

Contents

Preface	xxxvii
---------------	--------

Author	xli
--------------	-----

1 Introduction to biotechnology 1

Learning objectives	1
---------------------	---

1.1 What is biotechnology?	1
----------------------------	---

1.1.1 Definitions of biotechnology	2
------------------------------------	---

1.2 Animal biotechnology	4
--------------------------	---

1.3 Agricultural biotechnology	6
--------------------------------	---

1.4 Medical biotechnology	6
---------------------------	---

1.5 Industrial biotechnology	7
------------------------------	---

1.6 Environmental biotechnology	7
---------------------------------	---

1.7 Other emerging fields of biotechnology	8
--	---

1.7.1 Nanobiotechnology	8
-------------------------	---

1.7.2 Bioinformatics	9
----------------------	---

1.7.3 Pharmacogenomics	9
------------------------	---

1.7.4 Regenerative medicine	9
-----------------------------	---

1.7.5 Therapeutic proteins	10
----------------------------	----

1.7.6 Biorobotics	11
-------------------	----

1.7.7 Biomimetics	11
-------------------	----

1.8 History of biotechnology	12
------------------------------	----

1.8.1 Ancient biotechnology	13
-----------------------------	----

1.8.2 Modern biotechnology	13
----------------------------	----

1.9 Human Genome Project	14
--------------------------	----

1.9.1 Need for Human Genome Project	16
-------------------------------------	----

1.10 Major scientific discoveries in biotechnology	16
--	----

1.11 Biotechnology as the science of integration	18
--	----

1.12 Bio-revolution	19
---------------------	----

1.13 Ethical and regulatory issues in biotechnology	21
---	----

1.14 Future of biotechnology	21
------------------------------	----

Problems	23
----------	----

Section A: Descriptive type	23
-----------------------------	----

Section B: Multiple choice	23
----------------------------	----

Section C: Critical thinking	24
------------------------------	----

Assignment	24
Online resources	24
References and further reading	25

2 Genes and genomics 27

Learning objectives	27
2.1 Introduction	27
2.2 Cell as the building block of life	28
2.3 Classification of cells	28
2.3.1 Prokaryotic cell	28
2.3.2 Eukaryotic cell	30
2.4 Extracellular organization	30
2.4.1 Cell membrane	30
2.4.2 Cell capsule	31
2.4.3 Flagella	31
2.5 Intracellular organization	32
2.5.1 Cytoplasmic constituents	32
2.5.1.1 Mitochondria and chloroplasts	32
2.5.1.2 Ribosomes	32
2.5.1.3 Endoplasmic reticulum	33
2.5.1.4 Golgi apparatus	33
2.5.1.5 Lysosomes and peroxisomes	33
2.5.1.6 Centrosome	33
2.5.1.7 Vacuoles	33
2.5.1.8 Cytoskeleton	33
2.5.2 Nuclear constituents	34
2.5.2.1 Deoxyribonucleic acid	34
2.5.2.2 Ribonucleic acid	37
2.5.2.3 Messenger ribonucleic acid	38
2.5.2.4 Transfer RNA	38
2.5.2.5 Ribosomal RNA	39
2.5.2.6 Small nuclear RNA	39
2.5.2.7 Nucleolus	40
2.5.2.8 Chromatin	40
2.6 Macromolecules	40
2.6.1 Proteins	40
2.6.2 Carbohydrates	41
2.6.2.1 Simple sugars	42
2.6.2.2 Complex carbohydrates	42
2.6.3 Lipids	43
2.6.4 Epigenetics	43
2.6.4.1 Historical perspective	44
2.6.4.2 Clinical implications	45

2.7	Genes and genetics	47
2.7.1	Mendelian genetics	47
2.7.2	Modern genetics	49
2.8	Cell division	50
2.8.1	Meiosis	50
2.8.2	Mitosis	51
2.9	DNA replication	53
2.9.1	Role of DNA polymerase in replication	53
2.9.2	DNA replication within the cell	55
2.9.2.1	Replication fork	55
2.9.2.2	Leading strand	55
2.9.2.3	Lagging strand	56
2.9.3	Regulation of DNA replication	56
2.9.4	Termination of replication	57
2.10	DNA interactions with proteins	57
2.11	DNA-modifying enzymes	58
2.11.1	Topoisomerases and helicases	59
2.12	DNA methylation	59
2.13	DNA mutation	60
2.14	Tools of biotechnology	61
2.14.1	Polymerase chain reaction	61
2.14.1.1	Principle and practice of PCR	63
2.14.1.2	PCR optimization	65
2.14.2	Types of PCR reactions	65
2.14.2.1	Allele-specific PCR	65
2.14.2.2	Polymerase cycling assembly	65
2.14.2.3	Asymmetric PCR	65
2.14.2.4	Helicase-dependent amplification	66
2.14.2.5	Hot-start PCR	66
2.14.2.6	AFLP-PCR	66
2.14.2.7	Alu PCR	66
2.14.2.8	Colony PCR	67
2.14.2.9	Inverse PCR	67
2.14.2.10	Ligation-mediated PCR	67
2.14.2.11	Methylation- specific PCR	67
2.14.2.12	Miniprimer PCR	67
2.14.2.13	Multiplex ligation- dependent probe amplification	68
2.14.2.14	Multiplex PC	68
2.14.2.15	Nested PCR	68

