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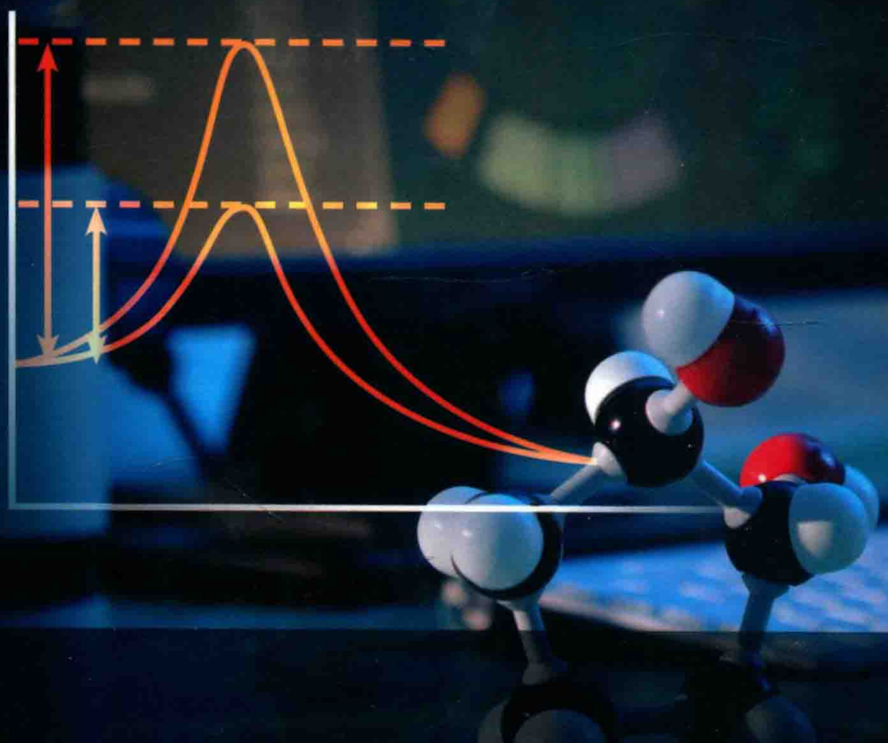
Problem Solving in Enzyme Biocatalysis



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Problem Solving in Enzyme Biocatalysis

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Preface

You shall bring forth your work as a mother brings forth her child: out of the blood of your heart. Each act of creation shall leave you humble, for it is never as great as your dream and always inferior to that most marvelous dream which is Nature.

"Decalogue of the Artist," Gabriela Mistral, Chilean Nobel Laureate

This book is primarily intended for chemical and biochemical engineering students, but also for biochemists, chemists, and biologists dealing with biocatalytic processes. It was purposely written in a format that resembles the Schaum's textbooks of my long gone college years. An abridged coverage of the subject is provided in each chapter, followed by a number of sample exercises in the form of solved problems illustrating resolution procedures and the main concepts underlying them, along with supplementary problems for the reader to solve, provided with the corresponding answers. Learning through problem solving is designed to be both challenging and exciting to students. There is no book on enzyme biocatalysis with this format and purpose, so we sincerely hope to have made a contribution to the toolbox of graduate and undergraduate students in applied biology, chemical, and biochemical engineering with formal training in college-level mathematics, organic chemistry, biochemistry, thermodynamics, and chemical reaction kinetics. The book pretends to be a complement of a previous book, *Enzyme Biocatalysis* (Springer, 2008), which I had the privilege and pleasure to edit.

Chapter 1 is an introduction, giving an updated vision of enzyme biocatalysis, its present status, and its potential development. Chapters 2 and 3 refer to enzyme kinetics in homogeneous and heterogeneous systems, respectively, considering both fundamental and practical aspects of the subject. Chapter 4 is devoted to enzyme reactor design and operation under ideal conditions, while Chapters 5 and 6 deal with the main causes of nonideal behavior (mass-transfer limitations and enzyme inactivation, respectively). Chapter 7 presents an overview of the optimization of enzyme reactor operation and different tools for optimization. The book is complemented by two documents. Appendix A refers to mathematical methods for those readers not sufficiently acquainted with the subject, while Epsilon Software Information presents a software program (εpsilon) for solving and representing enzyme reactor performance under different scenarios.

