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MATHEMATHICAL MODELING OF A BIOREACTOR PRODUCING EPO-hr OPERATING IN PERFUSION MODE

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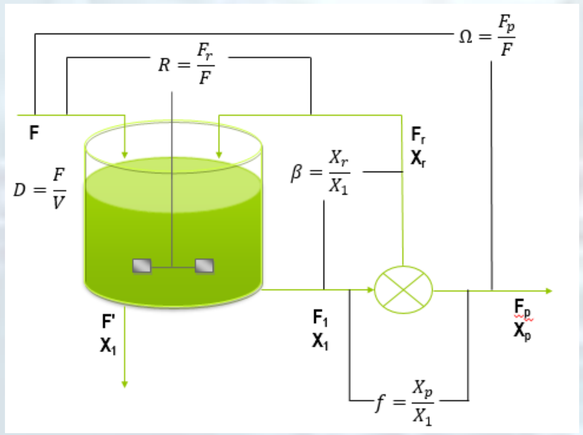
Abstract

The interest in the use perfusion of mode has increased in the last years, due to an increased awareness of perfusion advantages, some general improvement in equipment reliability, and a broadening of operational skills in the biomanufacturing industry. However, mathematical modeling of bioreactors in continuous mode with cell retention (perfusion mode) with continuous cell bleeding is still emerging, because this scheme has few applications in global biotechnology industry. The case of study was industrial fermentation process of CHO cells producing EPO-hr. The model involves a total of 7 equations and 19 variables. In order to fix the degrees of freedom were obtained experimentally from batch culture the kinetics parameters using logistic equations and was fixed operational parameters. It was determined that glutamine is limiting substrate and is related to specific growth rate through the model proposed by Monod. Validation of model was done by comparing different steady states with predicted values. On the other hand, a sensitivity analysis of the kinetic parameters and the influence of design and operating variables was performed. The variables with major impacts (up 2.5 fold) in volumetric productivity were concentration factor β and dilution rate D. Nevertheless a technological limitation for the perfusion equipment used was identify, for that reason another apparatus must be evaluate. It was further determined that optimum value of volumetric productivity can be reached is 4.5 fold, which is reached at a dilution rate (D) of 2.37 vvd and a concentration factor of 1.91. The feeding strategy could be works at 0.7 vvd with glutamine concentrated 2.55 times.

Perfusion Scheme



Formulation of Mathematical Model

Mass Balances

$$\beta = 1 + \frac{\Omega \cdot (1 - f)}{R}$$

$$\mu = \mathrm{D}(1 - \Omega - \Omega f)$$

$$x_1 = \frac{D}{q_s}(S_0 - S_1)$$

$$P_1 = P_0 + \frac{q_p \cdot x_1}{D}$$

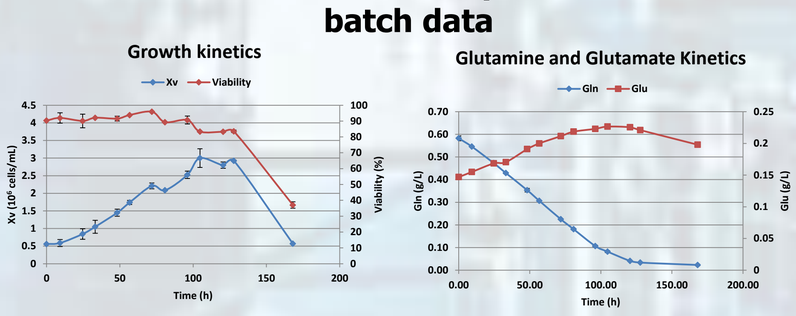
Kinetics Equations

- ✓ Monod-type equation
- ✓ q_s=f(μ)
- ✓ q_p=f(μ)

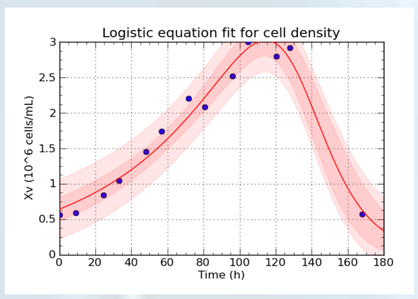
Limitations of the model

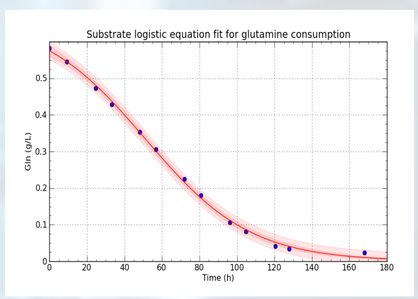
- ✓ Steady state
- ✓ All cells are in the same physiological state
- ✓ Perfect mixing