2.14.2.16	Overlap-extension PCR	68
2.14.2.17	Quantitative PCR	68
2.14.2.18	Reverse transcription PCR	69
2.14.2.19	Solid-phase PCR	69
2.14.2.20	Thermal asymmetric interlaced PCR	69
2.14.2.21	Touchdown PCR (step-down PCR)	69
2.14.2.22	Universal fast walking	69
2.14.3	Applications of PCR	70
2.14.3.1	Diagnostic assay	70
2.14.3.2	Genetic engineering	70
2.14.3.3	Forensic DNA profiling	70
Problems		71
Section A: Descriptive type		71
Section B: Multiple choices		71
Section C: Critical thinking		73
Assignment		73
References and further reading		73
3	Proteins and proteomics	75
Learning objectives		75
3.1	Introduction	75
3.2	Significance of proteins	76
3.2.1	Proteins for body functions	77
3.2.1.1	Nonessential amino acids	77
3.2.1.2	Essential amino acids	78
3.2.1.3	Other amino acids	79
3.2.2	Proteins as enzymes	80
3.2.3	Proteins in cell signaling and ligand binding	80
3.2.4	Structural proteins	81
3.3	Protein biosynthesis	82
3.3.1	Transcription stage	83
3.3.2	Transcription in prokaryotes and eukaryotes	83
3.3.3	Stages of transcription	84
3.3.3.1	Preinitiation	84
3.3.3.2	Initiation	85
3.3.3.3	Promoter clearance	86
3.3.3.4	Elongation	86
3.3.3.5	Termination stage	87
3.3.3.6	Reverse transcription	87
3.3.3.7	RNA splicing	87
3.3.3.8	Spliceosomal introns	88

3.3.3.9	Spliceosome formation and activity	88
3.3.3.10	Self-splicing	88
3.3.3.11	tRNA splicing	88
3.3.3.12	RNA export	88
3.3.4	Translation	89
3.3.4.1	Posttranslational modification	90
3.4	Protein structure	91
3.4.1	Protein prediction methods	93
3.5	Protein folding	93
3.5.1	Tools of protein folding	94
3.5.1.1	Circular dichroism	95
3.5.1.2	Dual polarization interferometry	95
3.5.1.3	Vibration circular dichroism of proteins	95
3.5.1.4	Protein folding with high time resolution	95
3.5.1.5	Energy landscape theory	95
3.6	Protein modification	96
3.6.1	Protein modification by phosphorylation	96
3.6.2	Protein modification by ubiquitination	96
3.6.3	Additional modifications	96
3.7	Protein transport	97
3.8	Protein dysfunction and degradation	97
3.9	Regulation of protein synthesis	101
3.9.1	Stages of gene expression	102
3.9.1.1	Operon model for gene regulation	102
3.9.1.2	General transcription factors	102
3.9.1.3	Enhancers	103
3.9.1.4	Induction and repression	103
3.10	Regulatory protein	105
3.11	Methods for protein analysis	105
3.11.1	Protein identification and quantification	106
3.11.1.1	Native gel	106
3.11.1.2	SDS-PAGE	106
3.11.1.3	QPNC-PAGE	107
3.11.1.4	Protomap	107
3.11.1.5	Northern blot	108
3.11.1.6	Western blot	110

3.11.1.7 Cellular techniques	111
3.11.1.8 Enzyme-linked immunosorbent assay	111
3.12 Protein purification	114
3.13 Tools of proteomics	115
3.13.1 Two-dimensional electrophoresis	115
3.13.2 Mass spectrometry	116
3.13.3 Protein microarray	117
3.13.4 Two-hybrid screening	118
3.13.5 Protein structure prediction	119
3.13.6 PEGylation	119
3.13.7 High-performance liquid chromatography	120
3.13.8 Shotgun proteomics	120
3.13.9 Top-down proteomics	120
Problems	120
Section A: Descriptive type	120
Section B: Multiple choice	121
Section C: Critical thinking	122
Assignment	122
References and further reading	122

4 Recombinant DNA technology 125

Learning objectives	125
4.1 Introduction	125
4.2 Making of recombinant DNA	126
4.2.1 Steps in making a recombinant DNA product	126
4.2.2 Methods involved in making recombinant DNA product	127
4.2.2.1 Transformation	127
4.2.2.2 Nonbacterial transformation	128
4.2.2.3 Phage introduction	128
4.3 Significance of recombinant DNA technology	128
4.4 Role of restriction enzymes in rDNA technology	129
4.4.1 Types of restriction enzymes	129
4.4.1.1 Type I restriction enzymes	129
4.4.1.2 Type II restriction enzymes	130
4.4.1.3 Type III restriction enzymes	131
4.4.2 Nomenclature of restriction enzymes	131

4.4.3	Recognition sequences for type II restriction enzymes	132
4.4.4	Cleavage pattern of type II restriction enzymes	133
4.4.5	Modification of cut ends	133
4.5	Steps in gene cloning	134
4.6	Synthesis of complete gene	134
4.7	Polymerase chain reaction and gene cloning	136
4.7.1	Comparison of PCR versus gene cloning	136
4.7.1.1	How PCR works	136
4.7.1.2	How cloning works	137
4.7.1.3	What are the differences between PCR and cloning?	137
4.8	Significance of vectors in recombinant DNA technology	137
4.8.1	Properties of good vectors	138
4.8.2	Cloning and expression vectors	139
4.8.3	Applications of viral vectors	139
4.9	Classification of vectors	140
4.9.1	Bacterial vectors	141
4.9.1.1	E. coli vectors	141
4.9.1.2	Plasmid vectors	141
4.9.2	Viral vectors	142
4.9.2.1	Retroviruses	142
4.9.2.2	Lentiviruses	144
4.9.2.3	Adenoviruses	145
4.9.2.4	Adeno-associated viruses	145
4.9.3	ARS vectors	146
4.9.4	Minichromosome vectors	146
4.9.5	Yeast artificial chromosome vectors	147
4.9.6	Vectors for animals	148
4.9.7	SV40 vectors	149
4.9.8	Bovine papillomavirus vectors	149
4.10	Integration of the DNA insert into the vector	150
4.10.1	Both ends cohesive and compatible	150
4.10.2	Both ends cohesive and separately matched	151
4.10.3	Both ends cohesive and unmatched	151
4.10.4	Both ends flush/blunt	152
4.10.5	One end cohesive and compatible, the other end blunt	152

