# 1
# Introduction

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## 1.1
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The European Federation of Biotechnology proposed a definition of biotechnology as "The integration of natural science and organisms, cells, parts thereof and molecular analogs for products and services." The Concise Oxford English Dictionary states "biotechnology is the exploitation of biological processes for industrial and other purposes especially the genetic manipulation of microorganisms for the production of antibiotics, hormone, and so on" [1].

Biochemical engineering has developed as a branch of chemical engineering, and deals with the design and construction of unit processes that involve biological molecules or organisms. Biochemical engineering is often taught as a supplementary option to students of chemical engineering or biological engineering courses because of the overlap in the curriculum and similarities in problem-solving techniques used in both professions. Its contribution is widely found in the food, feed, pharmaceutical, and biotechnological industries, and in water treatment plants.

Biological engineering or bioengineering is the application of the concepts, principles, and methods of biology to solve real-world problems using engineering methodologies and also its traditional sensitivity to the cost advantage and practicality. In this context, while traditional engineering applies physical and mathematical sciences to analyze, design, and manufacture inanimate tools, structures, and processes, biological engineering primarily utilizes knowledge of molecular biology to study, investigate, and develop applications of living organisms. In summary, biological engineers principally focus on applying engineering principles and the knowledge of molecular biology to study and enhance biological systems for varied applications.

Referring to the above review and brief discussion, it is proposed to have a section titled "Applied Bioengineering" be included in the Wiley Biotechnology Series. This section will deal with recent progress in all subjects closely related to "engineering and technologies" in the field of biotechnology; widening the coverage beyond conventional biochemical engineering and bioprocess engineering

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to include other biology-based engineering disciplines. The topics involved were selected specifically from the perspective of practical applications.

The volume "Applied Bioengineering" comprises five topics: enzyme technology, microbial process engineering, plant cell culture, animal cell culture, and environmental bioengineering. Each topic is figured in several chapters, though with more chapters pertaining to environmental bioengineering. This field has seen an increase in active research as mentioned below because of growing awareness and concern about conservation, remediation, and improvement of the environment.

The later part of this chapter provides a brief overview on the developments in bioengineering, referring to recent highly cited research.

## 1.2 Enzyme Technology

Recently, several attempts have been made to screen organic-solvent-tolerant enzymes from various microorganisms [2]. The ligninolytic oxidoreductases are being improved utilizing protein engineering by the application of different "omics" technologies. Enzymatic delignification will soon come into practical use in pulp mills [3]. Enzyme stabilization has been attempted using various approaches such as protein engineering, chemical modification, and immobilization [4].

Microbial glucose oxidase has garnered considerable interest because of its wide applications in chemical, pharmaceutical, food, beverage, clinical chemistry, biotechnology, and other industries. Novel applications of glucose oxidase in biosensors have further increased its demand [5]. Numerous oxidative biotransformation studies have demonstrated that enzymes have diverse characteristics and wide range of potential, and established applications [6]. Multienzymatic cascade reactions used in the asymmetric synthesis of chiral alcohols, amines, and amino acids, as well as for C–C bond formation, have been extensively studied [7].

## 1.3 Microbial Process Engineering

### 1.3.1 Bioreactor Development

Stirred-tank bioreactors are used in a large variety of bioprocesses because of their high rates of mass and heat transfer and excellent mixing. Theoretical predictions of the volumetric mass transfer coefficient have been recently proposed, and different criteria for bioreactor scale-up have been reported [8].

Miniaturized bioreactor (MBR) systems have made great advances both in function and in performance. The dissolved oxygen transfer performance of submilliliter microbioreactors and 1–10 ml mini-bioreactors has been well examined. MBRs have achieved considerably high $k_L a$ values and offer flexible instrumentation and functionality comparable to that of production systems at high-throughput screening volumes; furthermore, the superior integration of these bioreactors with automated fluid handling systems demonstrates that they allow efficient scale-up [9].

The pharmaceutical and biotechnology industries face constant pressure to reduce development costs and accelerate process development. A small scale bioreactor system enabling multiple reactions in parallel ($n \geq 20$) with automated sampling would provide significant improvement in development timelines. State-of-the-art equipment that facilitates high-throughput process developments includes shake flasks, microfluidic reactors, microtiter plates, and small-scale stirred reactors [10].

