Chapter

Potential of Biocatalysis in Pharmaceuticals

Snehi Soy, Riddhi Prabha and Vinod Kumar Nigam

Abstract

Biocatalysis has been continuously evolving as an essential tool which is playing a significant role in the industrial synthesis of chemicals, active pharmaceuticals, pharmaceutical intermediates, etc. where the high-yielding chemo-, regio-, and enantioselective reactions are needed. Despite its vital importance, industrial biocatalysis is facing certain limitations such as operational stability, economic viability, efficient recovery, and reusability. The limitations mentioned can be overcome by the isolation of specific enzyme producers from extreme environment by protein engineering, bioinformatics, and recombinant DNA technologies. Recently, chemoenzymatic pathway and biological cascade reactions have also been developed and designed to perform the synthesis of pharmaceuticals. In this chapter, we compile the broad applications of biocatalysts in the synthesis of pharmaceuticals.

Keywords: biocatalysis, biocatalyst, enantiomers, pharmaceuticals, substrate specificity, stability

1. Introduction

Biocatalysis is appropriately defined as the enzyme-based applications for the transformation of molecular substrate into several natural as well as synthetic chemicals [1, 2]. The enzymes used in the process are in the form of cell lysate, whole cells, or purified enzyme and are prepared either as recombinant expressed proteins in different host cells or expressed in their native cells itself [3]. The key players of biocatalysis are biocatalysts or enzymes that have been divided into six classes by the IUPAC nomenclature system based on the reactions they catalyze [4], as shown in **Table 1**.

Enzymes as biocatalysts are incredibly proficient and are always preferred to conventional chemical processes. It is due to the fact that enzyme-based biocatalysis has distinct advantages over chemical reactions such as (1) significant specificity towards catalyzed reactions and recognized substrates, (2) simplified synthetic route, (3) high yields with exceptional regio-, chemo-, and stereoselectivities, (4) minimum energy requirements, and (5) generation of less by-products and wastes [5–8]. Another preferred advantage includes whole bioprocess and bulk operations being carried out under mild conditions at elevated rates and with extreme specificity and with minimum environmental and physiological toxicity, thus making them an ideal candidate in the development and improvement of sustainable chemical processes [9–12].

Enzyme class	IUPAC code	Catalyzed reactions	Important subclasses
Hydrolases	EC3	Hydrolytic reactions and their reversal	Esterases, glycosidases, lipases, proteases, peptidases, amidases
Oxidoreductases	EC1	Redox reactions	Dehydrogenases, oxidases, oxygenases, peroxidases, reductases
Transferases	EC2	Functional group transformation, addition/elimination involving C-C, C-N, and C-C bond formation or breakage	C_1-transferases, glycosyltransferases, aminotransferases, phosphotransferases
Lyases	EC4	Elimination reactions	Aldolases, decarboxylases, dehydratase, few pectinases
Isomerases	EC5	Molecular isomerizations	Epimerases, racemases intramolecular transferases
Ligases/synthetases	EC6	Formation of a covalent bond joining two molecules together, coupled to hydrolysis of an ATP or analog	C-C, C-N, C-O, C-S ligases

Table 1.
IUPAC classification of enzymes based on reactions they catalyze.

However, despite holding tremendous potential, biocatalysis has an inevitable pitfall associated with it when extreme conditions of industrial processes are to be considered. An efficient biocatalyst needs to be compatible enough with specific properties such as thermostability, catalytic ability, substrate specificity, and operational stability in turbulent flow regimes, toxic, hazardous solvents, and substrate inhibition [13–21].

Thus, there is a need for the identification and production of stable biocatalysts with broad industrial applicability by exploring and screening novel microbes or identification of new genes with desired properties through the analysis of genes responsible for enzyme production and stability. Further enhancement of the enzyme properties can be done by applying protein engineering tools such as molecular docking, directed evolution, molecular modeling, and process engineering [22–25].