Writing is certainly a most exciting endeavor. Undoubtedly, writing about enzymes is beyond being the subject of our expertise, because enzymes are the catalysts of life, so writing about enzymes lies in the boundaries of writing about life. And life, as the Iraqi poet Abdul Wahab said, is about standing straight, never bent or bow, about remaining glad, never sad or low. We hope our readers will keep straight and glad while getting a little more acquainted with these catalysts of life.

A book is a journey from expectation to consolidation, an act of love from conception to birth. It has been a most rewarding experience to travel and conceive in the company of my

co-authors, two brilliant young colleagues of mine, Dr. Carlos Vera and Dr. Lorena Wilson, who were formerly my students and now fly well over my shoulders. My gratitude also to my colleague Dr. Raúl Conejeros, who authored Chapter 7 and beyond that contributed to spice this book throughout with mathematical modeling and elaborated thinking. Do not blame him too much; he is a good mix of wisdom and kindness. Our gratitude also to Dr. Felipe Scott for developing, together with Dr. Carlos Vera, the software Epsilon, presented in Epsilon Software Information to the development of the Epsilon Software.

A significant part of this book was written in Spain, at the Chemical Engineering Department of the Universitat Autònoma de Barcelona, where I was (again) warmly hosted while undertaking a sabbatical (my third one there) during the second semester of 2012. My personal gratitude to my colleagues there, Dr. Josep López and Gregorio Álvaro, who made me feel at home and who shared the ecstasy and the agony of this pregnancy.

My gratitude to the people at my institution, the Pontificia Universidad Católica de Valparaíso, who supported and encouraged this project, particularly to the rector, Professor Claudio Elórtegui, and the Director of my School of Biochemical Engineering, Dr. Paola Poirrier. Special thanks to Dr. Atilio Bustos, Head of the Library System, for his enthusiastic support and advice. My deepest appreciation also to the people at Wiley: Rebecca Stubbs and Sarah Tilley, and Shikha Pahuja at Thomson Digital, who were always helpful, warm, and supportive.

Last but not least, my gratitude to my life partner Dr. Fanny Guzmán, expert in peptide synthesis, loving care, and *savoir-vivre*.

Engineering is about products and processes. A book is both. We hope you will enjoy the product as much as we have enjoyed the process.

Andrés Illanes
Valparaíso, July 2013

Nomenclature

Symbol	Description	Dimensions	Chapter No.
A			
a	molar concentration of substrate A	$[ML^{-3}]$	2, 4
a	cube edge; major axis of oblate ellipsoid	[L]	3
a	constant.		Appendix A
A	surface area of catalyst particle	$[L^2]$	3
A	sectional area of reactor	$[L^2]$	5, 7
A	specific activity ratio of intermediate to initial enzyme species		6
A'	specific activity ratio of final (partly active) to initial enzyme species		6
A_S	surface area of catalyst	$[L^2]$	3
a_i	initial molar concentration of substrate A	$[ML^{-3}]$	4, 6
a_{sp}	catalyst-specific activity	$[IUM^{-1}]$	3, 4, 5, 6, 7
B			
b	molar concentration of substrate B	$[ML^{-3}]$	2, 4
b	minor axis of oblate ellipsoid		3
B	vector of parameter		7, Appendix A
B^*	optimum vector parameter		Appendix A
b_0, b_i, b_{ii}	zero-, first-, and second-order parameters for the surface-of-response model		7
B_1^*, B_2^*	vectors of first- and second-order regression coefficients of the surface-of-response model		7
b_i	initial molar concentration of substrate B	$[ML^{-3}]$	4, 6
b_i	parameter i		Appendix A
Bi	Biot number		3, 5
b_{ij}	second-order interaction parameters for the surface-of-response model		7
C			
c	molar concentration of enzyme–substrate complex (ES)	$[ML^{-3}]$	2
C	constant		4, 5, Appendix A
[C]	concentration of analyte C	$[ML^{-3}]$	2, 3, 4, 5, 6, 7, Appendix A

