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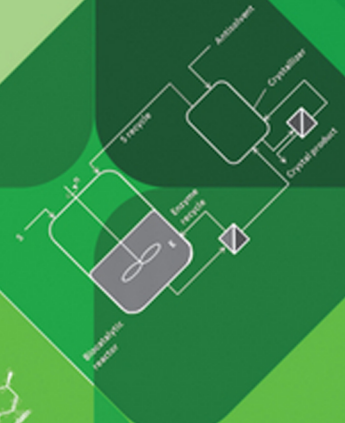

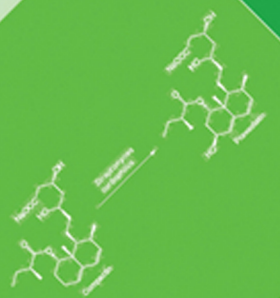

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Abbreviations

A5P	D-Arabinose-5-phosphate
ABP	Acyl-Co-A binding protein domain
ABTS	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid
ACN	Acetonitrile
ACP	Acyl-carrier protein domain
AcOEt	Ethyl acetate
Acyl-Co-A	Acetyl coenzyme A
AD	4-Androstene-3,17-dione
ADD	1,4-Androstadiene-3,17-dione
ADH	Alcohol dehydrogenase
ADH-hT	ADH from *Bacillus stearothermophilus*
ADP	Adenosine diphosphate
δ-ALA	δ-Aminolevulinic acid
AP	Area percentage
API	Active pharmaceutical ingredient
ArR-ωTA	ω-Transaminase from *Aspergillus terreus*
AST	Arylsulfotransferase
ATHase	Artificial transfer hydrogenases
ATP	Adenosine triphosphate
*Av*PAL	*Anabaena variabilis* phenylalanine ammonia lyase
a_w	Water activity
BBE	Berberine bridge enzyme
BDC	Benzoic acid decarboxylase
BHT	2,6-Di-tert-butyl-4-methylphenol
BIA	Benzylisoquinoline alkaloid
BM3	CYP102A1 from *Bacillus megaterium*
*Bm*GDH	*Bacillus megaterium* glucose 1-dehydrogenase
Boc	*t*-Butoxycarbonyl
BSA	Bovine serum albumin
BSTR	Batch-stirred tank reactor
BVMO	Baeyer–Villiger monooxygenase
b.y.	Baker's yeast
CALA	*Candida antarctica* lipase A
CALB	*Candida antarctica* lipase B
CDW	Cell dry weight
CEBR	Continuous expanded-bed reactors

CFBR	Continuous fluidized-bed reactor
CH_3-IMH	D,L-5-(3-Indolylmethyl)-3-N-methylhydantoin
CHMO	Baeyer–Villiger monooxygenase from *Acinetobacter sp.*
CLEA	Cross-linked enzyme aggregates
CLEC	Cross-linked enzyme crystals
CPE	Chloro-1-phenylethanol
CPFR	Continuous plug-flow reactor
CPO	Chloroperoxidase from *C. fumago*
CPR	Cytochrome P450 reductase
CRED	Carbonyl reductase
CSTR	Continuous stirred-tank reactor
CV-ωTA	ω-Transaminase from *Chromobacterium violaceum*
CYP	Cytochrome P450 monooxygenase
DAAO	D-amino acid oxidase
DAB	1,4-Diaminobutane
dC	2′-Deoxycytidine
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCP	Dicholoro-2-propanol
DCU	Dicyclohexylurea
ddAHU7P	1,2-Dideoxy-D-arabino-hept-3-ulose 7-phosphate
DEAE	Diethylaminoethanol (group in ion-exchange resin)
dF6P	1-Deoxy-D-fructose-6-phosphate
dG	2′-Deoxyguanosine
DHA	Dihydroxyacetone
DHAK	Dihydroxyacetone kinase
DHAP	Dihydroxyacetone phosphate
DHK	Dihydroxyacetone kinase
dH_2O	Distilled water
DIPE	Diisopropylether
DIPEA	Di-isopropyl ethylamine
DKR	Dynamic kinetic resolution
DMAP	4-Dimethylaminopyridine
DMAPP	Dimethylallyl pyrophosphate
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxides
DNA	Deoxyribonucleic acid
DSP	Downstream processing
DTT	Dithiothreitol
EDA	Ethyl diazoacetate
E	Enantiomeric ratio
EBA	Expanded-bed adsorption
EDTA	Ethylenediaminetetraacetic acid
ee	Enantiomeric excess
EEHP	Ethyl 2-ethoxy-3-(4-hydroxyphenyl)propanoate
ELSD	Evaporative light-scattering detector