4.11	Introduction of the recombinant DNA into the suitable host	152
4.12	Increased competence of <i>E. coli</i> by CaCl_2 treatment	152
4.13	Infection by recombinant DNAs packaged as virions	153
4.14	Selection of recombinant clones	153
4.15	Identification of clones having recombinant DNAs	154
4.16	Selection of clone containing a specific DNA insert	155
4.16.1	Colony hybridization	155
4.16.2	Other approaches for developing specific probes	155
4.16.3	Complementation	156
4.16.4	Unique gene products	156
4.16.5	Antibodies specific to the protein product	156
4.16.6	Colony screening with antibodies	157
4.16.7	Fluorescence-activated cell sorter	157
4.17	Applications of recombinant DNA technology	157
4.17.1	Genetically modified organisms	158
4.17.1.1	Transgenic microbes	158
4.17.1.2	Transgenic animals	159
4.17.1.3	Transgenic plants	159
4.17.1.4	Cisgenic plants	160
4.18	DNA sequencing	160
4.18.1	Maxam and Gilbert procedure	160
4.18.2	Enzymatic procedure	162
4.18.3	Automated DNA sequencing	163
4.18.4	Current challenges in DNA sequencing	164
4.18.5	Trends in DNA sequencing	165
4.18.5.1	High-throughput sequencing	165
4.18.5.2	In vitro clonal amplification	165
4.18.5.3	Parallelized sequencing	165
4.18.5.4	Sequencing by ligation	166
4.18.5.5	Microfluidic Sanger sequencing	166
4.18.5.6	Other sequencing technologies	166
4.19	Microarrays	166
4.20	DNA chips	167
4.21	Isolation of desired DNA	168

4.22 cDNA library	169
4.22.1 Problems in cDNA preparation	169
4.22.2 Isolation of mRNA	169
4.23 Preparation of cDNA	170
4.24 Genomic library	170
4.24.1 Construction of a genomic library	170
4.25 DNA libraries	171
4.26 Chemical synthesis gene	171
4.26.1 Phosphodiester approach	172
4.26.2 Phosphotriester approach	172
4.26.3 Phosphite triester approach	173
4.27 Applications of synthetic oligonucleotides	174
Problems	174
Section A: Descriptive type	174
Section B: Multiple choice	174
Section C: Critical thinking	176
Assignments	176
References and further reading	176

5 Microbial biotechnology 179

Learning objectives	179
5.1 Introduction	179
5.2 Structural organization of microbes	180
5.2.1 Structure	180
5.2.2 Intracellular organization	181
5.2.3 Extracellular organization	184
5.3 Microbial metabolism	186
5.3.1 Heterotrophic microbial metabolism	186
5.3.2 Fermentation	188
5.3.3 Aerobic respiration	189
5.3.4 Denitrification	189
5.3.5 Nitrogen fixation	190
5.4 Microbial growth	191
5.4.1 Phases of microbial growth	191
5.4.2 Factors that influence microbial growth	193
5.5 Microbial genetics	193
5.5.1 Mutations	194
5.5.1.1 Auxotrophic mutant	194
5.5.1.2 Resistant mutant	195
5.5.1.3 Metabolic mutant	195
5.5.1.4 Regulatory mutant	195
5.5.2 Spontaneous mutations	195
5.5.3 Induction of selective mutations	196
5.5.4 Induced mutations	197

5.6	Genetic recombination in bacteria	197
5.6.1	Bacterial transformation	198
5.6.2	Bacterial transduction	200
	5.6.2.1 Types of transduction	201
	5.6.2.2 Stages of transduction	204
5.6.3	Conjugation mechanism in gene recombination	204
5.6.4	Human microbiome project	205
5.7	Transposable genetic elements	207
5.7.1	Types of transposable genetic elements	207
	5.7.1.1 Insertion sequences	207
	5.7.1.2 Transposons	208
5.8	Use of <i>E. coli</i> in microbial cloning	208
5.8.1	Genetic simplicity	208
5.8.2	Growth rate	208
5.8.3	Safety	209
5.8.4	Conjugation and the genome sequence	209
5.8.5	Ability to host foreign DNA	209
5.9	Pathogenic bacteria	209
5.10	Application of microbes	210
5.10.1	Microbes and agriculture	211
5.10.2	Nitrogen fixers	211
5.10.3	Biopesticides and bioherbicides	211
5.10.4	Acetone butanol fermentations	212
5.10.5	Microbes in recovery of metals and petroleum	212
5.10.6	Microbes in the paper industry	212
5.10.7	Microbes in medicine	212
5.10.8	Microbes in synthetic energy fuels	213
5.10.9	Microbes and environment cleaning	213
5.11	Food microbiology	214
5.11.1	Microbes associated with food spoilage	214
5.11.2	Meat and fish	215
5.11.3	Poultry and eggs	215
5.11.4	Breads and bakery products	215
5.11.5	Other foods	215
5.11.6	Importance of microbes in foods	216
5.11.7	Food fermentation	216
5.12	Microbial biotechnology	217
5.12.1	Production of enzymes by microorganisms	218

Problems	218
Section A: Descriptive type	218
Section B: Multiple choice	219
Section C: Critical thinking	220
References and further reading	220

6 Agricultural biotechnology 221

Learning objectives	221
6.1 Introduction	221
6.2 Plant breeding	222
6.2.1 Classical breeding	224
6.2.2 Plant breeding by traditional techniques	224
6.2.2.1 Selection	224
6.2.2.2 Hybridization	224
6.2.2.3 Polyploidy	225
6.2.3 Modern plant breeding	225
6.2.3.1 In vitro cultivation	225
6.2.3.2 In vitro selection and somaclonal variation	226
6.2.3.3 Somatic hybrid plants	227
6.2.3.4 Breeding by restriction fragment length polymorphism	227
6.2.3.5 Plant breeding by gene transfer	229
6.2.3.6 Agrobacterium-mediated gene transfer	229
6.2.3.7 Particle bombardment	230
6.2.3.8 Electroporation and direct DNA entry into protoplasts	230
6.2.3.9 Transgene expression	230
6.2.3.10 Selection and plant regeneration	231
6.2.3.11 Reverse breeding and doubled haploids	233
6.2.3.12 Genetic modification	234
6.3 Plant diseases	234
6.3.1 Diseases caused by fungi	235
6.3.2 Diseases caused by oomycetes	235
6.3.3 Diseases caused by bacteria	236
6.3.4 Diseases caused by plant virus	236
6.3.5 Diseases caused by nematodes	237
6.3.6 Diseases caused by protozoa	237
6.3.7 Diseases caused by parasitic plants	237