2. Scenario of biocatalysis in pharmaceuticals industries and its pertinent applications

In 1992, Roger Sheldon estimated environmental impact factor (*E* factor) (kg waste/kg product) for several chemical industries, and an *E* factor of 25–>100 was noted in the pharmaceutical industries [26]. Thus, to reduce the harmful impact of pharmaceutical manufacturing processes and making it more sustainable, "green chemistry" has been increasingly adopted. An efficient biocatalytic process encompasses the "12 principles of green chemistry" to an extent which give it an edge over other technologies [27], as shown in **Figure 1**.

In Europe, a project CHEM21 was launched by the collaboration of both government and industries for the implementation of green technology in the chemical and pharmaceutical sectors [28–30]. The project was launched because of the replacement of biocatalysis over chemical in the synthesis of pharmaceuticals involving several redox reactions, chiral amine synthesis, and regio- and stereospecific hydroxylation of abundant compounds [18, 28, 31]. Since then biocatalysis has been

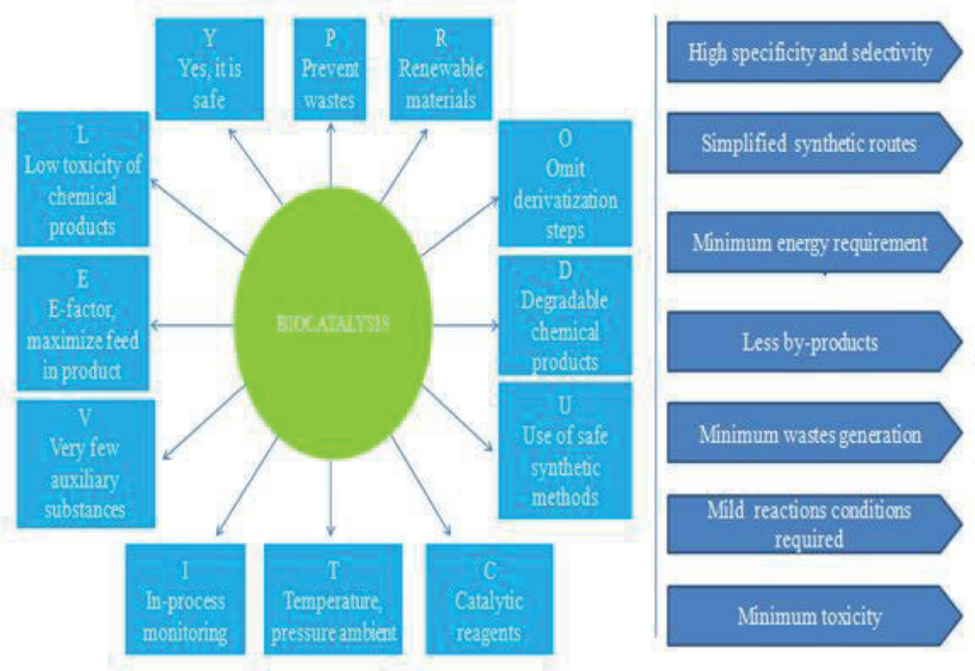

Figure 1.
Schematic representation of biocatalysis benefits embracing the principles of green chemistry.

profitably used for the production of pharmaceutically active chemicals and several blockbuster drugs at the industrial level and some of which are mentioned below:

- Sitagliptin—Sitagliptin, an antidiabetic compound, was successfully produced via biocatalytic approach. It finds application in the treatment of type II diabetes and is sold under the trade name "Januvia" by Merck [32, 33]. This work was accomplished by engineering R-selective transaminase (R-ATA, ATA-117) from *Arthrobacter* species by researchers at Codexis and Merck. The drug produced was having 99.95% enantiopurity even in the presence of 1 M i-PrNH_2 with 50% DMSO and at a temperature >40°C [33]. Conventionally, it was prepared using rhodium, a heavy metal as a catalyst. However, on comparing both processes, the biocatalytic method showed a massive reduction in waste as well as the use of heavy metal. Besides this, the overall yield and productivity were increased by 10 and 53% [34]. The R- and S-selective ATA was also used in the production of a variety of drugs such as niraparib and the production of an antagonist of orexin receptor with the formation of inhibitor of JAK kinase pathway [35–38].