Symbol	Description	Dimensions	Chapter No.
CA_i	annual cost of item i	[USD]	7
ce	coenzyme concentration	$[ML^{-3}]$	2
ce′	modified coenzyme concentration	$[ML^{-3}]$	2
D			
d	molar concentration of enzyme–inhibitor complex (EI)	$[ML^{-3}]$	2
D	differential operator		Appendix A
D_0	diffusion coefficient in water	$[L^2T^{-1}]$	3
D_{eff}	effective diffusion coefficient	$[L^2T^{-1}]$	3, 5, 6, 7
d_P	diameter of catalyst spherical particle	[L]	3, 6
E			
e	concentration of active enzyme	$[IUL^{-3}]$	2, 4, 5, 6, 7, Appendix A
E	enzyme activity	[IU]	4, 5, 6
E	relative error or enzyme species		Appendix A
e_0	initial concentration of active enzyme	$[IUL^{-3}]$	2, 6, 7
E_0	initial enzyme activity	[IU]	6
E_a	energy of activation in Arrhenius equation	$[ML^2T^{-2}]$	2, 5, 7
E_C	contacted enzyme activity	[IU]	3
ee_S	enantiomeric excess of the S-enantiomer		3
E_I	immobilized enzyme activity	[IU]	3
E_{ia}	energy of activation of inactivation in Arrhenius equation	$[ML^2T^{-2}]$	2, 3, 7
$E_{ia,\ M}$	energy of activation of inactivation in Arrhenius equation in the presence of a modulator	$[ML^2T^{-2}]$	7
E_L	enzyme activity lost by immobilization	[IU]	3
em	molar concentration of enzyme–modulator M complex	$[ML^{-3}]$	6
E_R	enzyme activity remaining in solution after immobilization	[UI]	3
E(t)	enzyme activity over time	[IU]	6
F			
f	molar concentration of enzyme–inhibitor–substrate complex (EIS)	$[ML^{-3}]$	2
f	size distribution frequency		3
f	number of factors assessed by the surface-of-response methodology		7
F	ratio of V_{max} to V'_{max}		2
F	reactor feed flow rate	$[L^3T^{-1}]$	4, 5, 6, 7
F_0	initial reactor feed flow rate	$[L^3T^{-1}]$	6

Symbol	Description	Dimensions	Chapter No.
F_0	Fisher-Snedecor distribution for a null hypothesis		7
f(x)	function of vector x		Appendix A
F(x)	vector of functions of x		Appendix A
f(x,y)	implicit function of vector x and dependent variable y		Appendix A
$F_{\alpha,g,h}$	Fisher–Snedecor distribution for a confidence level of $1-\alpha$ with g degrees of freedom in the numerator and h degrees of freedom in the denominator		7
H			
h	film volumetric mass-transfer coefficient for substrate	$[L^3T^{-1}]$	3, 6
h	packed-bed reactor length	[L]	7
h	integration step size		3,5, Appendix A
H	total reactor length	[L]	7
h'	film linear mass-transfer coefficient for substrate	$[LT^{-1}]$	3, 5
h^+	proton (molar) concentration	$[ML^{-3}]$	2, 3
h_0^+	proton (molar) concentration in the bulk medium	$[ML^{-3}]$	3
h_P	film volumetric mass transfer coefficient for product	$[L^3T^{-1}]$	Epsilon Software Information
I			
i	inhibitor molar concentration	$[ML^{-3}]$	2
J			
J	Jacobian matrix		Appendix A
J	substrate flow rate	$[MT^{-1}]$	3
J	objective function		7
K			
k	catalytic rate constant		2, 4, 5, 6, 7, Appendix A
K	equilibrium constant of dissociation of enzyme–substrate complex into enzyme and substrate	$[ML^{-3}]$	2
k_0	pre-exponential term in Arrhenius equation		2, 7
k_B	Boltzmann universal constant		3
k_D	first-order inactivation rate constant	$[T^{-1}]$	2,3,6, Appendix A

