNIFICANT EFFECT ON CHO-S CELL GROWTH

FreeStyleCHO medium 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	Tween 80 (X)	0	1

Plackett-Burman results



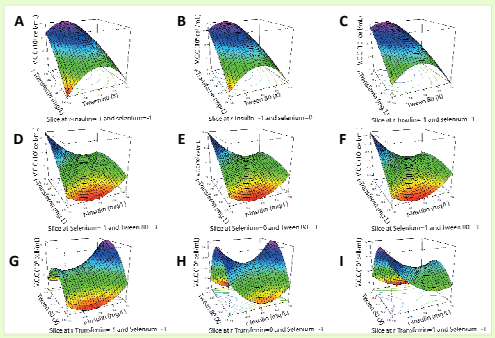
DEFINING OPTIMAL SUPPLEMENT LEVELS FOR CHO-S CELL GROWTH

r-Insulin, r-transferrin, selenium and tween 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)	0	10	20
Tween 80 (X)	0	2	4

Box-Behnken results



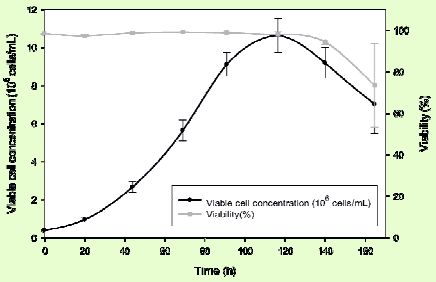
Data was fitted to a second order model:

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

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

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

VALIDATION OF THE MODEL



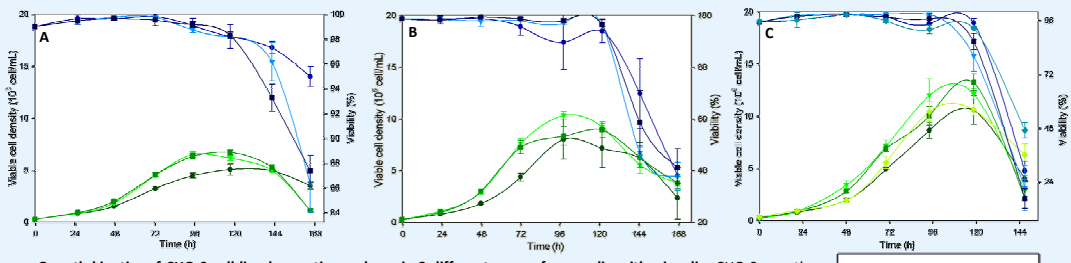
Growth kinetics of CHO-S cells in optimized cell culture conditions Cell density and viability are shown. Values presented are the mean ± SD (n=3). The maximum cell concentration attained was 10.6 x 10^6± 0.89 x 10^6 cells/mL, closer to the value predicted by the model (9.33 x 10.6x10^6 ± 1.23 x 10^6 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 FreeStyleCHO medium was achieved by adding three supplements: r-insulin (2 mg/L), r-transferrin (15.3 mg/L) and tween 80 (2.3 X).
- The maximum cell concentration was 60% higher than the control.
- The optimization of FreeStyleCHO medium was successfully achieved by means of Design of Experiments.

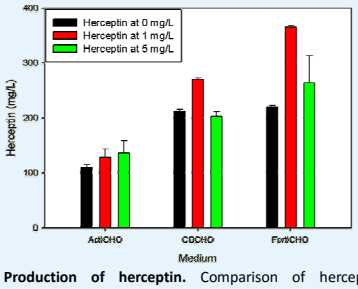
CHO-S HERCEPTIN PRODUCER CELL LINE

r-INSULIN SUPPLEMENTATION TO 3 COMERCIAL MEDIA



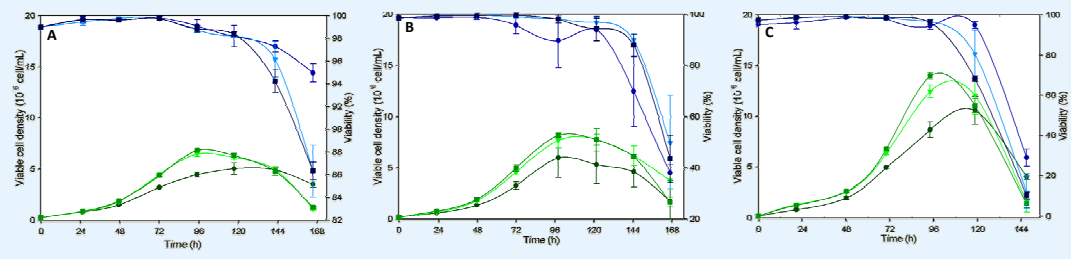
Growth kinetics of CHO-S cell line herceptin producer in 3 different serum-free media with r-insulin. CHO-S growth in ActiCHO (A), CDCHO (B) and FortiCHO (C) serum-free formulations with different concentration of r-insulin as indicated in the legend. Values presented are the mean ± SD (n=3). Cell density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

HERCEPTIN PRODUCTION



Production of herceptin. Comparison of herceptin 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 producer with bolus addition of r-insulin. CHO-S growth in ActiCHO (A), CDCHO (B) and FortiCHO (C) chemically defined media, 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	Herceptin improvement
ActiCHO	Initial	45%	16%
	Bolus	53%	-
CDCHO	Initial	28%	27%
	Bolus	37%	-
FortiCHO	Initial	31%	66%
	Bolus	53%	-

Media supplementation with r-insulin. Percentage of improvement comparing viable cell density at 96h and herceptin 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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