- Boceprevir—This is a product of chiral amine synthesis and is marketed by Merck under trade name Victrelis. It is used for the treatment of chronic hepatitis C infections. In the production process, monoamine oxidase (MAO) from fungus *Aspergillus niger* was used for the asymmetrical amine oxidation of bicyclic proline intermediate [39]. The biocatalytic process increased yield by 150%, with an overall reduction in raw materials and side products as waste. At present, engineered monoamine oxidase (MAO) is also used in the production of another hepatitis C drug, telaprevir [34, 40], and various other synthetic drugs such as solifenacin, levocetirizine along with few natural alkaloid products (confine, harmicine, elegance, and leptaflorine).

- Montelukast—Montelukast or Singulair (trade name) is an anti-asthmatic drug marketed by Merck [41]. The engineered keto-reductase (KRED) was used for the production of montelukast, which displayed significant enantioselectivity

(99.9%) and was stable in 70% organic solvent and temperature of 45°C [24]. The biocatalytic method was advantageous in the sense that it omitted the use of hazardous chemical catalyst chlorodiisopinocampheylborane (DIP-CI), which was conventionally used. Several other drugs such as atorvastatin, crizotinib, duloxetine, and phenylephrine were also developed by biocatalytic process using KRED from bacterium *Lactobacillus kefir* [29].

- Atorvastatin—It comes from the statin family and is marketed under the trade name Lipitor by Pfizer. This drug reduces cholesterol levels by inhibiting the synthesis of cholesterol in the liver [42]. Atorvastatin production is also carried out by employing KRED for the production of hydroxy nitrile, an important intermediate. It is a multienzyme process involving glucose dehydrogenase (GDH), KRED, and halohydrindehalogenase (HHDH). Thus, the process is environmentally as well as economically feasible.

- Pregabalin—Pregabalin, a lipophilic GABA (γ-aminobutyric acid) analog, finds use in the treatment of various central nervous system ailments including neuropathic pain, fibromyalgia, epilepsy, and anxiety [43, 44]. Its production was carried out by biocatalytic conversion of *rac*-2-carboxyethyl-3-cyano-5-methylhexanoic acid ethyl ester to 2-carboxyethyl-3-cyano-5-methylhexanoic acid using lipolase. A heat-promoted decarboxylation of 2-carboxyethyl-3-cyano-5-methylhexanoic acid yielded (*S*)-3-cyano-5-methylhexanoic acid ethyl ester, which is a principal known precursor of pregabalin [45]. The mentioned chemoenzymatic synthesis route not only produced increased yields of pregabalin (40–45%) but also eliminated wastes and usage of organic solvent.

- 7-ACA (7-aminocephalosporic acid)—Cephalosporin has been extensively used as semisynthetic antibiotics; it acts on bacterial cell wall (peptidoglycan) synthesis. 7-Aminocephalosporanic acid (7-ACA), the critical intermediate or precursor for the production cephalosporins, is biocatalytically produced by

Biocatalysts	Microbial sources	Pharmaceutical compounds	References
Lipase B	*Candida antarctica*	Reboxetine	[49]
Carbonyl reductase (YlCR2)	*Yarrowia lipolytica*	Statins	[50]
Oxidase	*P. simplicissimum*	Pinoresinol	[51]
Acyltransferase (LovD)	Whole-cell *Escherichia coli* strain overexpressing LovD	Simvastatin	[52, 53]
Engineered cyclohexanone monooxygenase	—	Armodafinil	[54]
(+)-γ-lactamases	*Bradyrhizobium japonicum* USDA 6	Carbovir, abacavir, melogliptin	[5, 55]
Immobilized lipase	*Thermomyces lanuginosus*	Rasagiline mesylate (active ingredient of AZILECT®)	[56]
Expressing tyrosine phenollyase	*Erwinia herbicola* cells	L-DOPA	[57]
E. coli cells expressing cellobiose 2-epimerase	*Caldicellulosiruptor saccharolyticus*	Lactulose	[58]

