Introduction to Biochemical Engineering

Second Edition

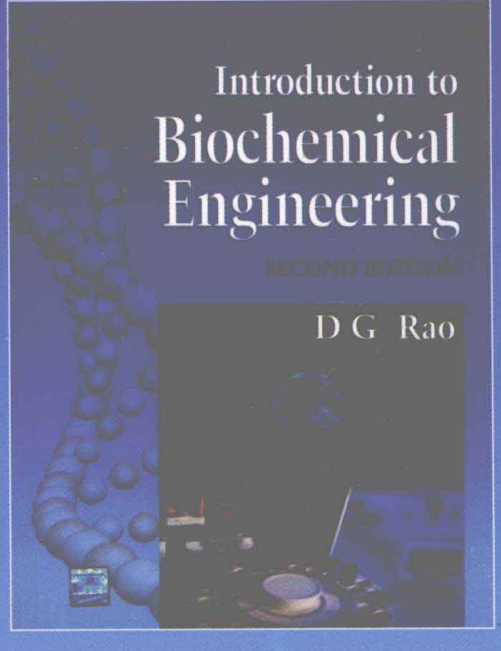
生物工程导论

（第2版）

（英文改编版）

[美] 拉奥（D G Rao） 主编

李春 改编



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编者序言

生物化工是一门以实验研究为基础，理论和工程应用并重，综合了生物分子工程、细胞工程、代谢工程、酶工程、工程设计、过程控制和化学反应工程的多学科交叉学科。这门学科的研究发展非常迅猛，近年来已经成为化学、化学工程、轻工、食品、制药、材料、环保等领域的重要基础之一。生物化工运用化学工程的原理和方法，对实验室所取得的生物技术成果加以开发，使之成为工业产品而为社会服务。它主要是依据生物系统的生命活动与代谢特征进行过程设计、分子操纵与过程优化，实现物质转化与加工过程的高效、清洁和可持续性。尤其对当今社会所面临的资源、能源、环境、健康和食品等重大问题起到积极的作用。

当前能源紧张和环保问题日益成为制约世界经济可持续发展的主要瓶颈，迫切需要构建一个稳定、经济、清洁和安全的能源供应体系。生物能源是仅次于煤炭、石油和天然气而居于世界能源消费总量第四位的能源，并有望成为未来可持续能源系统的重要组成部分。生物化工在生物能源技术的开发和应用中占有极其重要的地位，超过80%的生物能源领域的研究都是以生物化工技术和平台为基础的，而这种新能源的发展将越来越依赖生物化工技术。同时，在大气污染治理、水污染控制、有毒有害物质的降解、清洁可再生能源的开发、废物资源化、污染环境的修复等各个方面，生物化工技术依然发挥着极为重要的作用。人们已经越来越意识到，生物化工技术和产业的发展，为从根本上解决环境问题提供了技术保障。

以改善人类健康为目标的医药产业，早已将生物技术融入新药开发和药理研究等领域。而以生物化工为特征的生物制药是近 10 年来发展最快的高新技术产业之一，它的产生和发展给药物的研发和生产带来了一场革命，推动了整个产业的发展。由于传统的新药研制方法难度越来越大，研制开发成本不断上升，成功率也越来越低。因此，在世界有影响的制药公司中，目前有 70%的项目是使用生物化工技术和平台来开发，而由此所形成高速成长的生物医药产业也是生物化工技术在应用方面用得最多和最广的领域。未来的十年，生物化工领域的创新与开发会使整个医药工业面临更大规模的更新改造。

同时，生物化工的发展还将有力地推动化工生产技术的变革和进步，产生巨大的经济效益和社会效益。生机勃勃的生物化工领域前景广阔，预计未来将有 20%～30%的化学工艺过程会被生物过程所取代，生物产业将成为 21 世纪的重大产业之一。因此，具有化学、化工、生物、食品以及医药类专业背景的读者，掌握生物化工基础知识对于将来的工作和发展是非常必要的。