6.4	Applications of molecular and genetic tools in agriculture	238
6.4.1	Expression of viral coat protein to resist infection in agriculture	238
6.4.2	Expression of bacterial toxin in agriculture using molecular techniques	238
6.5	Herbicide-tolerant plants	239
6.6	Pigmentation in transgenic plants	240
6.7	Altering the food content of plants	241
6.8	Gene transfer methods in plants	241
6.9	Target cells for gene transformation	241
6.10	Vectors for gene transfer	242
6.10.1	Structure and functions of Ti and Ri plasmids	242
6.11	Transformation techniques using <i>Agrobacterium</i>	245
6.11.1	Requirements of transgenic plants	245
6.11.2	Explants used for transformation	245
6.11.3	Marker genes for selection and scoring of cells	246
6.11.4	Neomycin phosphotransferase gene	246
6.12	Glucuronidase gene	246
6.13	Agroinfection and gene transfer	247
6.14	DNA-mediated gene transfer	247
6.14.1	Microinjection and macroinjection	248
6.15	Electroporation for gene transfer	248
6.16	Liposome-mediated gene transfer	249
6.17	Gene transformation using pollen	249
6.18	Application of transgenic plants	250
6.18.1	Detoxification or degradation of herbicides	250
6.18.2	Crystal (cry) proteins	250
6.18.3	Toxic action of cry proteins	251
6.18.4	Expression of cry genes in plants	251
6.18.5	Insect resistance to cry proteins	251
6.18.6	Virus resistance	252
6.18.7	Drought resistance	252
6.18.8	Modification of seed protein quality	253
6.18.8.1	Introduction of an appropriate transgene	253

6.18.8.2	Modification of endogenous genes	254
6.18.8.3	Successful examples	254
6.18.9	Cosuppression of genes	254
6.18.10	RNA-mediated interference	255
6.18.11	Biochemical production in plants	256
6.18.12	Plant-derived vaccines	256
6.18.12.1	Edible vaccines	256
6.18.12.2	Recombinant and subunit vaccines	257
6.18.13	Hirudin: A polypeptide	258
6.18.14	Phytase as an enzyme	258
6.18.15	Polyhydroxybutyrate biodegradable plastic substrate	258
6.19	How safe are transgenic plants?	259
6.20	Bioengineered plants	259
6.20.1	Golden rice	260
6.20.2	Tomato "Flavr Savr"	262
6.21	Genetically modified maize	263
6.22	Terminator technology	265
6.22.1	Types of terminator technology	265
6.22.1.1	V-GURT	265
6.22.1.2	T-GURT	266
6.22.2	Benefits of terminator technology	266
6.22.3	Concerns of terminator technology	266
Problems		267
Section A: Descriptive type		267
Section B: Multiple choice		267
Section C: Critical thinking		268
Assignment		268
References and further reading		269

7 Animal biotechnology 271

Learning objectives	271
7.1 Introduction	271
7.2 History of the use of animals in research	273
7.3 Drug testing in animals is mandatory	274
7.4 Most commonly used animals in research	274
7.4.1 Invertebrates	275
7.4.2 Fish and amphibians	276
7.4.3 Vertebrates	276
7.4.3.1 Rodents	276
7.4.3.2 Cats and dogs	276

7.4.3.3	Primates and nonprimates	277
7.5	Application of animal models	277
7.5.1	Use of animals in basic research	278
7.5.2	Use of animals in applied research	279
7.5.2.1	Genetic diseases	279
7.5.2.2	Virology	279
7.5.2.3	Neurological disorders	279
7.5.2.4	Organ transplantation	280
7.5.2.5	Drug efficacy testing	280
7.5.2.6	Toxicological analysis	281
7.5.2.7	Cosmetics testing	283
7.6	Animal models	284
7.6.1	Caenorhabditis elegans	284
7.6.2	Drosophila melanogaster	286
7.6.3	Laboratory mouse	287
7.6.4	Rhesus monkey	287
7.6.5	Xenopus laevis	288
7.6.6	Zebrafish	289
7.6.7	Bioengineered mosquito	290
7.7	Animal biotechnology	291
7.7.1	Use of animals in antibody production	291
7.7.1.1	Monoclonal antibodies in diagnostic applications	291
7.7.1.2	Monoclonal antibodies in therapeutic applications	291
7.7.1.3	Recombinant antibodies	293
7.7.2	In vitro fertilization and embryo transfer	293
7.7.2.1	Embryo transfer in cattle	293
7.7.3	Animal cell culture products	295
7.7.4	Animal cloning	295
7.7.4.1	Cloning of extinct and endangered species	296
7.7.5	Transgenic animals	297
7.7.5.1	Transgenic cow	300
7.7.5.2	Transgenic pigs	300
7.7.5.3	Transgenic goat	301
7.7.5.4	Transgenic sheep	301
7.7.5.5	Transgenic chickens	302
7.7.5.6	Transgenic primates	303
7.8	Biotechnology and fish farming	303
7.8.1	Mariculture	303
7.8.2	Polyculture	304

7.8.3	Aquatic biotechnology	304
7.8.3.1	Transgenic fish	304
7.8.3.2	Green fluorescent protein	305
7.8.3.3	Antifreeze proteins	305
7.8.3.4	Transgenic salmon	306
7.8.3.5	Transgenic mussel	307
7.8.3.6	Fugu fish	307
7.8.3.7	Squalus acanthias	308
7.8.3.8	Limulus polyphemus	308
7.9	Regulations of animal testing	309
7.10	Alternatives to animal testing	310
7.10.1	In vitro cell culture technique	310
7.10.2	Synthetic membranes	311
7.10.3	Statistics instead of animal testing	311
7.10.4	Newer scanning techniques	311
7.10.5	Computer models	312
Problems		312
Section A: Descriptive type		312
Section B: Multiple choices		313
Section C: Critical thinking		314
Assignment		314
References and further reading		314