Table 2.
List of biocatalysts and their microbial source employed for the synthesis of pharmaceutical drugs.

enzymatic deacylation of cephalosporin-C (CPC). A two-step enzymatic process utilizes D-amino acid oxidase (DAAO) and 7-β-(4-carboxybutanamido)-cephalosporanic acid acylase (GLA) for two consecutive reactions. Also, a single-step conversion from CPC to 7-ACA has been reported [46]. It has been successfully applied for the conversion of CPC to 7-ACA at industrial level [47]. Similarly, 6-aminopencillanic acid has been reported for the synthesis of semisynthetic penicillins using penicillin acylase [48].

Some other noteworthy examples and recent progress being made in pharmaceutical synthesis using enzymes from various sources are represented in **Table 2**.

3. Conclusion

Biocatalysis has made a remarkable journey so far and has been successfully applied for the numerous biotransformation processes in several industries. It has benefitted nearly all sectors, particularly chemical and pharmaceuticals. The flourishing development of economically viable and sustainable chemoenzymatic processes highly depends on the broader availability and applicability of enzymes with robust performance irrespective of extreme conditions. Recent surveys have shown that most of the biocatalysts are being used in the synthesis of pharmaceuticals or drugs or intermediates replacing some of the chemical processes, but their stability, selectivity, and specificity are of prime concern.

4. Future prospects

Based on the literature available on the role of biocatalysts in the drug/pharmaceutical synthesis, biocatalysts with improved desired characteristics can be achieved by a multifaceted approach, as shown in **Figure 2**.

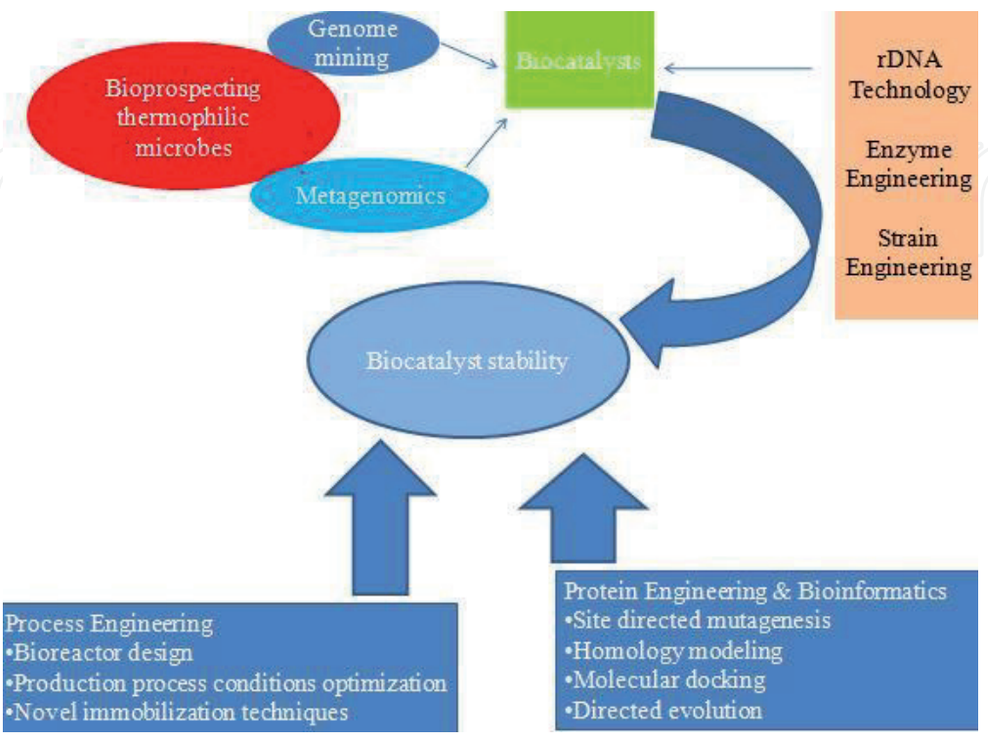

Figure 2.
Schematic representation of improving the operational stability of biocatalyst and enhancing its performance.