ER	Ene-reductases
ESI-MS	Electrospray ionization – mass spectrometry
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
F6P	D-Fructose-6-phosphate
FAD	Flavin adenine dinucleotide
$FADH_2$	Flavin adenine dinucleotide, reduced form
FBR	Fluidized-bed reactor
FDH	Formate dehydrogenase
FID	Flame ionization detection
FMN	Flavin mononucleotide
FMO	Flavin monooxygenase enzyme
FPLC	Fast protein liquid chromatography
FSA	D-Fructose-6-phosphate aldolase
FTIR	Fourier-transform infrared spectroscopy
αG1P	α-D-Glucose 1-phosphate
G3P	D-Glyceraldehyde-3-phosphate
GA	Glycolaldehyde
GABA	γ-Aminobutyric acid
GC	Gas chromatography
GC-FID	Gas chromatography – flame ionization detection
GC-MS	Gas chromatography – mass spectrometry
GDE	Gas diffusion electrode
GDH	Glucose dehydrogenase
Gly-Gly	Glycyl-glycine buffer
GM	Glucose-milk medium
GPC	Gel permeation chromatography
GPDH	α-Glycerophosphate dehydrogenase
HA	Hydroxyacetone
Hal	Halogenase
HapD	Thiamine diphosphate-dependent enzyme from *Hahella chejuensis*
HB	Hydroxybutanone
Hct	Hectochlorin biosynthesis enzyme
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
Hhe	Halohydrin dehalogenase
HLADH	Horse liver alcohol dehydrogenase
HNB	Hefe–Nährbouillon
4-HPAA	4-Hydroxyphenylacetaldehyde
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography with diode-array detection
HPLC-RI	High-performance liquid chromatography with refractive index
HRP	Horse radish peroxidase
IL	Ionic liquid
INT	Iodonitrotetrazolium salt
IPA	Isopropyl alcohol
IPAc	Isopropyl acetate

IPTG	Isopropyl β-D-1-thiogalactopyranoside
IRED	Imine reductase
IS	Internal standard
ISPR	*In situ* product removal
KDO	3-Deoxy-D-*manno*-oct-2-ulosonic acid
α-KG	α-Ketoglutarate
KPi	Potassium phosphate buffer
KR	Kinetic resolution
KRED	Ketoreductase
LB	Luria–Bertani medium
LDH	Lactose dehydrogenase
LK-ADH	ADH from *Lactobacillus kefir*
LSADH	*Leifsonia* sp. alcohol dehydrogenase
M9-N	M9 minimal salts microbial growth medium
MALDI	Matrix-assisted laser desorption/ionization
MAO-N	Monoamine oxidase from *Aspergillus niger*
MBR	Membrane bioreactor
MCF	Siliceous mesocellular foam
MeCN	Acetonitrile
MeOH	Methanol
MES	2-(N-Morpholino)ethanesulfonic acid
MOPS	4-Morpholinepropanesulfonic acid
MS	Mass spectrometry
MPLC	Medium-pressure liquid chromatography
MPTA	α-Methyl-α-trifluoromethylphenylacetic acid
MTBE	Methyl *t*-butyl ether
MWCO	Molecular weight cut-off
m/z	Mass-to-charge ratio
NAAAR	*N*-Acetyl amino acid racemase
NAD+	β-Nicotinamide adenine dinucleotide
NADH	β-Nicotinamide adenine dinucleotide, reduced form
NADPH	β-Nicotinamide adenine dinucleotide 2′-phosphate, reduced form
NADP+	β-Nicotinamide adenine dinucleotide 2′-phosphate
NCS	(*S*)-Norcoclaurine synthase
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometer
*N*CβPhe	*N*-Carbamoyl-β-phenylalanine
NMR	Nuclear magnetic resonance spectroscopy
OD	Optical density
ω-OHFA	ω-Hydroxy fatty acid
OxM	Oxygenase medium
4-OT	Oxalocrotonate tautomerase
OYE	Old yellow enzyme
P450cam	Cytochrome P450 camphor-hydroxylating
PAD	Phenolic acid decarboxylase
PAGE	Polyacrylamide gel electrophoresis