Symbol	Description	Dimensions	Chapter No.
K_D	dissociation constant of enzyme–substrate complex into enzyme and substrate	$[ML^{-3}]$	2
$k_{D,0}$	pre-exponential term in Arrhenius equation	$[T^{-1}]$	2, 7
k_{D1}	first-order inactivation rate constant in first stage of inactivation	$[T^{-1}]$	6
k_{D2}	first-order inactivation rate constant in second stage of inactivation	$[T^{-1}]$	6
$k_{D,M}$	first-order inactivation rate constant of enzyme–modulator M complex	$[T^{-1}]$	6
$k_{D,M,0}$	pre-exponential term in Arrhenius equation in the presence of modulator M	$[T^{-1}]$	7
K_{eq}	equilibrium constant		2
k_i	Runge–Kutta method coefficients		Appendix A
K_I	inhibition constant	$[ML^{-3}]$	3, 4
K_{IC}	dissociation constant of enzyme–competitive inhibitor complex	$[ML^{-3}]$	2, 6, 7
K_{INC}	dissociation constant of enzyme–noncompetitive inhibitor complex	$[ML^{-3}]$	2, 4, 6
K'_{INC}	dissociation constant of enzyme–substrate–noncompetitive inhibitor tertiary complex	$[ML^{-3}]$	2, 4
K_M	Michaelis–Menten constant	$[ML^{-3}]$	2, 3, 4, 5, 6, 7, Appendix A
K_{MA}	dissociation constant of secondary complex EA into E and A	$[ML^{-3}]$	2, 6
K'_{MA}	dissociation constant of tertiary complex EAB into EB and A	$[ML^{-3}]$	2
K_{MAP}	apparent Michaelis–Menten constant	$[ML^{-3}]$	2, 3
K_{MB}	dissociation constant of secondary complex EB into E and B	$[ML^{-3}]$	2
K'_{MB}	dissociation constant of tertiary complex EAB into EA and B	$[ML^{-3}]$	2, 6
$K_{M,P}$	dissociation constant of EP into E and P (reversible reaction)	$[ML^{-3}]$	2
$K_{M,S}$	dissociation constant of ES into E and S (reversible reaction)	$[ML^{-3}]$	2
K_P	electrostatic partition coefficient at the matrix–medium interface		3
K_P	inhibition constant by product		4, Epsilon Software Information

Symbol	Description	Dimensions	Chapter No.
K_S	dissociation constant of the inactive tertiary complex ESS into ES and S		2, 3, 4, 5, 6, 7, Appendix A
$K_{p,S}$	partition coefficient for substrate		3
K_{p,h^+}	partition coefficient for protons		3
L			
L	optical path in spectrophotometer cell		2
L	catalytic slab width	[L]	3, 5, 7
L_{eq}	equivalent catalyst particle length	[L]	3, 7
L_R	packed-bed reactor length	[L]	5
M			
m_{cat}	concentration of biocatalyst	$[ML^{-3}]$	4, 5, 6
M_{cat}	mass of immobilized enzyme catalyst	[M]	3, 4, 5, 6, 7
m_i	characteristic equation root		Appendix A
MW	molecular weight		7
N			
n	stoichiometric coefficient of product with respect to substrate		4
n	number of observations		7, Appendix A
N	number of sections		Appendix A
N_1	lumped modulation factor in first stage of enzyme inactivation		6
n_{1P}	modulation factor by product in first stage of inactivation		6
n_{1S}	modulation factor by substrate in first stage of inactivation		6
N_2	lumped modulation factor in second stage of enzyme inactivation		6
n_{2P}	modulation factor by product in second stage of inactivation		6
n_{2S}	modulation factor by substrate in second stage of inactivation		6
n_M	modulation factor by modulator M		6, 7
N_R	number of staggered reactors		6
$N_{t1/2}$	number of half-lives of catalyst use		6
O			
OD	optical density		2
P			
p	product molar concentration	$[ML^{-3}]$	2, 3, 4, 7
p	number of parameters		7, Appendix A