Several tools and techniques represented above will enhance the biocatalytic stability, activity, enzyme-substrate affinity, and thermostability and will lead to higher yield. Also, the incorporation of artificial metabolic pathways, cell factory design, and nanotechnology approaches will further aid towards a suitable biocatalytic process. It will also ensure the quality and productivity of the drugs manufactured by optimizing safe process development. Thus, we envision that biocatalysis will be a more radical approach that is going to feat the arena of pharmaceutical manufacturing as well as other sectors such as bioenergy and waste treatment that are far more challenging at present.

Conflict of interest

The authors declare that there are no conflicts of interests whatsoever.

Author details

Snehi Soy, Riddhi Prabha and Vinod Kumar Nigam*
Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

*Address all correspondence to: vknigam@bitmesra.ac.in

References

[1] Hughes G, Lewis JC. Introduction: Biocatalysis in industry. Chemical Reviews. 2018;**118**:1-3. DOI: 10.1021/acs.chemrev.7b00741

[2] Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M, Witholt B. Industrial biocatalysis today and tomorrow. Nature. 2001;**409**:258-268. DOI: 10.1038/35051736

[3] Truppo MD. Biocatalysis in the pharmaceutical industry: The need for speed. ACS Medicinal Chemistry Letters. 2017;**8**:476-480. DOI: 10.1021/acsmedchemlett.7b00114

[4] Sheldon RA, Brady D. Broadening the scope of biocatalysis in sustainable organic synthesis. ChemSusChem. 2019;**12**:2859-2881. DOI: 10.1002/cssc.201900351

[5] Sun H, Zhang H, Ang EL, Zhao H. Biocatalysis for the synthesis of pharmaceuticals and pharmaceutical intermediates. Bioorganic & Medicinal Chemistry. 2018;**26**(7):1275-1284. DOI: 10.1016/j.bmc.2017.06.043

[6] Madhavan A, Sindhu R, Binod P, Sukumaran RK, Pandey A. Strategies for design of improved biocatalysts for industrial applications. Bioresource Technology. 2017;**245**:1304-1313. DOI: 10.1016/j.biortech.2017.05.031

[7] Bommarius AS, Paye MF. Stabilizing biocatalysts. Chemical Society Reviews. 2013;**42**:6534-6565. DOI: 10.1039/C3CS60137D

[8] DiCosimo R, McAuliffe J, Poulose AJ, Bohlmann G. Industrial use of immobilized enzymes. Chemical Society Reviews. 2013;**42**:6437-6474. DOI: 10.1039/C3CS35506C

[9] Woodley JM. Accelerating the implementation of biocatalysis in industry. Applied Microbiology and Biotechnology. 2019;**103**(12):4733-4739

[10] Sheldon RA, Brady D. The limits to biocatalysis: Pushing the envelope. Chemical Communications. 2018;**54**:6088-6104. DOI: 10.1039/C8CC02463D

[11] Sheldon RA, Woodley JM. The role of biocatalysis in sustainable chemistry. Chemical Reviews. 2018;**118**:801-838. DOI: 10.1021/acs.chemrev.7b00203

[12] Ni Y, Holtmann D, Hollmann F. How green is biocatalysis? To calculate is to know. ChemCatChem. 2014;**6**:930-943. DOI: 10.1002/cctc.201300976

[13] Grigoras AG. Catalase immobilization—A review. Biochemical Engineering Journal. 2017;**117**:1-20. DOI: 10.1016/j.bej.2016.10.021

[14] Mehta J, Bhardwaj N, Bhardwaj SK, Kim KH, Deep A. Recent advances in enzyme immobilization techniques: Metal-organic frameworks as novel substrates. Coordination Chemistry Reviews. 2016;**322**:30-40. DOI: 10.1016/j.ccr.2016.05.007