8 Environmental biotechnology 317

Learning objectives	317
8.1 Introduction	317
8.1.1 Ecosystem	317
8.1.2 Biomes	318
8.1.3 Wilderness	318
8.1.4 Geological activity	319
8.1.5 Oceanic activity	319
8.2 Factors affecting the environment	320
8.2.1 Global warming	320
8.2.1.1 Carbon footprint	320
8.2.1.2 Destruction of forests	320
8.2.1.3 Air pollution	322
8.2.1.4 Major carbon dioxide emission countries	324
8.2.1.5 Greenhouse effect	324
8.2.1.6 Acid rain	324
8.2.1.7 Ocean acidification	325
8.2.1.8 Health hazards due to pollution	325
8.3 Environment protection by biotechnology	326
8.3.1 Bioremediation	327

8.3.1.1	<i>Mycoremediation</i>	329
8.3.2	<i>Wastewater treatment</i>	329
8.3.2.1	<i>Pretreatment phase</i>	330
8.3.2.2	<i>Screening phase</i>	330
8.3.2.3	<i>Sedimentation phase</i>	331
8.3.2.4	<i>Secondary treatment phase</i>	331
8.3.2.5	<i>Activated sludge</i>	332
8.3.2.6	<i>Filter beds</i>	332
8.3.2.7	<i>Biological aerated filters</i>	332
8.3.2.8	<i>Nutrient removal</i>	332
8.3.2.9	<i>Nitrogen removal</i>	333
8.3.2.10	<i>Phosphorus removal</i>	333
8.3.2.11	<i>Disinfection of wastewater</i>	334
8.3.2.12	<i>Sludge disposal</i>	334
8.3.3	<i>Biofuels</i>	335
8.3.3.1	<i>Biodiesel</i>	337
8.3.3.2	<i>Biogas</i>	338
8.3.3.3	<i>Bioalcohols</i>	338
8.3.3.4	<i>Bioethers</i>	339
8.3.3.5	<i>Syngas</i>	339
8.3.3.6	<i>Solid biofuels</i>	339
8.3.3.7	<i>Second-generation biofuels</i>	340
8.3.3.8	<i>Third-generation biofuels</i>	340
8.3.3.9	<i>International biofuel efforts</i>	340
8.3.3.10	<i>Future of biofuels</i>	340
8.3.4	<i>Biodegradable plastic</i>	341
8.3.5	<i>Biodegradation by bacteria</i>	342
8.3.6	<i>Oil-eating bacteria</i>	343
8.3.7	<i>Biostimulation and bioaugmentation</i>	345
8.3.8	<i>Bioleaching</i>	346
8.3.9	<i>Single-cell protein and biomass from waste</i>	347
8.3.10	<i>Vermitechnology</i>	347
8.3.11	<i>Biosorption</i>	348
8.3.11.1	<i>Bacteria</i>	348
8.3.11.2	<i>Fungi</i>	349
8.3.11.3	<i>Algae</i>	350
8.3.12	<i>Genetically engineered organisms</i>	350
	<i>Problems</i>	350
	<i>Section A: Descriptive type</i>	350

Section B: Multiple choice	350
Section C: Critical thinking	352
Debate	352
Field visit	352
References and further reading	352
9 Medical biotechnology	355
Learning objectives	355
9.1 Introduction	355
9.1.1 Vaccine development	355
9.1.1.1 Killed vaccines	356
9.1.1.2 Attenuated vaccines	356
9.1.1.3 Toxoid vaccines	357
9.1.1.4 Subunit vaccines	357
9.1.1.5 Conjugate vaccines	357
9.1.1.6 Experimental vaccines	358
9.1.1.7 Valence vaccines	358
9.1.1.8 Vaccine production	358
9.1.1.9 Making of influenza vaccines	360
9.1.1.10 Large-scale production of vaccines	362
9.1.1.11 Economics involved in vaccine production	364
9.1.1.12 Trends in vaccine research	364
9.1.1.13 Issues related to vaccines	366
9.1.1.14 Synthetic peptides as vaccines	366
9.2 Antibody production	367
9.2.1 Applications of monoclonal antibodies	368
9.2.1.1 Diagnostic test	369
9.2.1.2 Cancer treatment	369
9.2.2 Hybridoma technology	369
9.2.2.1 Purification of monoclonal antibodies	370
9.2.3 Recombinant monoclonal antibodies	372
9.2.4 Constraints in making monoclonal antibodies	372
9.3 Therapeutic proteins	373
9.3.1 Growth factors	374
9.4 Stem cell transplantation	375
9.4.1 Adult stem cells	375

9.4.1.1	Dental pulp-derived stem cells	377
9.4.1.2	Hematopoietic stem cells	377
9.4.1.3	Mammary stem cells	377
9.4.1.4	Mesenchymal stem cells	378
9.4.1.5	Neural stem cells	378
9.4.1.6	Olfactory adult stem cells	379
9.4.1.7	Clinical applications of stem cells	379
9.4.1.8	Cancer treatment	380
9.4.1.9	Spinal cord injury	381
9.4.1.10	Corneal repair	381
9.4.2	Embryonic stem cells	382
9.4.2.1	How were embryonic stem cells discovered?	382
9.4.2.2	Cell line contamination	382
9.4.2.3	Immunorejection	384
9.4.2.4	Alternative approach to creating embryonic stem cells	384
9.4.2.5	Embryonic stem cells as models for human genetic disorders	385
9.4.2.6	First clinical trial	385
9.5	Bioengineered skin	386
9.6	Bioengineered organ transplantation	387
9.7	Gene therapy	387
9.7.1	Ex vivo gene therapy	388
9.7.1.1	Gene therapy using viral vectors	389
9.7.1.2	Nonviral methods of DNA transfer	393
9.7.2	In vivo gene therapy	396
9.7.3	Problems with gene therapy	397
9.7.3.1	Short-lived nature of gene therapy	397
9.7.3.2	Immune response	397
9.7.3.3	Virus-induced toxicity	397
9.7.3.4	Not for multigene disorders	397
9.7.3.5	Induced mutagenesis	398
9.7.4	Genetic counseling	398
9.8	Molecular diagnostics	399
9.8.1	DNA fingerprinting	399