Symbol	Description	Dimensions	Chapter No.
p_0	molar concentration of product in the bulk reaction medium	$[ML^{-3}]$	3
P_C	contacted protein	[M]	3
P_I	immobilized protein	[M]	3
P_R	protein remaining in solution after immobilization	[M]	3
p_S	molar concentration of product at the biocatalyst surface	$[ML^{-3}]$	3
p_{sp}	specific protein load		3
Q			
q	coupled analyte concentration	$[ML^{-3}]$	2
q	number of regression variables		7
R			
r	variable radius of spherical particle	[L]	3
R	ideal gas constant	$[ML^2T^{-2}\theta^{-1}]$	2, 3, 7
r'	rate of transformation of substrate into product		3
R^2	coefficient of determination		Appendix A
r_a	agitation rate	$[T^{-1}]$	3
R^2_{adj}	adjusted coefficient of determination		Appendix A
R_F	allowable flow-rate fluctuation as a result of downstream operations		6
R_P	radius of catalyst spherical particle	[L]	3, 5
S			
s	substrate concentration within the catalyst	$[ML^{-3}]$	2, 3, 4, 5, 7, Appendix A
s_0	molar concentration of substrate in the bulk reaction medium	$[ML^{-3}]$	3
s_{0i}	molar initial (or inlet) substrate concentration in the bulk reaction medium	$[ML^{-3}]$	5
s^2	residual mean square for n − p degrees of freedom		Appendix A
S(B)	sum of square error		Appendix A
s_i	initial (or inlet) substrate molar concentration	$[ML^{-3}]$	2, 4, 6, 7
s_S	molar concentration of substrate at the biocatalyst surface	$[ML^{-3}]$	3, 7
SS_E	sum of squares due to residuals (error)		7
SS_R	sum of squares due to regression (model)		7

Symbol	Description	Dimensions	Chapter No.
$SS_{R\ k/,p-k}$	regression sum of squares for k variables, given that p minus k variables are already in the model		7
$SS_{R,p-k}$	regression sum of squares for the reduced model containing p minus k variables		7
T			
t	time	[T]	2, 3, 4, 5, 6, 7, Appendix A
T	absolute temperature	[θ]	2, 3, 7
t_0	initial time	[T]	7
$t_{1/2}$	half-life of enzyme catalyst	[T]	2, 6
t_C	total time of one cycle of reactor operation	[T]	6
t_{dless}	dimensionless time of reactor operation		4
t_f	final time	[T]	7
T_{lo}	temperature lower bound	[θ]	7
$t(n-p, \alpha/2)$	upper $\alpha/2$ quantile of Student's t-distribution for $n-p$ degrees of freedom		7, Appendix A
t_s	reactor staggering time	[T]	6
T_{up}	temperature upper bound	[θ]	7
U			
u	control variable		7
u_{lo}	control variable lower bound		7
u_{up}	control variable upper bound		7
V			
v	initial reaction rate	$[ML^{-3}T^{-1}]$	2, 4, 5, 6, Appendix A
v	model parameter vector or vector whose elements are different from zero		7
V	reactor volume	$[L^3]$	7
v′	enzymatic reaction rate	$[MT^{-1}]$	3
v″	reaction rate per unit volume of catalyst	$[ML^{-3}T^{-1}]$	3
V_{bed}	bed volume of packed-bed reactor	$[L^3]$	4, 5
$V(b_i)$	variance of the parameter b_i		Appendix A
VC(B)	variance–covariance matrix of B		Appendix A
V_{eff}	effective volume of reaction	$[L^3]$	4, 5, 6, 7
$v_{effective}$	effective reaction rate	$[ML^{-3}T^{-1}]$	5
V_{max}	maximum reaction rate	$[ML^{-3}T^{-1}]$	2, 4, Appendix A
V_{maxAP}	maximum apparent reaction rate of product formation	$[ML^{-3}T^{-1}]$	2

