PERFUSION CELL CULTURE PROCESSES FOR BIOPHARMACEUTICALS

Master the design and operation of perfusion cell cultures with this authoritative reference. Discover the current state of the art in the design and operation of continuous bioreactors, with emphasis on mammalian cell cultures for producing therapeutic proteins. Topics include the current market for recombinant therapeutic proteins, current industry challenges, and the potential contribution of continuous manufacturing. The volume provides coverage of every step of process development and reactor operation, including small-scale screening to lab-scale and scale-up to manufacturing scale. Illustrated through real-life case studies, this is a perfect resource for groups active in the cell culture field, as well as graduate students in areas such as chemical engineering, biotechnology, chemistry, and biology, and to those in the pharmaceutical industry, particularly biopharma, biotechnology, and food or agro industry.

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Perfusion Cell Culture Processes for Biopharmaceuticals

Process Development, Design, and Scale-Up

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Abbreviations

ATF	alternating tangential flow
ATP	adenosine triphosphate
BPOG	Biophorum Operations Group
BR	benchtop bioreactor
CCC	critical coagulation concentration
CD	chemically defined
CFB	concentrated fed-batch
CFD	computational fluid dynamics
CGI	chemical growth inhibitor
CMP-Neu5Ac	cytidine diphosphate N-acethyneuraminic acid
COG	cost of goods
CPP	critical process parameter
CQA	critical quality attributes
$\mathbf{CSPR_{min}}$	minimum cell-specific perfusion rate
CSPR	cell-specific perfusion rate
CSTR	continuous stirred tank reactor
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DWP	deepwell plate
EGI	environmental growth inhibitor
ER	endoplasmic reticulum
ESS	explained variance
Fc	fragment crystallisable region
FDA	Food and Drug Administration
FucT	$\alpha - 1, 6$ fucosyltransferase
G0	no galactose molecule attached
G1	one galactose molecule attached
G2	two galactose molecules attached
GalT	$\beta - 1, 4$ Galactosyltransferase
GDP-Fuc	guanosine diphosphate fucose
GnTI	$\alpha - 1, 3$ N-acetylglucosaminyl transferase I
GnTII	$\alpha - 1, 6$ N-acetylglucosaminyl transferase II
HMW	high molecular weight
HS	high seeding fed-batch
IgG	immunoglobulin G
IPC	in-process control

List of Abbreviations

LCA	life-cycle assessment
LMW	low molecular weight
LS	low seeding fed-batch
LV	latent variable
mAb	monoclonal antibody
MALDI-TOF	matrix assisted laser desorption ionisation – time of flight
ManI	$\alpha - 1,2$ mannosidase I
ManII	$\alpha - 1,6$ mannosidase II
MAN	Mannose
MCSGP	multicolumn countercurrent solvent gradient purification
MIR	mid-infrared
MS	mass spectroscopy
msBR	micro-scale bioreactor
MVDA	multivariate data analysis
NIH	National Institute of Health
NIPALS	non-linear iterative partial least square
NIR	near infrared
NPV	net present value
NS	nucleotide activated sugar
NTP	nucleotide triphosphate
OS	oligosaccharide
OTR	oxygen transfer rate
PAT	process analytical technology
PCA	principle component analysis
PDE	partial differential equation
PF	perfusion
PFR	plug flow reactor
PID	proportional integral derivative
PLS	partial least square
PTM	post-translational modification
PMMA	poly(methyl methacrylate)
QbD	quality by design
relRMSEP	relative root mean square error in prediction
REMSECV	root mean square error in cross-validation
RMSEP	root mean square error in prediction
ROS	radical oxydative species
RT	Rushton turbine
RTD	residence time distribution
RV	reactor volume
SCADA	supervisory control and data acquisition
SialT	$\alpha - 1,6$ sialyltransferase
ST	shake tube
STD	standard deviation
SVD	single value decomposition
TCA	tricarboxylic acid cycle
TFF	tangential flow filtration

List of Abbreviations

TMP	transmembrane pressure
TSS	total variance
U	uridine
UDP-Gal	uridine diphosphate galactose
UDP-GlcNAc	uridine diphosphate N-acetylglucosamine
UFDF	ultrafiltration diafilatration
UV	ultraviolet
VCD	viable cell density
$\mathbf{VCD}_{\mathbf{max}}$	maximum viable cell density
VVD	vessel volume per day