9.8.2	Techniques of DNA profiling	401
9.8.2.1	Restriction fragment length polymorphism analysis	401
9.8.2.2	PCR analysis	402
9.8.2.3	Short tandem repeat analysis	402
9.8.2.4	Amplified fragment length polymorphism analysis	403
9.8.2.5	Y chromosome analysis	403
9.8.2.6	Mitochondrial DNA analysis	404
9.8.2.7	Confirmation of genetic relationship	404
9.8.2.8	Fake DNA evidence	404
9.8.2.9	Criminal DNA data	405
9.9	Artificial blood	405
9.10	Organ transplant	407
9.10.1	History of organ transplant	407
9.10.2	Types of transplants	409
9.10.2.1	Autograft	409
9.10.2.2	Allograft	410
9.10.2.3	Isograft	410
9.10.2.4	Xenograft	411
9.11	Cloning	411
9.11.1	Molecular cloning	411
9.11.2	Clonal cell technology	412
9.11.3	Clonal embryo	413
9.11.4	Reproductive cloning	413
9.11.5	Human Cloning	414
Questions		415
Section A: Descriptive type		415
Section B: Multiple choice		415
Section C: Critical thinking		417
Assignment		417
References and further reading		417

10 Nanobiotechnology 421

Learning objectives	421
10.1 Introduction	421
10.2 Nanotechnology	421
10.3 Nanobiotechnology	423
10.4 Applications of nanobiotechnology	424
10.4.1 Nanomedicine	424
10.4.1.1 Drug delivery	425

10.4.1.2	Cancer diagnostics	428
10.4.1.3	In vivo drug imaging	431
10.4.1.4	Nanonephrology	432
10.4.1.5	Gene therapy using nanotechnology	433
10.4.1.6	Antimicrobial techniques	434
10.4.2	Neuro-electronic interfaces	434
10.4.3	Molecular nanotechnology	435
10.4.4	Nanorobots	435
10.4.5	Cell repair machines	435
10.4.6	Nanosensors	436
10.4.7	Nanoparticles	438
10.4.7.1	Classification of nanoparticles	439
10.4.7.2	Characterization of nanoparticles	439
10.4.7.3	Nanoparticles and safety issues	439
10.5	Nanotechnology in the food industry	440
10.6	Water pollution and nanotechnology	441
10.7	Research trends	441
	Problems	442
	Section A: Descriptive type	442
	Section B: Multiple choice	442
	Section C: Critical thinking	443
	References and further reading	443

11 Product development in biotechnology 445

	Learning objectives	445
11.1	Introduction	445
11.2	Methods of scientific inquiry	446
11.2.1	Characterizations of scientific investigation	447
11.2.2	Scientific inventions	448
11.3	Commercialization of scientific discovery	449
11.4	Business plan	449
11.4.1	Project feasibility	450
11.4.1.1	Market research	450
11.4.1.2	Significance of a project	450
11.4.1.3	Technical outline	450
11.4.1.4	Time plan	450
11.4.1.5	Project cost	450
11.4.1.6	Legal and regulatory issues	451
11.4.1.7	Quality of the product	451

11.5	Biotechnology product development	451
11.5.1	Infrastructure requirements	451
11.5.1.1	Research and development facility	453
11.5.1.2	Animal testing facility or preclinical lab	454
11.5.1.3	Bioequivalence lab	456
11.5.1.4	Clinical trial center	456
11.5.1.5	Manufacturing plant	457
11.5.1.6	Formulation lab	457
11.5.1.7	Quality assurance and quality control lab	458
11.6	Phases of biotechnology product development	458
11.6.1	Preclinical studies	459
11.6.2	Phase 0	459
11.6.3	Phase I	460
11.6.3.1	Single ascending dose	460
11.6.3.2	Multiple ascending doses	461
11.6.3.3	Food effect	461
11.6.4	Phase II	461
11.6.5	Phase III	461
11.6.6	Phase IV	462
11.7	Biotechnology entrepreneurship	463
11.7.1	Starting a biotechnology company	463
11.7.1.1	Grants	463
11.7.1.2	Private investors	464
11.7.1.3	Angel investors	464
11.7.1.4	Venture capitalists	464
11.7.1.5	Bank loans	464
11.8	Biotechnology industry: Facts and figures	464
11.8.1	Capital investment in biotechnology	466
11.8.2	Mergers and acquisitions of biotechnology companies	467
11.9	Formation of a new biotechnology company	467
11.10	Successful bioentrepreneurship	468
11.11	Biotechnology products and intellectual property rights	468

11.11.1	Patenting, licensing, and partnership in the biotechnology industry	469
11.11.2	Intellectual property protection	470
11.11.3	Intellectual property rights for plants	471
11.11.4	Patents and biotechnology products	472
11.11.5	International treaties	473
11.11.5.1	Patent Cooperation Treaty	474
11.11.5.2	World Intellectual Property Organization	475
11.11.5.3	Agreement on Trade-Related Aspects of Intellectual Property Rights	475
11.11.5.4	Issues with biotechnology patents	476
11.11.5.5	Patent infringements	477
11.12	Biotechnology stock investment: Pros and cons	478
11.12.1	Products in the pipeline	479
11.12.2	Collaboration and merger	479
11.12.3	Experienced management	479
11.12.4	Cash flow	480
11.13	Marketing trends in biotechnology	480
11.14	Role of regulators in biotechnology product development	480
11.14.1	World Health Organization	480
11.14.2	International Conference on Harmonization	481
11.14.3	United States Food and Drug Administration	482
11.14.4	Good laboratory practice	484
11.14.4.1	GLP requirements	484
11.14.4.2	National legislation	484
11.14.4.3	Facilities	485
11.14.4.4	GLP inspection and enforcement	486
11.14.5	Good manufacturing practice	486
11.14.5.1	Cleanroom facility	487

11.14.5.2 Cleanroom classifications	487
11.14.5.3 GMP enforcement	487
11.15 Certification and accreditation	488
11.15.1 International Organization for Standardization	488
11.15.1.1 ISO 14644-1	489
11.15.1.2 ISO 14698-1	489
11.15.1.3 ISO 14698-2	489
11.15.2 British Standard 5295	489
Problems	490
Section A: Descriptive type	490
Section B: Multiple choice	490
Section C: Critical thinking	491
Field trip	491
References and further reading	491