An expert panel organized by the $M^3C$ Working Group of the European Section of Biochemical Engineering Science (ESBES) reviewed the prevailing methods of monitoring of MBRs and identified the need for further development [11]. Their recommendations includes combining online analytics such as chromatography or mass spectrometry with bioreactors, preferably using noninvasive sensors such as optical or electronic ones. The sensors to be used online in these bioreactors should be selected on the basis of three criteria: (i) detection limits in relation to analytes, (ii) stability in relation to the testing period, and (iii) the possibility for miniaturization to the volume ranges and dimensions of the microfluidic system applied in the bioreactors. In addition, mathematical models based on soft sensor principles should be exploited to reduce the number of sensors.

### 1.3.2 Measurement and Monitoring

Biosensors for detection of cellobiose, lactose, and glucose based on various cellobiose dehydrogenases from different fungal producers, which differ with respect to their substrate specificity, optimum pH, electron transfer efficiency, and surface-binding affinity; therefore, promising a wide range of new applications [12].

Infrared sensors are ideal tools for bioprocess monitoring, because they are noninvasive, of no-time-delay, and harmless on the bioprocess itself, and furthermore, simultaneous analyses of several components are possible. Therefore, directly monitoring of substrates, products, metabolites, and the biomass itself is possible [13]. The panel of the $M^3C$ Working Group of ESBES recommended the use of soft sensors in bioprocess engineering [14]. In the Food and Drug Administration's (FDA) proposed and promoted process analytical technology (PAT) initiative, intending to collaborate with industry to promote the integration of new manufacturing technologies with pharmaceutical production [15]. The

program aimed to design, develop, and operate processes consistently ensuring a predefined quality at the end of the manufacturing process [15]. An advanced monitoring and control system has been developed, based on different inline, online and at-line measurements for substrates and products. Observation of cell viability by inline measurement of radio frequency impedance and online determination of intracellular recombinant target protein using the reporter protein T-sapphire green fluorescent protein (GFP) could allow real-time monitoring of critical process states [16].

### 1.3.3 Modeling and Control

Stoichiometric models of cell metabolism have been developed with the use of information about reaction stoichiometry embedded in metabolic networks and the assumption of a pseudo-steady state. Stoichiometric models have been used to estimate the metabolic flux distribution under given circumstances in the cell at some given moment (metabolic flux analysis) and to predict it on the basis of some optimality hypothesis (flux balance analysis). Mechanistic models based on deterministic principles, recently, have been interested in substantially. Gernaey *et al*. [17] highlighted the utility of models with respect to the selection of variables required for the measurement, control, and process design. In the near future, mechanistic models will play key roles in the development of next-generation fermentation, especially in the frame of multiobjective decision making. One of the key issues in the process engineering of microbial production processes is the control of culture conditions to maximize production. In a repeated batch or fed-batch fermentation, optimizing the trajectory to maximize productivity and yield is desired. For example, temperature profiles for a temperature induction system, based on optimal control theory [18] or on past industrial experience, should be monitored, and then a model predictive control (MPC) system should be designed. Among the methodologies and practical application of bioprocess controls, the online optimized control for continuous culture, cascade control for mixed cultures, and supervision and fault detection have been developed [19].

### 1.3.4 Solid-State Fermentation

Solid-state fermentation (SSF), which has long been used in fermented foods production, ethanol fermentation, fungi cultivation, etc, is currently considered superior to submerged fermentation for use in modern bioprocessing because of the recent improvements in the design. Mathematical models based on mechanistic equations give insight into how microscale processes like the interaction of growth with intraparticle diffusion of enzymes, hydrolysis products, and oxygen can potentially limit the overall performance of a bioreactor [20]. Bioremediation,

bioleaching, biopulping, and so on, are the major applications of SSF in new bioprocesses. Utilization of agroindustrial residues as substrates in SSF processes open a way for efficient use of under- or unutilized residues. In future, SSF technology will steadily develop if rationalization and standardization continue as per the current trend [21].

## 1.4 Plant Cell Culture

Switchgrass is a promising natural feedstock for the production of biofuels and other value-added materials from biomass due to its high productivity, low requirements for agricultural inputs, and positive environmental impacts. Pretreatment of switchgrass is required to improve the yields of fermentable sugars. Depending on the type of pretreatment, glucose yields range from 70% to 90% and xylose yields from 70% to 100% after hydrolysis. Following pretreatment and hydrolysis, ethanol yields range from 72% to 92% of the theoretical maximum [22].