改编者已从事生物化工及生物工程领域的教学和科研工作近 20 年，自 2005 年开始针对生物工程、生物技术等专业特点、现阶段学生就业压力较大和部分同学对专业内容及其对社会经济发展所起作用不甚清楚的形势下，在北京理工大学一年级的学生中开设了《生物工程与技术导论》的课程，经过 7 年的实践，取得良好的教学效果。通过讲授使本专业的学生能

够很快了解生物工程与技术专业的全貌，搞清楚后续专业课程之间的关系，领悟生物工程领域发展的现状和动态及其对社会和经济发展的影响，尤其是在当今社会面临的能源危机、资源危机和环境危机中所发挥的作用。通过该课程学习提高学生对专业的理解，激发其对专业的兴趣和爱好。对于其他相关专业的学生则可拓展其视野、优化其知识结构、提高其科学素养。

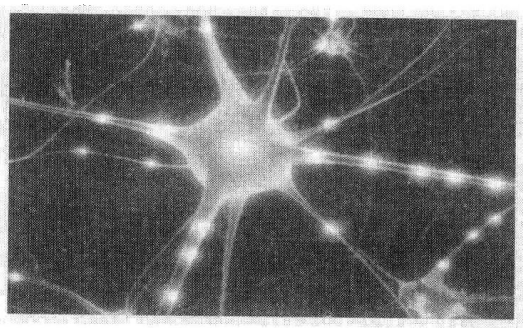
本书是改编者在 D G Rao 的原著《Introduction to Biochemical Engineering》基础上，经过重新组织、删减和修改出版的。经过改编，既保留了原作的主要内容和思想，删减了一些与生物化工联系较远的内容，又针对目前国际生物化工领域的热点研究进行了适当补充，以使其内容更加适应符合国内生物化工双语教学需要。本书的主要章节包括：生物化工的基础生物学部分“微生物学概论（第二章）”和“生物化学基础（第三章）”；驱动生物反应过程的“酶学与酶工程（第四章）”、“发酵（第五章）”、“微生物生长动力学与生化反应器”（第六章）；保障生物反应顺利进行的“理想生物反应器（第七章）”、“多反应体系（第八章）”、“生物化工过程的传质传热（第九、十章）”、“非均质反应体系（第十一章）”和“生物反应器和发酵工程（第十二章）”；有关产品与废弃物处理的“产物回收（第十三章）”和“废弃物处理（第十四章）”；评价生物化工过程的“生物反应器的设计和分析（第十五章）”和 “生物过程经济学（第十六章）”等。

作为一本相关学科入门的英文教材或重要的参考书，既要考虑基本知识的传授，又要力求反映当前本学科所取得的重要理论成果与进展。本书既可作为高等院校生物工程、化学工程、制药工程和环境工程等专业的导论教材，化学、生物和食品等专业的拓展教材，也可供从事相关学科教学、科研和生物化工产业管理者学习参考。

本书也是北京理工大学生物工程、生物技术专业开设的《生物工程与技术导论》课程配套的英文辅助教材，不但有助于学生对专业英语的学习，同时也可提高学生的国际化视野。

最后，本书在改编过程中，由于改编者水平有限，难免出现错误或者描述不当的地方，敬请批评指正！

李　春
北京理工大学
2011 年 6 月



Preface

I am thankful to all the readers for the encouragement they have given to the first edition of the book.

Based on the interaction with various students and faculty in the last five years, the present edition is revised bringing in a few changes and additions. Two new chapters are included on **Heat Transfer**, one dealing with heat transfer in bioprocessing and the other on heat transfer equipment. This was felt necessary as the earlier edition did not deal with heat transfer. Also a third chapter on **Bioprocess Economics** was added at the end. Abiochemical-engineering textbook is not complete without a mention of bioprocess economics which is so vital for any project on engineering operations.

The topic of Enzyme Kinetics in Chapter 9 was merged with Chapter 4 which exclusively deals with Enzymes. Similarly, Chapter 6 on Media Design for Fermentation was merged with Chapter 5 dealing with Fermentation. Kinetics of Homogeneous Reactions was shifted to Chapter 7 on **Microbial Growth and Biochemical Reactors**.