12 Industrial biotechnology 493

Learning objectives	493
12.1 Introduction	493
12.2 Fermenter or bioreactor	494
12.3 Principle of fermentation	495
12.3.1 Aerobic fermentation	496
12.3.1.1 Submerged culture method	496
12.3.1.2 Semisolid/solid- state methods	496
12.3.2 Anaerobic fermentation	497
12.3.2.1 Batch fermentation process	498
12.3.2.2 Continuous fermentation process	499
12.4 Production of biomolecules using fermenter technology	499
12.4.1 Gluconic acid	499
12.4.2 Citric acid	499
12.4.3 Acetone butanol	500
12.4.4 Itaconic acid	500
12.4.5 Gibberellic acid	500
12.4.6 Lactic acid	501
12.4.7 Amino acids	501
12.4.8 Enzymes	502
12.4.9 Proteases	502
12.4.10 Amylases	503
12.5 Development process of microbial products	503

12.5.1	Isolation and screening of microorganisms	503
12.5.1.1	Isolation of microorganisms	504
12.5.1.2	Screening of microorganisms	505
12.5.2	Inoculum development	506
12.5.3	Culture media	506
12.5.4	Contamination	506
12.5.5	Sterilization	507
12.5.5.1	Heating	507
12.5.5.2	Radiation	507
12.5.5.3	Chemicals	508
12.5.5.4	Filtration	508
12.5.6	Strain improvement	508
12.5.6.1	Mutant selection	508
12.5.6.2	Selective isolation of mutants	509
12.6	Upstream bioprocess	510
12.6.1	Industrial microbial culture	510
12.6.2	Mammalian cell culture	512
12.6.2.1	Manipulation of cultured cells	512
12.6.2.2	Generation of hybridomas	513
12.6.3	Nonmammalian culture methods	513
12.6.3.1	Industrial plant cell culture	513
12.6.3.2	Bacterial/yeast culture methods	514
12.6.3.3	Viral culture methods	514
12.7	Downstream bioprocess	514
12.7.1	Stages in DSP	514
12.7.1.1	Removal of insolubles	515
12.7.1.2	Product isolation	515
12.7.1.3	Product purification	515
12.7.1.4	Product polishing	515
12.8	Bioprocess automation	515
12.8.1	Modeling individual unit operations	516
12.8.2	Simple mass balance	517
12.8.3	Mass and heat balance	517
12.8.4	Rate-based model	517
12.8.5	Batch processing	517
12.8.6	Continuous processes	518

12.8.7	Dynamic simulation	518
12.8.8	Water consumption	518
12.8.9	Waste recycling	518
12.9	Industrial application of microbes	519
12.9.1	Corynebacterium	519
12.9.2	Bacillus	519
12.9.3	Saccharomyces cerevisiae	520
12.9.4	Pseudomonas	520
12.9.5	Clostridium	521
12.9.6	Thermophiles	521
12.10	Industrial production of healthcare products	522
12.10.1	Antibiotic manufacturing	522
12.10.1.1	Penicillin production	522
12.10.1.2	Cephalosporins production	523
12.10.1.3	Streptomycin production	523
12.10.2	Steroids production	526
12.10.3	Textile production	526
12.10.4	Microbial synthesis of vitamin B12	527
Problems		527
Section A: Descriptive type		527
Section B: Multiple choice		528
Section C: Critical thinking		529
Laboratory assignment		529
References and further reading		529

13 Ethics in biotechnology 531

Learning objectives	531
13.1 Introduction	531
13.2 Genetically modified foods and plants	531
13.3 Use of animals as experimental models	534
13.3.1 Disadvantages of animal testing	536
13.3.2 Attacks on researchers	536
13.3.3 Regulations for animal testing in the United States	537
13.3.4 Role of animal welfare groups	537
13.3.5 Future of animal testing	538
13.3.6 Decide for yourself	538
13.4 Use of humans as experimental models	539
13.4.1 Bioethical implications	540

13.5	Xenotransplantation	541
13.6	Genetic screening	543
13.7	Biometrics	544
13.8	DNA fingerprinting	545
13.9	Organ donation and transplantation	545
13.10	Euthanasia	546
13.11	Neuroethics	547
13.12	Assisted reproductive technology	547
13.13	Embryonic stem cell research	548
13.13.1	In favor of embryonic stem cell research	550
13.13.2	Against embryonic stem cell research	550
13.13.3	Current status	551
13.14	Human cloning	551
	Questions	553
	Section A: Descriptive type	553
	Section B: Multiple choice	553
	Section C: Critical thinking	553
	Assignments	554
	References and further reading	555

14 Careers in biotechnology 557

	Learning objectives	557
14.1	Introduction	557
14.2	Education and investment in biotechnology	557
14.3	Research and development in biotechnology	561
14.4	Biotechnology industry and products	563
14.5	Biotechnology status in the United States	563
14.6	Career opportunities in biotechnology	564
14.7	Entry-level job positions in biotechnology	566
14.7.1	Research and development division	566
14.7.1.1	Glass washer	566
14.7.1.2	Laboratory assistant	566
14.7.1.3	Research associate	567
14.7.1.4	Research assistant	567
14.7.1.5	Postdoctoral fellow	567
14.7.1.6	Media preparation technician	567
14.7.1.7	Greenhouse assistant	567
14.7.1.8	Plant breeder	567
14.7.2	Quality control	568