Plant cell factories constitute an alternative source of high-value-added phytochemicals such as the anticancer drug taxol (generic name paclitaxel), biosynthesized in *Taxus* spp. The production of taxol and related taxanes in *Taxus baccata*, (European yew), was investigated using cell suspension culture to develop mass production technology [23]. Antioxidants are an important group of preventive medicinal compounds as well as food additives that prevent the loss of easily oxidizable nutrients. The efficiency of *in vitro* production of antioxidants has been improved by media optimization, biotransformation, elicitation, *Agrobacterium* transformation, and scale-up [24].

## 1.5 Animal Cell Culture

The gel-like endothelial glycocalyx layer (EGL) that coats the luminal surface of blood vessels has garnered great interest recently. Among its, interesting functions, EGL modulate oncotic forces that regulate the exchange of water in microvessels [25].

Stem cells have emerged as the starting material of choice for bioprocesses to produce cells and tissues to treat degenerative, genetic, and immunological diseases. Fundamentals of bioprocess engineering, including bioreactor design and process control, need to be combined with principles of cellular systems biology to guide the development of next-generation technologies capable of producing cell-based products in a safe, robust, and cost-effective manner [26].

The insect cell baculovirus expression vector system (IC-BEVS) has been shown to be a powerful and convenient system for rapid and easy production of a virus-like protein. A rotavirus-like particle was produced by culturing the IC-BEVS using bioprocess engineering devices [27].

## 1.6 Environmental Bioengineering

Adsorption techniques are widely used to remove certain classes of pollutants from waters, especially those that are not easily biodegradable (e.g., biosorption of Cr(III) and Cr(VI) onto the cell surface of *Pseudomonas aeruginosa* [28], removal of Gryfalan Black RL metal complex dye by fungi [29], and methylene blue remediation by use of agricultural waste [30]). Cyanide removal using biological methods is more cost-effective than that using chemical and physical methods. Several microbial species can effectively degrade cyanide into less toxic products. Biological treatment of cyanide is possible under anaerobic and aerobic conditions [31].

A combined solar photo-Fenton and biological treatment was proposed for the decontamination of surface waters contaminated with pesticides and pharmaceutical wastewater [32, 33]. Currently there are global efforts towards development of water reuse technologies. Advanced oxidation processes (AOPs) with other bioremediation technologies has been developed for the removal of organic pollutants with high chemical stability and/or low biodegradability. Special emphasis is also placed on large-scale combination schemes developed in Mediterranean countries for treatment and reuse of nonbiodegradable wastewater [34]. "Produced water" is the largest waste stream generated in the oil and gas industries. The effect of discharging produced water in the environment has become a serious issue of environmental concern. Major research efforts in the future could focus on optimizing current technologies and combining physicochemical and/or biological methods of treatment [31]. Sewage contains various organic compounds, which should be recycled. The approach involves concentration of municipal effluents on its arrival at the water treatment plant, followed by anaerobic digestion of organics and maximum reuse of its mineral contents as nutrients. Because of the increasing economic and ecological value of the recovered nutrients, this new conceptual design for the treatment of "used water" will become a reality in the next decade [35]. The feasibility of using biological hydrolysis and the acidification for the treatment of different types of municipal sludge, from six major treatment plants located in Denmark, was investigated by batch and semicontinuous experiments. The results showed that fermentation of primary sludge produced greater amount of volatile fatty acids (VFAs) and generated significantly higher yield of COD- and VFAs than fermentation of other sludge types [36].

Bioaugmentation-assisted phytoextraction is a promising method for decontaminating soil containing metals. The system is composed of bacteria mainly plant-growth-promoting rhizobacteria, and fungi, mainly arbuscular mycorrhizal fungi, associated with hyperaccumulating or non-hyperaccumulating plants. This association was analyzed using a bioprocess engineering approach, and, in general, bioaugmentation increased metal accumulation by shoots [37].