In addition to the above, based on the response from students, a number of **worked examples** are included in various chapters. It is hoped that students who go through the worked examples will definitely appreciate the applicability of various engineering principles and calculations in the design and operation of fermentation processes. As has been done in the first edition, all efforts have been made to judiciously blend the chemical-reaction engineering principles for successful design and operation of fermenters.

The book is ideally suited for the UG students of biotechnology pursuing a BTech or masters degree(MSc) in biotechnology. Even though all efforts have been made to present the engineering principles without an overdose of mathematical derivations, knowledge of mathematics at the 10+2 level would definitely be an added advantage. The book is also ideally suited for students of BTech (Chemical Engineering) and MSc(Food Technology)for their first course in Biochemical Engineering. The book adequately covers bioprocessing principles and biochemical engineering. Various chapters in the book can be subdivided as follows:

Bioprocessing	(Ch 1→4→5→6→7→16)
Biochemical Engineering	(Ch 8→9→10→11→12→13→14→15→19→20)
Downstream Processing	(Ch 17→18)
Process Engineering Principles	(Ch 1→5→8→10→11→12→13→14)
Enzyme Engineering	(Ch 4→5→8→15)

Chapter 1 introduces the reader to bioprocessing, viz., the historical developments in bioprocessing, overview of traditional and modern applications of biotechnology and various steps outlined in bioprocessing. The understanding of biochemical engineering is strengthened with a brief discussion on the fundamentals of microbiology and biochemistry in **Chapters 2** and **3**. **Chapter 4** on Enzymes outlines the enzyme classification, and how enzymes specifically catalyse certain bioprocesses. The concept of biocatalysts is introduced here. Some common immobilization methods and industrial applications of enzymes are also described. **Chapter 5** introduces Fermentation with its requirements, range of processes, and different types of fermentation, viz., aerobic,anaerobic, solid state and submerged.Design and construction aspects are also highlighted. A brief mention about different types of fermenters is made at the end of this chapter.

Media design, their requirements, and various sources of carbon, nitrogen and the energy required for the medium are also discussed. Sterilization, which is an important component in any biochemical/biotechnological process, is discussed in **Chapter 6**. Various methods of sterilization of media. viz., batch and continuous, and of calculation of batch sterilization time are also explained. **Chapter 7**deals with the kinetics of microbial growth and biochemical reactions. The general principles of biochemical kinetics, and the methods to evaluate the rate and order of reaction are stressed in this chapter.

The biochemical engineering principles are highlighted in **Chapters 8-15** and **Chapter 19 Chapter 8** starts with a description of ideal reactors, viz., batch reactor, plug flow reactor and continuous flow reactor. Design features of these reactors are also highlighted in this chapter. **Chapter 9** classifies the biochemical reaction systems into simple, parallel, series reactions and a combination of these. This classification is akin to that done by Deindoerfer(1960).Efforts were made to cite examples of biochemical reactions that fall into each of these categories.Non-isothermal reaction systems and the criterion for achieving thermal stability are also discussed. After having discussed the ideal reactions and ideal reactor systems, non-ideal reactors(i.e., reactors with non-ideal flow)are highlighted in **Chapter 10**. This chapter is very important because of the fact that most of the fermenters behave non-ideally.

The concept of non-ideality was explained with RTD (residence time distribution)studies. **Chapter 11** discusses the rheology and mixing in fermentation broths. **Chapter 12** discusses Heat Transfer in Bioprocessing through the methods of conduction, convection and radiation.**Chapter 13** deals with Heat Transfer Equipment like double-pipe, shell and tube heat exchangers. **Chapter 14** highlights the oxygen mass transfer phenomena, which is an important aspect in the aerobic fermentation processes.