[15] Cao S, Xu P, Ma Y, Yao X, Yao Y, Zong M, et al. Recent advances in immobilized enzymes on nanocarriers. Chinese Journal of Catalysis. 2016;**37**:1814-1823. DOI: 10.1016/S1872-2067(16)62528-7

[16] Cipolatti EP, Valerio A, Henriques RO, Moritz DE, Ninow JL, Freire DMG, et al. Nanomaterials for biocatalyst immobilization—State of the art and future trends. RSC Advances. 2016;**6**:104675-104692. DOI: 10.1039/C6RA22047A

[17] Misson M, Zhang H, Jin B. Nanobiocatalyst advancements, and bioprocessing applications. Interface. 2015;**12**:20140891. DOI: 10.1098/rsif.2014.0891

[18] Choi JM, Han SS, Kim HS. Industrial applications of enzyme biocatalysis:

Current status and future aspects. Biotechnology Advances. 2015;**33**:1443-1454. DOI: 10.1016/j.biotechadv.2015.02.014

[19] Bezerra CS, Lemos CMGDF, Sousa MD, Goncalves LRB. Enzyme immobilization onto renewable polymeric matrixes: Past, present and future trends. Journal of Applied Polymer Science. 2015;**132**:1-15. DOI: 10.1002/app.42125

[20] Ansari SA, Husain Q. Potential applications of enzymes immobilized on/in nano materials: A review. Biotechnology Advances. 2012;**30**:512-523. DOI: 10.1016/j.biotechadv.2011.09.005

[21] Sheldon RA. Enzyme immobilization: The quest for optimum performance. Advanced Synthesis and Catalysis. 2007;**349**:1289-1307. DOI: 10.1002/adsc.200700082

[22] Chapman J, Ismail AE, Dinu CZ. Industrial applications of enzymes: Recent advances, techniques, and outlooks. Catalysts. 2018;**8**(6):238. DOI: 10.3390/catal8060238

[23] Denard CA, Ren H, Zhao H. Improving and repurposing biocatalysts via directed evolution. Current Opinion in Chemical Biology. 2015;**25**:55-64. DOI: 10.1016/j.cbpa.2014.12.036

[24] Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, Robins K. Engineering the third wave of biocatalysis. Nature. 2012;**485**:185-194. DOI: 10.1038/nature11117

[25] Bornscheuer UT, Pohl M. Improved biocatalysts by directed evolution and rational protein design. Current Opinion in Chemical Biology. 2001;**5**:137-143. DOI: 10.1016/S1367-5931(00)00182-4

[26] Sheldon RA. Organic synthesis; past, present and future. Chemistry and Industry. 1992;**23**:903-906

[27] Sheldon RA. The E factor 25 years on: The rise of green chemistry and sustainability. Green Chemistry. 2017;**19**:18-43. DOI: 10.1039/C6GC02157C

[28] Aldridge S. Industry backs biocatalysis for greener manufacturing. Nature Biotechnology. 2013;**31**:95-96. DOI: 10.1038/nbt0213-95

[29] Huisman GW, Collier SJ. On the development of new biocatalytic processes for practical pharmaceutical synthesis. Current Opinion in Chemical Biology. 2013;**17**:284-292. DOI: 10.1016/j.cbpa.2013.01.017

[30] Tomsho JW, Pal A, Hall DG, Benkovic SJ. Ring structure and aromatic substituent effects on the pKa of the benzoxaborole pharmacophore. ACS Medicinal Chemistry Letters. 2012;**3**:48-52. DOI: 10.1021/ml200215j

[31] Lutz S, Liu LF, Liu YC. Engineering kinases to phosphorylate nucleoside analogs for antiviral and cancer therapy. Chimia. 2009;**63**:737-744. DOI: 10.2533/chimia.2009.737

[32] Desai AA. Sitagliptin manufacture: A compelling tale of green chemistry, process intensification, and industrial asymmetric catalysis. Angewandte Chemie. 2011;**50**:1974-1976. DOI: 10.1002/anie.201007051