## 1.7
## Composition of the Volume

Chapters in the subsequent part of the volume of "Applied Bioengineering" provide reviews on the selected topics, outlining the progress of current researches supplemented with unique perspectives and meaningful discussions. The volume consists of five sections after the introduction. The first section, on enzyme technology, presents an overview of the history and current trends, followed by detailed discussion on the topics: molecular engineering of enzymes, development of biocatalytic processes, and development of enzymatic reactions in miniaturized reactors. The second section, microbial process engineering, presents an overviews on bioreactor development and process analytical technology, omics-integrated approach for metabolic state analysis of microbial processes, and control of microbial processes. The third section, on plant culture and engineering, comprises three articles: one on contained molecular farming using plant cell and tissue cultures, one on bioprocess engineering of plant cell culture, and one on the role of bacteria in phytoremediation. The fourth section, on animal cell culture, contains three articles one on cell line development for biomanufacturing processes; medium design, culture management and the PAT initiative; and advanced bioprocess engineering: fed-batch and perfusion processes. Finally, the fifth section, on environmental bioengineering, contains five articles: treatment of industrial and municipal wastewater, treatment of solid waste, energy recovery from organic waste, microbial removal and recovery of metal resources from wastewater, and sustainable use of phosphorus through bio-based recycling.

### References


1 Smith, J.E. (2009) A concise history of biotechnology – Some key determinants, *Biotechnology – Vol. 1*, UNESCO-EOLSS, pp. 321–360.

2 Doukyu, N. and Ogino, H. (2010) Organic solvent-tolerant enzymes. *Biochem. Eng. J.*, **48**, 270–282.

3 Martinez, A.T., Ruiz-Duenas, F.J., Martinez, M.J., del Rio, J.C. *et al.* (2009) Enzymatic delignification of plant cell wall: From nature to mill. *Curr. Opin. Biotechnol.*, **20**, 348–357.

4 Iyer, P.V. and Ananthanarayan, L. (2008) Enzyme stability and stabilization-aqueous and non-aqueous environment. *Process Biochem.*, **43**, 1019–1032.

5 Bankar, S.B., Bule, M.V., Singhal, R.S., and Ananthanarayan, L. (2009) Glucose oxidase – An overview. *Biotechnol. Adv.*, **27**, 489–501.

6 Burton, S.G. (2003) Oxidizing enzymes as biocatalysts. *Trends Biotechnol.*, **21**, 543–549.

7 Ricca, E., Brucher, B., and Schrittwieser, J.H. (2011) Multi-enzymatic cascade reactions: Overview and perspectives. *Adv. Synth. Catal.*, **353**, 2239–2262.

8 Garcia-Ochoa, F. and Gomez, E. (2009) Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol. Adv.*, **27**, 153–176.

9 Kirk, T.V. and Szita, N. (2013) Oxygen transfer characteristics of miniaturized bioreactor systems. *Biotechnol. Bioeng.*, **110**, 1005–1019.

10 Bareither, R. and Pollard, D. (2011) A review of advanced small-scale parallel bioreactor technology for accelerated process development: Current state and future need. *Biotechnol. Progr.*, **27**, 2–14.

11 Gernaey, K.V., Baganz, F., Franco-Lara, E., Kensy, F. *et al.* (2012) Monitoring and control of microbioreactors: An expert opinion on development needs. *Biotechnol. J.*, **7**, 1308–1314.

12 Ludwig, R., Ortiz, R., Schulz, C., Harreither, W. *et al.* (2013) Cellobiose dehydrogenase modified electrodes: Advances by materials science and biochemical engineering. *Anal. Bioanal.Chem.*, **405**, 3637–3658.

13 Landgrebe, D., Haake, C., Hopfner, T., Beutel, S. *et al.* (2010) On-line infrared spectroscopy for bioprocess monitoring. *Appl. Microbiol. Biotechnol.*, **88**, 11–22.

14 Luttmann, R., Bracewell, D.G., Cornelissen, G., Gernaey, K.V. *et al.* (2012) Soft sensors in bioprocessing: A status report and recommendations. *Biotechnol. J.*, **7**, 1040–1048.

15 Lopes, J.A., Costa, P.F., Alves, T.P., and Menezes, J.C. (2004) Chemometrics in bioprocess engineering: Process analytical technology (PAT) applications. *Chemom. Intell. Lab. Syst.*, **74**, 269–275.

16 Kaiser, C., Pototzki, T., Ellert, A., and Luttmann, R. (2008) Applications of PAT-process analytical technology in recombinant protein processes with *Escherichia coli*. *Eng. Life Sci.*, **8**, 132–138.

17 Gernaey, K.V., Lantz, A.P., Tufvesson, P., Woodley, J.M. *et al.* (2010) Application of mechanistic models to fermentation and biocatalysis for next-generation processes. *Trends Biotechnol.*, **28**, 348–354.