In a way, **Chapter 14** is a treatment of homogeneous systems with the dissolution of oxygen in the fermentation broths, while heterogenous systems involving immobilized bioreactor systems are highlighted in **Chapter 15**. The diffusional limitations and how the mass transfer affects the immobilized cell reactions are also highlighted. Thus, Chapter 15 discusses the engineering applications of enzymes dealt in **Chapter 4**, and may be considered as an extension to the same. Bioreactors and fermentation processes are discussed in **Chapter 16**. This chapter blends the bioreactor operations with fermentation processes. Various bioseparation steps (downstream processing steps) for separation and purification of the metabolic products are discussed in **Chapter 17**. General principles of effluent treatment with the concept of Central Effluent Treatment Plants (CETP) are discussed in **Chapter 18**. In the biochemical engineering aspect, the design and analysis of bioreactors are discussed in **Chapter 19**. The scale-up criterion for bioreactors is also discussed in this chapter. The choice of different bioreactors is highlighted based on the physico-chemical properties of the fermentation broths. Finally, Bioprocess Economics is discussed in **Chapter 20**.

I am greatly indebted to the publishers, Tata McGraw Hill Education Pvt. Ltd., New Delhi, for their constant encouragement during the revision, and their efforts in bringing out the book in its present form by providing excellent editorial support. I am grateful to the various authors and publishers who have accorded permissions to utilise their materials in this book.

The numerous reviewers who took out time to review the book also deserve a special mention. Their names are mentioned below.

T R Sreekrishnan	*IIT Delhi, Delhi*
Ashish M Gujrathi	*BITS Pilani, Rajasthan*
Susmita Dutta	*NIT Durgapur, West Bengal*
Amalesh Sirkar	*HIT Haldia, West Bengal*
Sameer Jadhav	*IIT Bombay, Mumbai, Maharashtra*
T Panda	*IIT Madras, Chennai, Tamil Nadu*
P Ravichandran	*St Joseph's College of Engineering, Chennai, Tamil Nadu*
A V N Swamy	*JNTU College of Engineering, Anantpur, Andhra Pradesh*

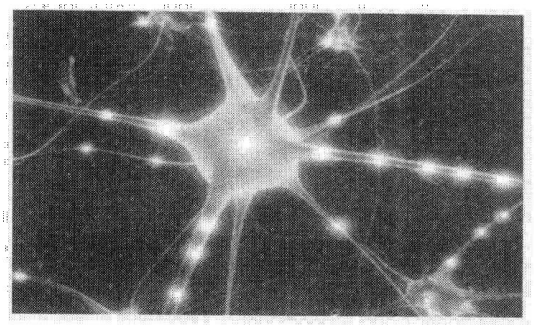
A heartfelt thanks to my young daughters, Asha Latha and Priyanka, who have cheerfully typed the additional chapters and worked examples from my illegibly handwritten manuscripts. I would also like to acknowledge the help I have received from the staff of JNTU, particularly Prof. M Lakshmi Narasu, Head, Centre for Biotechnology and Mr D Kiran Kumar for helping me at different times during the

course of preparation of this book. A special thanks to Mrs V Rohini for helping me in the writing of Chapter 2. My thanks are also due to a few of my relatives and friends who constantly encouraged me in this endeavour. Last but not the least, I wish to appreciate the role of my wife, Sai Leela, for her ungrudging endurance during the period of writing this book.

It has always been my endeavour to keep the contents of the book crisp and easily comprehensible. I feel that the contents in the book are by no means exhaustive, and am afraid that certain mistakes and omissions may have inadvertently crept in. Readers may kindly indicate them to me for future improvement. Any suggestions for improving this book are most welcome. Users of the book can contact me at dgrao1950@rediffmail.com.

Dated 3rd July, 2009

D G Rao



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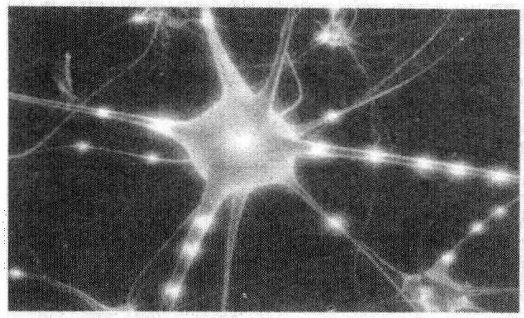

Chapter 1

Introduction to Bioprocessing Fundamentals

1.1 HISTORICAL DEVELOPMENTS OF BIOPROCESSING TECHNOLOGY

Biotechnology is the art and science of converting reactants (substrate) into useful products by the action of microorganisms or enzymes. Whether we are aware of it or otherwise, microorganisms have been honestly serving the mankind. Thus, any process in which microbes or living organisms play a vital role in getting transformation of the feed into useful products is termed as **bioprocessing.** It could be by way of converting milk into curds, or fruit juices into wines, or sugars into alcohol or the application of yeast in bread-making etc.