14.7.2.1	Quality control analyst	568
14.7.2.2	Quality control engineer	568
14.7.2.3	Environmental health and safety specialist	568
14.7.2.4	Quality assurance auditor	569
14.7.2.5	Validation engineer	569
14.7.2.6	Validation technician	569
14.7.3	Clinical research	569
14.7.3.1	Clinical research administrator	569
14.7.3.2	Clinical coordinator	570
14.7.3.3	Clinical programmer	570
14.7.3.4	Biostatistician	570
14.7.3.5	Clinical data specialist	570
14.7.3.6	Drug experience coordinator	571
14.7.3.7	Clinical research associate	571
14.7.3.8	Animal handler	571
14.7.3.9	Animal technician	571
14.7.3.10	Technical writer	571
14.7.4	Product and development	572
14.7.4.1	Product development engineer	572
14.7.4.2	Production planner/scheduler	572
14.7.4.3	Manufacturing technician	572
14.7.4.4	Packaging operator	573
14.7.4.5	Manufacturing research associate	573
14.7.4.6	Instrument/calibration technician	573
14.7.4.7	Biochemical development engineer	573

	14.7.4.8	Process development associate	574
	14.7.4.9	Assay analyst	574
	14.7.4.10	Manufacturing engineer	574
14.7.5		Regulatory affairs	574
	14.7.5.1	Regulatory affairs specialists	574
	14.7.5.2	Documentation coordinator	575
	14.7.5.3	Documentation specialist	575
14.7.6		Information systems	575
	14.7.6.1	Library assistant	575
	14.7.6.2	Scientific programmer/analyst	575
14.7.7		Marketing and sales	576
	14.7.7.1	Market research analyst	576
	14.7.7.2	Systems analyst	576
	14.7.7.3	Sales representative	576
	14.7.7.4	Customer service representative	576
	14.7.7.5	Technical service representative	577
14.8		Administration	577
	14.8.1	Technical recruiter	577
	14.8.2	Human resource representative	577
	14.8.3	Patent administrator	577
	14.8.4	Patent agent	578
14.9		Which job is good for me?	578
14.10		Why are R&D jobs the most challenging?	580
	14.10.1	Medical and diagnostics sectors	580
	14.10.1.1	Detecting and treating hereditary diseases	580
	14.10.1.2	Heart disease	580
	14.10.1.3	Cancer	580
	14.10.1.4	AIDS	580
	14.10.1.5	Veterinary medicine	581
	14.10.1.6	Vaccines	581
	14.10.1.7	Monoclonal antibodies	581

14.10.1.8	Growth hormones	581
14.10.2	Agricultural sector	581
14.10.2.1	Crop yield	581
14.10.2.2	Protein and oil content of seeds	582
14.10.2.3	Environmental conditions	582
14.10.2.4	Disease and pest resistance	582
14.10.3	Law enforcement sector	582
14.10.4	Product manufacturing sector	582
14.10.5	Microbial engineering sector	583
14.11	Salary and incentives in biotechnology	583
	Problems	584
	Assignments	585
	Field visit	585
	References and further reading	585

15 Laboratory tutorials 587

15.1	Laboratory experiments	587
15.1.1	Controlled experiment	588
15.1.2	Observational experiment	589
15.2	Laboratory safety	589
15.3	Good laboratory practices for biotechnology labs	591
15.4	General laboratory techniques	593
15.4.1	Pipetting technique	593
15.4.1.1	Plastic pipette	593
15.4.1.2	Plastic pipette pump	593
15.4.1.3	Pipette with a bulb	594
15.4.1.4	Micropipette	594
15.4.2	Centrifugation technique	595
15.4.3	Spectrophotometer technique	596
15.4.4	Aseptic techniques	597
15.5	General principles of animal handling	598
15.6	Animal anesthesia	599
15.6.1	Anesthesia by gas	599
15.6.2	Injectable anesthesia	600
15.6.2.1	Intravenous method	600
15.6.2.2	Intramuscular method	600
15.6.2.3	Intraperitoneal method	601

	15.6.2.4 Subcutaneous method	601
15.6.3	Animal euthanasia	601
	15.6.3.1 Criteria for euthanasia	601
	15.6.3.2 Surgical operation	602
	15.6.3.3 Use of a CO ₂ chamber	602
15.7	Animal histology	602
15.8	Blood collection in animals	605
15.9	Histology and microscopy	607
	15.9.1 Immunocytochemistry technique	607
	15.9.1.1 Direct immunofluorescence	607
	15.9.1.2 Indirect immunofluorescence	609
15.10	Separation techniques	610
	15.10.1 Agarose gel electrophoresis (basic method)	610
	15.10.2 How much percentage gel will be made?	610
	15.10.3 Which gel tank to use?	611
	15.10.4 How much DNA should be loaded?	611
	15.10.5 Which comb?	611
15.11	Microbiology techniques	614
	15.11.1 Isolation of pure culture	614
	15.11.2 Streaking bacteria for single colonies	616
	15.11.3 Gram-staining procedure	616
15.12	Biochemistry techniques	617
	15.12.1 Estimation of fat in milk samples	617
	15.12.2 Protein quantification by Bradford assay	618
	15.12.3 Indirect ELISA	619
	15.12.4 Sandwich ELISA	621
	15.12.5 Sonication of bacteria	621
15.13	Molecular techniques	622
	15.13.1 Isolation of genomic DNA from blood	622
	15.13.2 Isolation of DNA from fresh or frozen tissue	623
	15.13.3 Preparation of genomic DNA from bacteria	626

15.13.4	DNA isolation procedure	626
15.13.5	Polymerase chain reaction	627
15.13.6	Semiquantitative RT-PCR	630
15.13.6.1	Role of housekeeping gene transcript	630
15.13.6.2	Isolation of total RNA	631
15.14	Genetic techniques	633
15.14.1	Preparation of human metaphase chromosomes	633
15.14.2	Structural analysis of human chromosomes by karyotype	635
15.14.3	DNA amplification fingerprinting protocol	637
15.14.4	Single-strand conformation polymorphism technique	638
15.15	Agricultural biotechnology	640
15.15.1	Plant DNA isolation	640
15.15.2	Plant regeneration by protoplast fusion	641
15.15.3	Simplified Arabidopsis transformation	643
15.15.4	Agrobacterium-mediated gene transfer via hypocotyls	644
15.15.5	Isolation of DNA from onion	644
15.15.6	Isolation of DNA from wheat germ	645
15.16	Microbial biotechnology	646
15.16.1	Gram-positive/Gram-negative staining	646
	References	647

Index	649
-------------	-----