[33] Savile CK, Janey JM, Mundorff EC, Moore JC, Tam S, Jarvis WR, et al. Biocatalytic asymmetric synthesis of chiral amines from ketones applied to a sitagliptin manufacture. Science. 2010;**329**:305-309. DOI: 10.1126/science.1188934

[34] Ghislieri D, Turner NJ. Biocatalytic approaches to the synthesis of enantiomerically pure chiral amines. Topics in Catalysis. 2013;**57**:284-300. DOI: 10.1007/s11244-013-0184-1

[35] Chung CK, Bulger PG, Kosjek B, Belyk KM, Rivera N, Scott ME, et al.

Process development of C-N cross coupling and enantioselective biocatalytic reactions for the asymmetric synthesis of niraparib. Organic Process Research & Development. 2013;**18**:215-227. DOI: 10.1021/op400233z

[36] Girardin M, Quellet SG, Gauvreau D, Moore JC, Hughes G, Devine PN, et al. Convergent kilogram scale synthesis of dual orexin receptor antagonist. Organic Process Research & Development. 2013;**17**:61-68. DOI: 10.1021/op3002678

[37] Frodsham L, Golden M, Hard S, Kenworthy MN, Klauber DJ, Leslie K, et al. Use of ω-transaminase enzyme chemistry in the synthesis of a JAK2 kinase inhibitor. Organic Process Research & Development. 2013;**17**:1123-1130. DOI: 10.1021/op400133d

[38] Meadows RE, Mulholland KR, Schurmann M, Golden M, Kierkels H, Meulenbroeks E, et al. Efficient synthesis of (S)-1-(5-fluoropyrimidin-2-yl)ethylamine using an ω-transaminase biocatalyst in a two-phase system. Organic Process Research & Development. 2013;**17**:1117-1122. DOI: 10.1021/op400131h

[39] Li T, Ambrogelly A, Brennan T, Gloor G, Huisman G, et al. Efficient, chemoenzymatic process for manufacture of the bicyclic [3.1.0] proline intermediate based on amine oxidase catalyzed desymmetrization. Journal of the American Chemical Society. 2012;**134**:6467-6472. DOI: 10.1021/ja3010495

[40] Znabet A, Polak MM, Janssen E, de Kanter FJ, Turner NJ, Orru RV, et al. A highly efficient synthesis of telaprevir by strategic use of biocatalysis and multicomponent reactions. Chemical Communications (Camb). 2010;**46**:7918-7920. DOI: 10.1039/C0CC02823A

[41] Liang J, Lalonde J, Borup B, Mitchell V, Mundorff E, Trinh N, et al. Development of a biocatalytic process as an alternative to the (−)-DIP-CI-mediated asymmetric reduction of a key intermediate of montelukast. Organic Process Research and Development. 2010;**14**:193-198. DOI: 10.1021/op900272d

[42] Ma SK, Gruber J, Davis C, Newman L, Gray D, Wang A, et al. A green-by-design biocatalytic process for atorvastatin intermediate. Green Chemistry. 2010;**12**:81-86. DOI: 10.1039/B919115C

[43] de María PD, de Gonzalo G, Alcántara AR. Biocatalysis as useful tool in asymmetric synthesis: An assessment of recently granted patents (2014-2019). Catalysts. 2019;**9**:802. DOI: 10.3390/catal9100802

[44] Rosenthal K, Lutz S. Recent developments and challenges of biocatalytic processes in the pharmaceutical industry. Current Opinion in Green and Sustainable Chemistry. 2018;**11**:58-64. DOI: 10.1016/j.cogsc.2018.03.015

[45] Martinez CA, Hu S, Dumond Y, Tao J, Kelleher P, Tully L. Development of a chemoenzymatic manufacturing process for Pregabalin. Organic Process Research & Development. 2008;**12**:392-398. DOI: 10.1021/op7002248