Greeks were known to have produced wine as early as 7000 BC, and Egyptians were baking bread in 4000 BC. However, an understanding of the role played by the living cells was not emanated until the **'cell theory'** was proposed in 1838 by Schleiden and Schwann. But, the foundations of modern biotechnology as a distinct field were laid as the outcome of microbiology in the late 19^{th} century.

The emergence of **Biochemical Engineering** is of more recent origin, since the biological industries did not recognize the importance of engineering inputs until the experience of penicillin manufacture. It is the manufacture of antibiotics which gave a boost to the biochemical industries. Even though the world's first successful commercial production of citric acid was commenced by Pfizer in 1923, nothing worth mentioning happened subsequently for some years. The origin of antibiotics can be traced to the first classical isolation of *penicillin* strain as a contaminant by Alexander Fleming in 1928 while he was working with *Staphylococcus aureus*. A mould of the *penicillium* family occurred as a contaminant in a petridish around which the *Styphylococcus* organism could not grow at all. This tremendous invention, however, did not receive much attention till the Second World War when there was a great demand for penicillin to heal the wounds of the war soldiers. It is at this stage again that a chance discovery of a new strain of penicillin, viz. *Penicillium chrysogenum*, gave 200 times more yield of penicillin than the Fleming's mould which was cultured in deep sterile aerated tanks.

Thus, the need arose to operate the submerged fermentation tanks with aeration under sterile conditions, and it became the job of the biochemical engineers to provide such sterile atmosphere and sterile air to meet the requirements of the submerged fermentation process. Aiba et al. (1965) observed "*perhaps the most notable contribution the engineer made was in the advancement of sterile techniques, or the "contamination-free" philosophy, in the design and operation of the fermentation vessel and its associated maze of pip-ing*". Thus, the penicillin production ushered in a new era of biotechnology, even though the first successful fermentation plant for production of citric acid from sugar by using *Aspergillus niger* was established in 1923 by Pfizer.

The chronological developments in biotechnology was classified by Atkinson (1974) into three stages:

- the period of ignorance (pre-1800)
- the period of discovery (1800–1900)
- the period of industrial development (post-1900)

However, Stanbury and Whitaker (1993) gave a more exhaustive division into five stages which are shown in Table 1.1. This is similar to the one proposed by Perlman (1977) as "*A century of growth of industrial fermenters*" and summarised by Sengha (1993).

Table 1.1 *The stages in the chronological development of fermentation industry*

Stage	*Main products*	*Vessels*	*Process control*	*Culture method*	*Quality control*	*Pilot plant facilities*	*Strain selection*
1 (Pre-1900)	Alcohol	Wooden upto 1500 barrels capacity. Copper used in later breweries,	Use of thermometer, hydrometer and heat exchanger	Batch	Virtually nil	Nil	Pure yeast cultures used at the Carlsberg brewery (1896) Fermentations inoculated with 'good' vinegar
	Vinegar	Barrels, shallow trays, trickle filters		Batch	Virtually nil	Nil	
2 (1900-1940)	Baker's yeast, glycerol, citric acid and acetone/butanol	Steel vessels upto 2000 hectalitres for acetone, butanol. Air spargers used for bakers' yeast. Mechanical stirring used in small vessels	pH electrodes with off-line control. Temperature control	Batch and fed-batch systems	Virtually nil	Virtually nil	Pure cultures used
3 (1940-till date)	Penicillin, streptomycin, other antibiotics, gibberellin, amino acids, nucleotides, transformations, enzymes	Mechanically aerated vessels, operated aseptically true fermenters	Sterilizable pH and oxygen electrodes. Use of control loops which were later computerized	Batch and fed-batch common. Continuous culture introduced in brewing and for some primary metabolites	Very important	Becomes common	Mutation and selection programmes essential
4 (1960-till date)	Single-cell protein using hydrocarbon and other feed stocks	Pressure cycle and pressure jet vessels developed to overcome gas and heat-exchange problems	Use of computer linked control loops	Continuous culture + medium recycle	Very important	Very important	Genetic engineering of production strains
5 (1979-till date)	'Foreign' compounds, not normally produced by microbial cells, e.g. insulin, interferon	Fermenters developed in stages 3 and 4	Control and sensors developed in Stages 3 and 4	May be batch, fed-batch or continuous	Very important	Very important	Introduction of foreign genes into microbial hosts using genetic engineering techniques