PAL	Phenylalanine ammonia lyase
PAPS	Adenosine-3′-phospho-5′-phosphosulfate
PAS	Sulfatase from *Pseudomonas aeruginosa*
PBR	Packed-bed reactor
PCR	Polymerase chain reaction
PE	Petrol ether
PEP	Phosphoenolpyruvic acid
PFAM	Protein family
PheDU	Phenyldihydrouracil
PhoN-Sf	Phosphatase from *Shigella flexneri*
PigD	Thiamine diphosphate-dependent enzyme from *Serratia marcescens*
PK	Pyruvate kinase
PLP	Pyridoxal 5′-phosphate
PMMA	Poly(methyl methacrylate)
PMSF	Phenylmethylsulfonyl fluoride
PNP	Purine nucleoside phosphorylase
PPi	Pyrophosphate anion $P_2O_7^{4-}$
PPM	Phosphopentomutase
PPTase	4′-Phosphopantetheinyl transferase
PTFE	Polytetrafluoroethylene
PYR	Pyruvate
prnA	Tryptophan 7-halogenase
R&D	Research and development
rac	Racemic
Rf	Retention factor
*Rg*PAL	*Rhodotorula glutinis* phenylalanine ammonia lyase
RK	Ribokinase
*Rm*Qred	Quinuclidinone reductase of *Rhodotorula mucilaginosa*
ROH	Generic alcohol
RPE	Ribulose-5-phosphate epimerase
RPI	Ribose-5-phosphate isomerase
PVDF	Polyvinylidene difluoride
pyrH	Tryptophan 5-halogenase
*Rm*Qred	Quinuclidinone reductase of *Rhodotorula mucilaginosa*
rpm	Revolutions per minute
rt	Room temperature
Rt	Retention time
Sav	Streptavidin
SDR	Short-chain alcohol dehydrogenase
SDS	Sodium dodecyl sulfate
SeAAS	Thiamine diphosphate dependent enzyme from *Saccharopolyspora erythraea*
SFC	Supercritical fluid chromatography
Sfp	Phosphopantheteinyl moiety transfer protein from *Bacillus subtilis*
SFPR	Substrate feeding product removal
SIM	Single-ion monitoring

TA	Transaminase
TA-CV	ω-Transaminase from *Chromobacterium violaceum*
TB	Terrific broth
TEAA	Triethylamine acetate
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TFA	Trifluoroacetic acid
THIQ	Tetrahydroisoquinoline
THNR	Tetrahydroxynaphthalene reductase
TLC	Thin-layer chromatography
TOF	Time-of-flight
TPI	Triosephosphate isomerase
Tris	Tris(hydroxymethyl)aminomethane
TycF	Thioesterase from *Bacillus brevis*
U	Units
UF	Ultrafiltration
UHPLC-UV	Ultra high-performance liquid chromatography, ultraviolet
UV	Ultraviolet
VCD	Vibrational circular dichroism
VCPO	Vanadium chloroperoxidase
v/v	Volume/volume
vvm	Gas volume flow per unit of liquid volume per minute (vessel volume per minute)
wcw	Wet cell weight
wt	Wild type
w/v	Weight/volume
w/w	Weight/weight
2xYT	2xYT Medium for microbial growth
YPD	Yeast extract peptone dextrose medium