18 Shimizu, K. and Matsuoka, Y. (2015) *Fundamentals of Systems Analysis and Modeling of Biosystems and Metabolism*, Hindawi Publishing Co. (Bentham e-Books).

19 Wardani, A.K., Egawa, S., Nagahisa, K., Shimizu, H. Shioya, S. (2006) Robustness of the cascade pH and DO control in a symbiotic nisin production process system of *L. lactis* and *K. marxianus, J. Biosci. Bioeng.*, **101**, 274–276.

20 Mitchell, D.A., von Meien, O.F., Krieger, N., and Dalsenter, F.D.H. (2004) A review of recent developments in modeling of microbial growth kinetics and intraparticle phenomena in solid-state fermentation. *Biochem. Eng. J.*, **17**, 15–26.

21 Singhania, R.R., Patel, A.K., Soccol, C.R., and Pandey, A. (2009) Recent advances in solid-state fermentation. *Biochem. Eng. J.*, **44**, 13–18.

22 Keshwani, D.R. and Cheng, J.J. (2009) Switchgrass for bioethanol and other value-added applications: A review. *Bioresour. Technol.*, **100**, 1515–1523.

23 Malik, S., Cusido, R.M., Mirjalili, M.H., Moyano, E. *et al.* (2011) Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: A review. *Process Biochem.*, **46**, 23–34.

24 Matkowski, A. (2008) Plant in vitro culture for the production of antioxidants – A review. *Biotechnol. Adv.*, **26**, 548–560.

25 Weinbaum, S., Tarbell, J.M., and Damiano, E.R. (2007) The structure and function of the endothelial glycocalyx layer. *Annu. Rev. Biomed. Eng.*, **9**, 121–167.

26 Kirouac, D.C. and Zandstra, P.W. (2008) The systematic production of cells for cell therapies. *Cell Stem Cell*, **3**, 369–381.

27 Palomares, L.A. and Ramirez, O.T. (2009) Challenges for the production of virus-like particles in insect cells: The case of rotavirus-like particles. *Biochem. Eng. J.*, **45**, 158–167.

28 Kang, S.-Y., Lee, J.-U., and Kim, K.-W. (2007) Biosorption of Cr(III) and Cr(VI) onto the cell surface of *Pseudomonas aeruginosa*. *Biochem. Eng. J.*, **36**, 54–58.

29 Aksu, Z. and Karabayir, G. (2008) Comparison of biosorption properties of different kinds of fungi for the removal of Gryfalan Black RL metal-complex dye. *Bioresour. Technol.*, **99**, 7730–7741.

30 Rafatullah, M., Sulaiman, O., Hashim, R., and Ahmad, A. (2010) Adsorption of methylene blue on low-cost adsorbents: A review. *J. Hazard. Mater.*, **177**, 70–80.

31 Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L.C., Biak, D.R.A. *et al.* (2009) Review of technologies for oil and gas produced water treatment. *J. Hazard. Mater.*, **170**, 530–551.

32 Zapata, A., Velegraki, T., Sanchez-Perez, J.A., Mantzavinos, D. *et al.* (2009) Solar photo-Fenton treatment of pesticides in water: Effect of iron concentration on degradation and assessment of ecotoxicity and biodegradability. *Appl. Catal., B*, **88**, 448–454.

33 Sirtori, C., Zapata, A., Oller, I., Gernjak, W. *et al.* (2009) Decontamination industrial pharmaceutical wastewater by combining solar photo-Fenton and biological treatment. *Water Res.*, **43**, 661–668.

34 Oller, I., Malato, S., and Sanchez-Perez, J.A. (2011) Combination of advanced oxidation processes and biological treatments for wastewater decontamination – A review. *Sci. Total Environ.*, **409**, 4141–4166.

35 Verstraete, W., Van de Caveye, P., and Diamantis, V. (2009) Maximum use of resources present in domestic "used water". *Bioresour. Technol.*, **100**, 5537–5545.

36 Ucisik, A.S. and Henze, M. (2008) Biological hydrolysis and acidification of sludge under anaerobic conditions. *Water Res.*, **42**, 3729–3738.

37 Lebeau, T., Braud, A., and Jezequel, K. (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: A review. *Environ. Pollut.*, **153**, 497–522.