1.2 OVERVIEW OF TRADITIONAL AND MODEN APPLICATIONS OF BIOTECHNOLOGY

The traditional applications of biotechnology/bio-processing are mostly in the areas of foods, bakery products and alcoholic beverages. The earliest known application was in the production of wine from fruit juices. Fermentation of milk to make curd was also being practiced in India from time immemorial. However, baking of bread by leavening the dough of wheat flour using yeast has brought biotechnology into day-to-day use. Most of the traditional processes in which the microorganisms were playing a vital role in bringing about the bioconversion/biotransformation were not well-recognized until the cell theory was proposed in 1838, and subsequently until Louis Pasteur proposed his classical theory in the middle of the 19th century that some diseases in animals and plants were caused by microorganisms. This discovery was a turning point in the medical history, and laid the foundation of **Microbiology.**

The modern fermentation industry has its roots in modern microbiology and also in the ancient technology. Particularly, when it comes to brewing, the breweries still use the old recipes. The improvement is only in the technological practices. Hoogerheide (1977) felt that the brewing industry had accepted the technological progress, but the scientific progress only with hesitation. The era of modern applications of biotechnology was ushered in with the commercial production of **antibiotics,** particularly penicillin, during the Second World War. Subsequently, the tentacles were spread over to diverse areas of health and hygiene, for the production of vaccines, enzymes, various fermented foods, organic acids, etc. The world market of various fermented foods is of the order of $30 billion out of which one-third is the share of US alone during 1990s.

The commercial products of fermentation are expanding day by day. Even during 1960-1970, about two dozens of antibiotics, and a large number of enzymes, polysaccharides, flavour-enhancing compounds were included in the list of fermented products. The area of antibiotics continues to dominate the scene. More than 10000 antibiotics and similar bioactive natural substances are known which are obtained from microorganisms. Out of them, about 100 products have been found applications.

Perlman (1977) gave a list of 145 fermentation industries of which most are multinationals having their operations in more than one country and manufacturing a large number of products.

1.3 INTERADISCIPLINARY APPROACH TO BIOPROGROCESSING

Bioprocessing or biotechnological operations are truly interrelated with a large number of faculties in science. Particularly the wedding of engineering sciences with life sciences is an interesting feature of biotechnology. Until the time of Louis Pasteur in 1857, alcoholic fermentation was considered to be a chemical process in which the sugars were getting converted to alcohol. Subsequently, it was realized that the conversion was affected by microorganisms which were the silent workers. This has opened up a wide range of interrelationships between various disciplines in science.

The commercial success of production of the penicillin during the Second World War period showed the path of advantages of engineering miracles. Subsequently, the biochemical engineers, who were responsible for providing an ideal and contamination-free environment, were considered as a part of bioprocessing.

The recent advances in **genetic engineering,** which help genetically engineer a cell to produce

- large amounts of recombinant proteins
- metabolites other than the proteins

have opened up new vistas of mathematical modeling of the cell as a micro batch reactor. The cell can be forced, by applying genetic engineering principles, to produce high amounts of the limiting enzymes. The over-production of a particular enzyme or protein does not help the organism in any way. On the contrary, it may disrupt the cell metabolic process, which may ultimately lead to the cell death (Georgiou, 1988). Hence, to optimize the production, the culture has to be maintained and forced to produce the desired enzymes without being perished in the process.