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Considerations for the Application of Process Technologies in Laboratory- and Pilot-Scale Biocatalysis for Chemical Synthesis

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1.1 Introduction

The development and implementation of an efficient new biocatalytic process relies upon successful communication between the scientists establishing the chemical reaction (organic chemists, process chemists, analysts, etc.), those developing the biocatalyst (microbiologists, biochemists and molecular biologists, analysts, etc.), and those scaling up the process (process, biochemical, and chemical engineers). The working relationship between the first two groups has strengthened enormously in recent years, but nevertheless successful scale-up also requires process engineering involvement from an early stage. In the pharmaceutical industry, it is easy to argue that the rate of attrition of new target molecules is such that any consideration for scale-up should be delayed for as long as possible. However, the reality is that to address the process aspects too late is equally problematic. The problem is exacerbated by many of the chemical reactions of greatest commercial interest in transforming non-natural substrates. In some cases, the selectivity of an enzyme is not compromised, but its activity is nearly always found to be lower than on a

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comparable natural substrate. Additionally, the conditions under which these enzymes are expected to operate in industry are also very often far from those found in nature, further affecting their activity and stability. Therefore, this necessitates improvements not only to the biocatalyst, but also to the reactor and process, such that a suitable system can be designed and implemented for scale-up. This demands effective communication and dialog between the various scientists at an early stage of process development.

This chapter is written with the intent of giving chemists, biologists, and engineers a basic idea of the concepts involved in implementing a biocatalytic reaction for development, scale-up, and, ultimately, production of a target product. Frequently, information relevant to these fields is scattered, and therefore a deliberate attempt has been made here to bring it together into a single compilation to help in disseminating the available knowledge to those working in all aspects of biological chemical conversions.

It is hoped that this will give a better understanding to scientists working in unifying these fields for efficient process development. For example, a biologist should be able to use this chapter to help understand the importance of setting commercial targets to measure the success of the biocatalysts they have developed. Likewise, process chemists can appreciate the key differences between the application of chemical catalysis and biological catalysis. Additionally, this chapter aims to guide chemists and biologists in designing experiments to obtain relevant data that might help in a smooth transition from a laboratory proof-of-concept to a scalable chemical synthesis with product isolation. Engineers will also abstract the differences between biochemical and conventional chemical transformations.

The aim of this chapter is to provide readers with an understanding of the important tools and technologies available for use in biocatalysis. Specifically, the technologies that can be implemented at laboratory and pilot scale will be addressed. Quantitative information will be provided when possible for application of these technologies, which will hopefully guide the reader to make educated decisions on how to efficiently operate their processes. The purpose, therefore, is not to answer all questions, but to give a quick overview of the different characteristics and considerations for the said technologies.

Finally, it is of vital importance to acknowledge that this text is based on the contributions and experiences of many scientists and engineers (both in academia and in industry) from different spheres involved in the establishment of fundamental and applied research of the discussed technologies.

1.2 Process Intensification and Proposed Scale-Up Concept

The arguments for the application of biocatalysis as a catalytic tool in organic synthesis and production are numerous, but are perhaps most usually focused on the exquisite selectivity that biocatalysts offer [1]. Clearly, the rationale for implementation depends upon the industrial sector and the value of the product to be produced.

One of the central challenges in the development and implementation of new enzymatic processes in industry is translating an established laboratory-scale reaction into a commercial process. The first step in that journey should be to establish suitable conditions for the reaction, in particular the required selectivity and product purity. This is mainly the work of organic and process chemists. The enhancement of enzyme properties is also a major