Determination of kinetics parameters from batch data



Check of experimental data consistency and fit of logistic equations





$$X_V = \frac{9.05 \cdot 10^3}{e^{0.057t} + 1.41 \cdot 10^4 e^{-0.016t}}$$

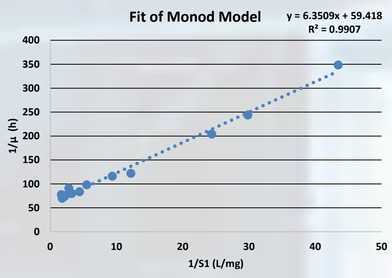
$k_{d\,max}$ μ_{max}

$$Gln = \frac{4.15}{e^{0.036t} + 6.22}$$

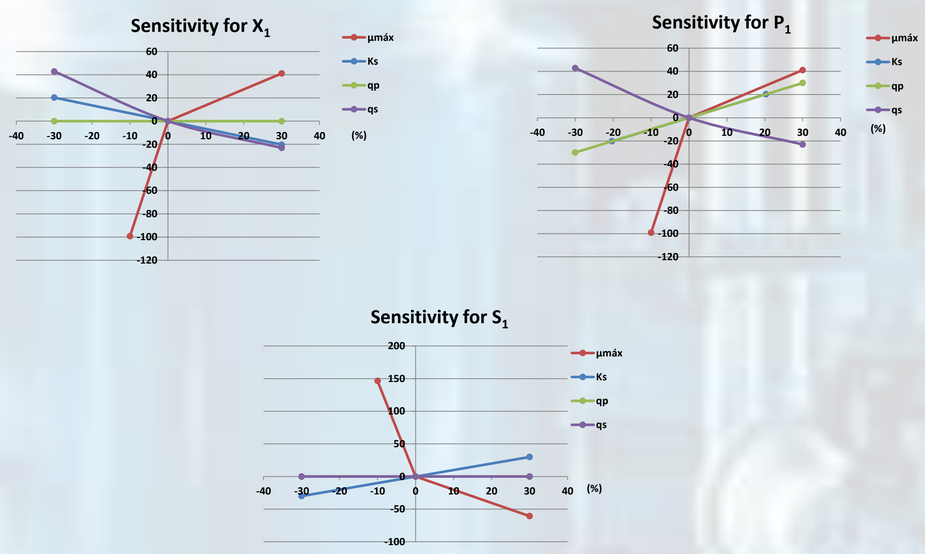
$$\mu = 0.016 \frac{Gln}{0.106 + Gln}$$

In all experimental points the index consistency (h) is lower than critical λ^2 (5.99) for 95% of confidence level

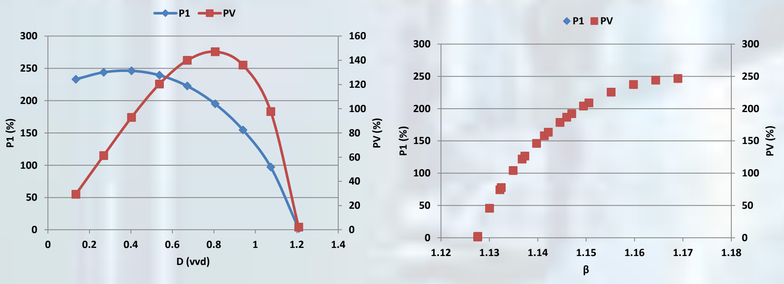
Fitting of Monod-type models



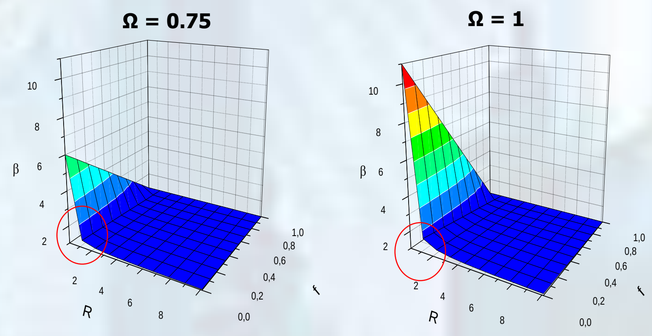
Sensitivity Analysis for experimental variables determined



Influence on volumetric productivity



Identification of technological limitation



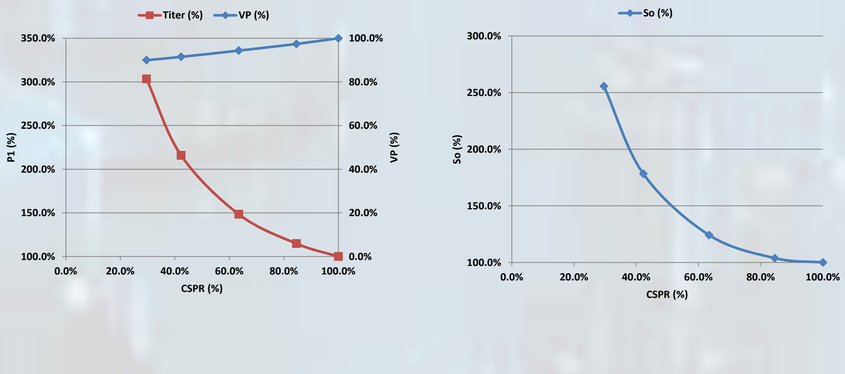
Optimization of volumetric productivity

D (vvd)	β (-)	μ (h^{-1})	S_1 (mg/L)	X_1 (%)	P_1 (%)	PV (%)	f (-)	Ω (-)	R (-)
2.37	1.91	0.009	75.30	503.92	205.56	454.71	0.01	0.95	1.00

Constraints
$D \leq D_{crit}$
$0.1 \leq f \geq 1$
$0 \leq \Omega \geq 0.95$
$R \geq 1$

$$D_{crit} = \frac{\mu_{max}\left(\frac{S_0}{K_s + S_0}\right)}{1 + \Omega(f - 1)}$$

Feeding strategy for diminish CSPR



Conclusions

- ❑The mathematical model describes steady states of perfusion process from a glutamine limiting substrate model
- ❑Output variables are sensitive to maximum specific growth rate and specific glutamine consumption rate.
- ❑The optimum value for volumetric productivity could be obtained for a dilution rate of 0.8 vvd.
- ❑The main limitation to obtain higher volumetric productivities is the impossibility of operate at very low recirculation rates, but a practical value of β =2 could be beneficial.
- ❑The theoretical maximum value of volumetric productivity (4.5 fold) is predicted at a combination of f=0.1; Ω=0.95; β=1.91 and D=2.37 vvd.
- ❑The feeding strategy could be works at 0.7 vvd with glutamine concentrated 2.55 times. The limiting factor for diminishing CSPR is the decreasing in volumetric productivity.

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