An advanced knowledge of cell growth and cell morphology, which are the subject matters of microbiology, biochemistry and cell physiology, is a useful tool for precisely controlling the biotechnological process. Modern biotechnological techniques such as recombinant DNA techniques, gene manipulation, cell fusion and tissue culture offer useful tools to improve the existing processes. Sophisticated medicines, kits, cultured human tissues and organs, biochips for new-age computers, environmentally compatible pesticides, and powerful pollutant-degrading microbes herald a revolution in the role of biology in the industry (Doran, 1995).

A successful scale-up technique can alone translate a scientific invention into a commercial reality. Since the behavior of biological systems is always complex, their scale-up is not that easy. Hence a rigorous evaluation and monitoring of various process parameters in sterile environmental conditions can alone result in realizing the commercial reality of the bioprocess. Application of mathematical modeling principles, backed by adequate pilot plant information will help in commercialization of the process.

Thus, the application of engineering principles to the biological processes to achieve commercial success is the subject matter of biochemical engineering, and constitutes the core substance matter for this textbook.

1.4 OUTLINES OF LINTEGRATED BIOPROCESS

Integrated bio-processing consists of various steps, some of them sequential and others concurrent. They are shown in Fig.1.1, and will be discussed here briefly in general. The specific details will be discussed separately in respective chapters.

Step 1. Isolation of the strain

The very first step is the identification and isolation of the strain of the microorganism which brings out the desired bioconversion. This is generally the task of a microbiologist. This basic work which involves some shake flask experimentation, etc., will be the basis for taking up any process. The techno-economic viability of the process will be taken up subsequently if the results of Step 1 are encouraging.

Step 2. Preservation of the strain

Preservation of the strain for future use is the basic requirement for the success of commercial ventures. The high-yielding strains, identified and isolated in Step 1, will be preserved for future use. It is essential that the strain is not only preserved, but its ability to perform and yield the products should also be preserved, without which the whole exercise is set at naught. The strains can be stored:

(i) at refrigerated temperatures (2–6℃)
(ii) at frozen storage temperatures (−18 to −80℃)
(iii) by freeze drying (lyophilization)

Step 3. Growth of inoculums

Before using the strain in the fermenter, the inoculum is cultured for growth. The preserved strain/culture is revived by growth in shake flasks or by solid-state fermentation on the solid-surfaces (Section 5.6.1).

The standard growth time for lyophilized cultures is approximately 4-10 days, whereas the frozen cultures and refrigerated cultures take different times for different cultures, viz. bacteria, actinomycetes and fungi (Crueger and Crueger, 1989), as shown in Table 1.2.

Step 4. Prefermentation culturing

Before the cells are admitted into the fermenter for performing bioconversions, they are initially grown separately with nutrients and the substrate so that the cells can multiply and proliferate. This will help increase the cell density. The optimal cell concentrations in a fermenter are as follows (Crueger and Crueger, 1989)