[46] Nigam VK, Kundu S, Ghosh P. Single-step conversion of cephalosporin-C to 7-aminocephalosporanic acid by free and immobilized cells of *Pseudomonas diminuta*. Applied Biochemistry and Biotechnology. 2005;**126**:13. DOI: 10.1007/s12010-005-0002-8

[47] Tang CD, Shi HL, Jiao ZJ, Shi HF, Yao LG, Xu JH, et al. Exploitation of cold-active cephalosporin C acylase by computer-aided directed evolution and its potential application in low-temperature biosynthesis of

7-aminocephalosporanic acid. Journal of Chemical Technology & Biotechnology. 2018;**93**:2925-2930. DOI: 10.1002/jctb.5647

[48] Hormigo D, López-Conejo MT, Serrano-Aguirre L, García-Martín A, Saborido A, de la Mata I, et al. Kinetically controlled acylation of 6-APA catalyzed by penicillin acylase from Streptomyces lavendulae: Effect of reaction conditions in the enzymatic synthesis of penicillin V. Biocatalysis and Biotransformation. 2019;**13**:1-10. DOI: 10.1080/10242422.2019.1652274

[49] Hayes ST, Assaf G, Checksfield G, Cheung C, Critcher D, Harris L, et al. Commercial synthesis of (s,s)-reboxetine succinate: A journey to find the cheapest commercial chemistry for manufacture. Organic Process Research & Development. 2011;**15**:1305-1314. DOI: 10.1021/op200181f

[50] Xu Q, Tao WY, Huang H, Li S. Highly efficient synthesis of ethyl (S)-4-chloro-3-hydroxybutanoate by a novel carbonyl reductase from *Yarrowia lipolytica* and using mannitol or sorbitol as cosubstrate. Biochemical Engineering Journal. 2016;**106**:61-67. DOI: 10.1016/j.bej.2015.11.010

[51] Ricklefs E, Girhard M, Koschorreck K, Smit MS, Urlacher VB. Two-step one-pot synthesis of pinoresinol from eugenol in an enzymatic cascade. ChemCatChem. 2015;**7**:1857-1864. DOI: 10.1002/cctc.201500182

[52] Xie XK, Watanabe K, Wojcicki WA, Wang CCC, Tang Y. Biosynthesis of lovastatin analogs with a broadly specific acyltransferase. Chemistry & Biology. 2006;**13**:1161-1169. DOI: 10.1016/j.chembiol.2006.09.008

[53] Hoyos P, Pace V, Alcántara AR. Biocatalyzed synthesis of statins: A sustainable strategy for the preparation of valuable drugs. Catalysts. 2019;**9**:260. DOI: 10.3390/catal9030260

[54] Ang EL, Alvizo O, Behrouzian B, et al. Biocatalysts and methods for the synthesis of armodafinil. US20160264945 A1. 2016

[55] Gao S, Zhu S, Huang R, Lu Y, Zheng G. Efficient synthesis of the intermediate of abacavir and carbovir using a novel (+)-γ-lactamase as a catalyst. Bioorganic & Medicinal Chemical Letters. 2015;**25**:3878-3881. DOI: 10.1016/j.bmcl.2015.07.054

[56] Fonseca TdS, Silva MRd, de Oliveira MdCF, Lemos TLGd, Marques RdA, de Mattos MC. Chemoenzymatic synthesis of rasagiline mesylate using lipases. Applied Catalysis A: General. 2015;**492**:76-82. DOI: 10.1016/j.apcata.2014.12.015

[57] Patel RN. Synthesis of chiral pharmaceutical intermediates by biocatalysis. Coordination Chemistry Reviews. 2008;**252**:659-701. DOI: 10.1016/j.ccr.2007.10.031

[58] Wang M, Yang R, Hua X, Shen Q, Zhang W, Zhao W. Lactulose production from lactose by recombinant cellobiose 2-epimerase in permeabilised *Escherichia coli* cells. International Journal of Food Science and Technology. 2015;**50**:1625-1631. DOI: 10.1111/ijfs.12776