Symbol	Description	Dimensions	Chapter No.
V_{maxAP0}	pre-exponential term in Arrhenius equation	$[ML^{-3}T^{-1}]$	2
$V_{max,P}$	maximum reaction rate of product to substrate conversion (reversible reaction)	$[ML^{-3}T^{-1}]$	2
$V_{max,S}$	maximum reaction rate of substrate to product conversion (reversible reaction)	$[ML^{-3}T^{-1}]$	2
V'_{max}	maximum reaction rate of product formation from enzyme-substrate-inhibitor tertiary complex	$[ML^{-3}T^{-1}]$	2
V'_{max}	maximum reaction rate	$[MT^{-1}]$	3, Epsilon Software Information
V''_{max}	maximum reaction rate per unit volume of catalyst	$[ML^{-3}T^{-1}]$	3, 7
V_{proc}	total processed volume	$[L^3]$	6
V_R	reaction volume	$[L^3]$	5
v_s	solute molar volume	$[L^3M^{-1}]$	3
X			
x	variable width of catalytic slab	[L]	3
x	state variables		7
x	vector of independent variables		Appendix A
X	limiting substrate to product conversion		3, 4, 5, 6
X	matrix of independent variables		7, Appendix A
X_0	initial (steady-state) conversion of limiting substrate into product		6
x_c	coded variables		7
x_g	vector of values for the model variables $(p \times 1)$		7
x_h	highest value for uncoded input variable		7
x_i	input variable i		7
x_l	lowest value for uncoded input variable		7
Y			
y	dependent variable		Appendix A
Y	vector of observed values		7, Appendix A
$\hat{y}$	predicted response by the model		7
$\bar{Y}$	average of the observed values		7, Appendix A
Y_E	enzyme immobilization yield		3
y_i	output variable i		7
y_i	ith observation for the dependent variable		Appendix A

Symbol	Description	Dimensions	Chapter No.
$\hat{y}_i$	predicted response for the ith observation		7
Y_P	protein immobilization yield		3
Z			
z	dimensionless variable width of catalytic slab		3, 7, Appendix A
Z	valence of ionic species		3
Greek Letters			
α	ratio of rate constant of formation of B from E′B and transition rate from EA to E′ + B (ping-pong mechanism)		2, 4
α	Damkoehler number		3, 5, 6
α	significance level		7, Appendix A
α_S	Damkoehler number for substrate		Epsilon Software Information
α_P	Damkoehler number for product		Epsilon Software Information
β	dimensionless substrate concentration		3, 7, Appendix A
β_0	dimensionless substrate concentration in the bulk reaction medium		3, 5, Appendix A
β_{0i}	initial (or inlet) dimensionless substrate concentration		5
β_c	dimensionless substrate concentration within the catalyst at the center of the slab		7
β_i	initial (or inlet) dimensionless substrate concentration		6
β_S	dimensionless substrate concentration at the biocatalyst surface		3, 5
γ	dimensionless product concentration $(p \cdot K_P^{-1})$		3
γ_0	dimensionless product concentration in bulk reaction medium $(p_0 \cdot K_P^{-1})$		3, 5
γ_{0i}	initial (or inlet) dimensionless product concentration		5
γ_S	dimensionless product concentration at the biocatalyst surface $(p_S \cdot K_P^{-1})$		3
δ	stagnant liquid film width	[L]	3
δ	relative error tolerance		Appendix A
ΔH^0	standard enthalpy change of dissociation of ES into E and S	$[ML^2T^{-2}]$	2