Bacteria: 0.1–3.0%
Actinomycetes: 5–10%
Fungi: 5–10%

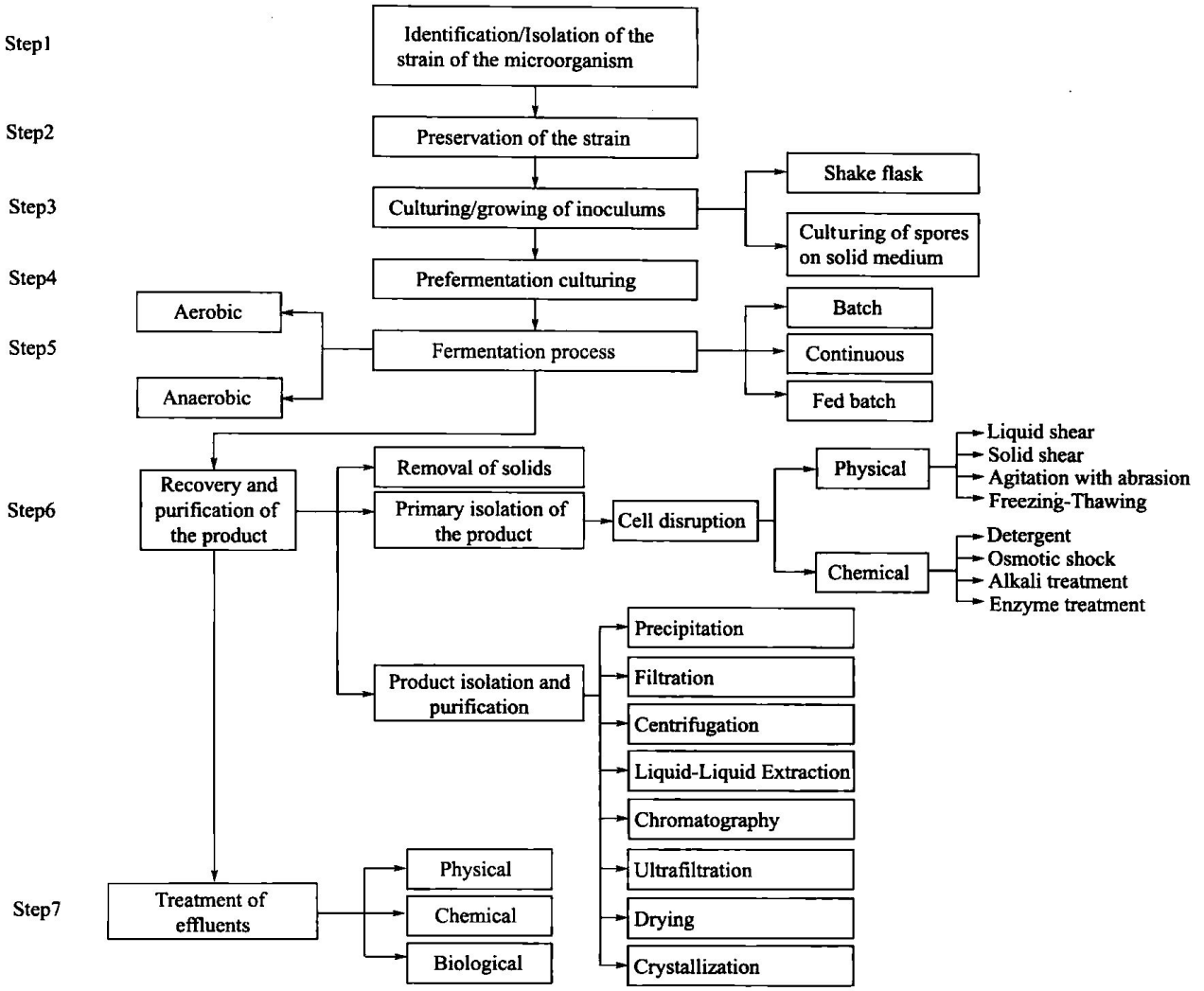

Fig. 1.1 *Integrated bioprocessing steps*

Table 1.2 *Growth time for frozen cultures and refrigerated cultures*

Culutres	*Growth time*	
	Frozen culture	*Refrigerated culture*
Bacteria	4-48 h	4-24 h
Actinomycetes	1-5 days	1-3 days
Fungi	1-7 days	1-5 days

Step 5. Fermentation

The major activity in industrial operation in the whole process is fermentation, in which biochemical engineers play an active role. Fermentation is the heart of the bio-processing operation. The fermenter sizes vary from 1 to 450m^3, depending upon the type of fermentation process. Based on the capacity, tonnage and nature of the fermentation product, it could be taken up in:

(i) batch fermenter

(ii) fed-batch fermenter

(iii) continuous fermenter.

The conditions of fermentation could be aerobic (with bubbling of air) or anaerobic (in the absence of air). Various fermentation operations will be dealt with in detail in the subsequent chapters.