Symbols

$(\bar{\epsilon}_T)_g$	Average total energy dissipation rate, $W \times m^{-3}$
$(\bar{\epsilon}_T)_{Ig}$	Average gasing energy dissipation rate, $W \times m^{-3}$
$(\bar{\epsilon}_T)_S$	Average stirring energy dissipation rate, $W \times m^{-3}$
$\bar{\epsilon}_T$	Specific energy dissipation rate, $W \times m^{-3}$
ΔC	Distance between two impellers, m
ΔC_{Gas}	Gas driving force, $mol \times L^{-1}$
Δ	Diagonal matrix of the non-zero singular values, –
$\hat{y}_{test,i}$	Model estimation of the y-value of the ith observation, –
λ	Eigenvalue, –
μ	Cell growth rate, d^{-1}
μ_d^{max}	Maximum cell death rate, d^{-1}
μ_d	Cell death rate, d^{-1}
μ_L	Dynamic viscosity, $kg \times m^{-1} \times s^{-1}$
μ_l	Cell lysis rate, d^{-1}
μ_{max}	Maximum cell growth rate, s^{-1}
ω_j	Width of the concentration profile of E_j, –
ω_k	Width of the concentration profile of TP_k, –
$\omega_{q,mAb}$	Width of the specific productivity as a function of pH, –
ρ_L	Liquid density, $kg \times m^{-3}$
σ_L	Liquid surface tension, $N \times m^{-1}$
τ	Average residence time, s
τ_{max}	Maximum tolerable stress, $N \times m^2$
τ_{Sep}	Average residence time in the cell retention device, s
θ_m	Characteristic mixing time, s
A	Bioreactor cross section, m
A	Number of principal components, –
a	Gas–liquid interfacial area per unit dispersion volume, m^{-1}
B	Bleed rate, $L \times d^{-1}$
B	PLS regression coefficient, –
C	Weights of matrix Y, –
C^*_{Gas}	Saturated gas concentration, $mol \times L^{-1}$
$C^*_{O_2}$	Oxygen concentration at saturation in liquid phase, $mg/L, ppm$
c_i^0	Initial molar concentration of species i, $mol \times L^{-1}$
C_{Gas}	Gas concentration in the reactor, $mol \times L^{-1}$
$C_{Harvest}$	Protein concentration in the harvest stream, $g \times L^{-1}$
c_i	Molar concentration of species i, $mol \times L^{-1}$
C_{O_2}	Oxygen concentration in liquid phase, mg/L, ppm

List of Symbols

C_P	Protein concentration, $g_{Protein} \times d^{-1}$
$C_{Reactor}$	Protein concentration in the reactor, $g \times L^{-1}$
D	Impeller diameter, m
d	Cell diameter, μm
D_i	Golgi diameter, μm
E	Residual matrix of the X space, –
$E(t)$	Residence time distribution, s
E_j^{max}	Peak concentration of glycosyltransferase j, $mol \times L^{-1}$
E_j	Glycosyltransferase j, –
F	Residual matrix of the Y space, –
f	Frequency, s^{-1}
F_i^{in}	Molar flowrate of species i entering the bioreactor, $mol \times d^{-1}$
f_{inh}	Term indicating inhibation, –
F_i	Molar flowrate of species i leaving the bioreactor, $mol \times d^{-1}$
f_{lim}	Term indicating nutrient limitation, –
$F_{T,k}$	Flowrate of sugar precursors into the Golgi, $mol \times s^{-1}$
G	Residual matrix of the regression model, –
g	Acceleration of gravity, $m^2 \times s^{-1}$
G_i	Rate of production of species i, $mol \times d^{-1}$
H	Bioreactor height, m
H_L	Filling height of the cell culture broth, m
I_n	Concentration of growth inhibitor n, $mol \times L^{-1}$
k	Reaction rate constant, $mol \times L^{-1} \times s^{-1}$
$K_{UDP-Gal,Gal}^{Gal}$	Equilibrium constant of the UDP-Gal equilibrium, $mol \times L^{-1}$
$k_{f,j}^{max}$	Maximum turnover rate of a specific reaction, s^{-1}
$K_{NS,k}^{MS}$	Equilibrium constant describing the equilibrium between monosaccharide in the medium and in the cytosol, $mol \times L^{-1}$
$K_{\mu,AMM}$	Ammonia growth inhibition constant, $mol \times L^{-1}$
$K_{d,AMM}$	Ammonia death inducing constant, $mol \times L^{-1}$
$K_{d,i}$	Dissociation constant of the specific donor-enzyme complex, $mol \times L^{-1}$
$K_{d,Mn^{2+}}$	Dissociation constant of the specific manganese-enzyme complex, $mol \times L^{-1}$
$K_{d,Nk}$	Dissociation constant of the nucleotide-enzyme complex, $mol \times L^{-1}$
$k_{f,j}$	Turnover rate constant, s^{-1}
k_L	Gas-liquid mass transfer coefficient, $m \times s^{-1}$
$k_L a$	Volumetric mass transfer coefficient, s^{-1}
K_n	Monod constant, $kg \times L^{-1}$
$k_{T,k}$	Transport turnover rate, s^{-1}
M	Measured torque on the impeller shaft, $n \times m$
M	Number of variables constituting the data matrix X, –
m_{AMM}	Ammonia-maintenance-related coefficient, $mol \times d^{-1}$
$m_{NS,k}$	Nucleotide-sugar-maintenance-related coefficient, $mol \times d^{-1}$
$m_{UDP-Gal}$	UDP-Gal maintenance coefficient, $mol \times d^{-1}$
MC	Medium consumption, $L_{Medium} \times g_{Protein}$
MS_k	Concentration of monosaccharide in the medium, mol
N	Agitation speed, s^{-1}