Symbol	Description	Dimensions	Chapter No.
ΔH_I^0	standard enthalpy change of dissociation of EI into E and I or EIS into ES and I	$[ML^2T^{-2}]$	2
ΔS^0	standard entropy change of dissociation of ES into E and S	$[ML^2T^{-2}\theta^{-1}]$	2
ΔS_I^0	standard entropy change of dissociation of EI into E and I, or EIS into ES and I	$[ML^2T^{-2}\theta^{-1}]$	2
ε	error		3
ε	molar extinction coefficient		2
ε	porosity of the catalyst matrix		3
ε	void fraction of catalyst bed		4, 5, 7
ε	error vector		7, Appendix A
E	enantioselectivity		3
ε_i	error for the ith observation		Appendix A
ζ	tortuosity of the catalyst matrix pores		3
η	local (or surface) effectiveness factor		3, 5
η_G	global (mean integral value) effectiveness factor in the catalyst particle		3, 5, 6, 7
η_P	global effectiveness factor under product inhibition		3
κ	ratio of Michaelis to uncompetitive inhibition constants ($K_M \times K_S^{-1}$)		3
λ	optical wavelength	L	2
λ	dimensionless reactor bed length		5
μ	viscosity of the solution	$[ML^{-1}T^{-1}]$	3
ν	dimensionless reaction rate ($v \times V_{max}^{-1}$)		3, 5, 6
π	volumetric productivity of reactor operation	$[ML^{-3}T^{-1}]$	4
π_{sp}	specific productivity of reactor operation	$[T^{-1}]$	4
ρ	dimensionless radius of the spherical catalyst particle		3, 5, Appendix A
ρ_{app}	apparent density of catalyst	$[ML^{-3}]$	4, 5, 6, 7
σ_i	standard deviation of b_i		Appendix A
τ	fluid residence time in the reactor	[T]	4, 5
$\varphi(t)$	enzyme decay function		6
Φ	Thièle modulus for substrate		3, 5, 6, Appendix A
Φ_P	Thièle modulus for product		3
Φ^R	Thièle modulus for R enantiomer		3
Φ^S	Thièle modulus for S enantiomer		3
Ψ	electrostatic potential of the support	$[ML^2T^{-2}Q^{-1}]$	3

Other

$\in$	electron charge		3

Epsilon Software Information

Available alongside this book is a copy of the software εpsilon which allows the simulation of the operation of enzymatic reactors. εpsilon has been designed to illustrate the main topics included in this book, offering an additional tool to improve the understanding of the design and operation of enzymatic reactors (specifically Chapters 4 to 6). Guidelines for software installation are given below:

i. Program Installing

Two situations may occur during installation:

- ***Matlab® is already installed in user's computer***
  The program was built using Matlab®'s compiler toolbox. Hence, if Matlab® is installed in the user's computer, open the distrib folder and run the file Epsilon_32.exe or Epsilon_64.ex, depending on the system's architecture.
- ***Matlab® is not installed in user's computer***
  First, open the Matlab® package (Epsilon_64_pkg or Epsilon_32_pkg, depending on the system's architecture). Two files will be created in the current directory. Second, open MCRInstaller.exe and follow the installer instructions. Once the installation is complete, open the newly created Epsilon_64.exe or Epsilon_32.exe.

ii. Program Description

This software was designed with a simple interface, with the purpose of allowing the comparison of reactor behavior under different scenarios (reaction kinetics and operation modes). The program considers reactor performance for biocatalysts having the most common enzyme kinetics (Michaelis-Menten, competitive and non-competitive inhibition by product and uncompetitive inhibition by substrate) and operating in usual reactor configurations (BSTR, CPBR and CSTR). The program also allows incorporating the effect of external diffusional restrictions (EDR) or catalyst inactivation during reactor operation. Figure 1 shows the interface of εpsilon.