List of Symbols

N	Number of observations of the data matrix X, –
N_A^{Golgi}	Ammonia-associated Golgi constant, $mol \times L^{-1}$
N_C	Number of species, –
N_i	Number of moles of species i, mol
N_k	Nucleotide k, –
N_R	Number of reactions, –
NR	Number of enzymatic reactions, –
NS_k	Nucleotide sugar k, –
OS_i	Oligosaccharide i, –
P	Perfusion rate, d^{-1}
P	Power input, W
P_0	Power number, –
p_a	Loading vector corresponding to the ath principal component, –
P_g	Gaseous power dissipation, W
$p_{m,a}$	Element of loading matrix P corresponding to ath principal component and mth variable, –
pK_A^{Golgi}	Apparent pK_A value of the Golgi, –
PR	Volumetric productivity, $g_{Protein} \times L_{Reactor} \times d^{-1}$
Q	Volumetric flowrate, $L \times d^{-1}$
Q^2	Relative variance explained in cross validation, –
Q_B	Bleed volumetric flowrate, $L \times d^{-1}$
Q_g	Volumetric gas flowrate, $L \times min^{-1}$
Q_H	Harvest volumetric flowrate, $L \times d^{-1}$
Q_{in}	Volumetric flowrate of nutrient feed addition, $L \times d^{-1}$
q_i	Specific production rate of species i, $mol \times L^{-1} \times d^{-1}$
q_{mAb}	Cell-specific productivity of monoclonal antibody, $g_{mAb} \times cell^{-1} \times d^{-1}$
Q_{out}	Volumetric flowrate of nutrient removal, $L \times d^{-1}$
Q_P	Perfusion volumetric flowrate of nutrient addition, $L \times d^{-1}$
q_p	Cell-specific productivity, $g_{Protein} \times cell^{-1} \times d^{-1}$
R	Overal rate of reaction, $mol \times L^{-1} \times d^{-1}$
r_i	Rate of production of species i, $mol \times L^{-1} \times d^{-1}$
R_j	Rate of the reaction j, $mol \times L^{-1} \times d^{-1}$
Re	Reynolds number, –
Re_{imp}	Reynolds impeller number, –
S	Covariance matrix, –
T	X-scores, –
T	Bioreactor diameter, m
t_a	Score vector corresponding to the ath principal component, –
TP_k^{max}	Peak concentration of the transport protein k, $mol \times L^{-1}$
TP_k	Transport protein k, –
U	Y-scores, –
U	Matrix of the left singular vector, –
V	Matrix of the right singular vector, –
V_{Bleed}	Bleed volume, L
$V_{Exchange}$	Exchange volume, L
$V_{Harvest}$	Harvest volume, L
$v_{i,j}$	Stoichiometric coefficient of species i in reaction j, –

List of Symbols

v_i	Stoichiometric coefficent of species i, –
$v_{NS,k}$	Incorporation rate of a nucleotide sugar, $mol \times s^{-1}$
V_R	Reactor volume, L
V_{Sep}	Volume of separation device, L
V_S	Gas superficial velocity, $m \times s^{-1}$
V_{tot}	Total volume, L
W^*	Adjusted weights of matrix X, –
X	Data matrix, –
X_d	Dead cell density, 10^6 $cells \times mL^{-1}$
X_l	Lysed cell density, 10^6 $cells \times mL^{-1}$
x_m	Vector of x observation of mth variable, –
$X_{V,meas}$	Measured cell density, 10^6 $cells \times mL^{-1}$
$X_{V,SP}$	Cell density set-point, 10^6 $cells \times mL^{-1}$
$X_{V,target}$	Cell density target, 10^6 $cells \times mL^{-1}$
X_V	Cell density, 10^6 $cells \times mL^{-1}$
Y	Yield, %
$Y_{\mu,AMM}$	Ammonia-growth-dependent yield coefficient, $1 \times mol^{-1}$
$Y_{NS,k}$	Nucleotide-sugar-growth-dependent yield coefficient, $1 \times mol^{-1}$
$y_{test,i}$	y-value of the ith observation in the external set, –
z_j^{max}	Localisation of the peak concentration of E_j, –
z_k^{max}	Localisation of the peak concentration of TP_k, –
CO_2	Carbon dioxyde
H_2O	Dihydrogen monoxyde
$HCO^{-}{}_{3}$	Bicarbonate ion
K	Potassium
Na	Sodium
$NaHCO_3$	Sodium bicarbonate
O_2	Dioxygen
OH^-	Hydroxyde ion