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Introduction

1.1 Biotechnology – Its Background and History

The office of Technology Assessment of the U.S. Congress defines biotechnology as "any technique that uses living organisms or their products to make or modify a(another) product, to improve plants or animals, or to develop microorganisms for specific uses." In its broadest terms, such a definition includes human beings, making biotechnology to be as old as the development of human skills such as to grow crops, harvest them, and use them as ingredients to cook their meals. In fact, a term Biotechnik, originally referring to social biology by Goldscheid in 1911, was used in the *Nature* journal of 1933 to print an editorial on Biotechnology (Bud, 1989; Goldcheid, 1911). Ironically, time has come a full circle as scientists are now examining gene expression patterns under different social conditions (Cole, 2014).

Biotechnology as a system of knowledge and application probably goes back to Vedic times, when the system of Ayurveda (literally meaning knowledge of life) was developed. Reference to the use of herbs for treating medical conditions are to be found in the earliest literature of India, over 14,000 years ago. According to BioREACH (which stands for Biotechnology Resource for Educational Advancement of Curriculum in High Schools at Arizona State University) some forms of biotechnology were being practiced by the ancients in Babylon, Egypt, and Rome in their selective breeding practices with livestock, over 10,000 years ago. There are instances where people around 6000 BC used fermentation to make wine and beer; and when the Chinese used lactic acid producing bacteria to produce yogurt around 4000 BC.

The modern term 'biotechnology' was first coined by a Hungarian engineer, Karl Ereky (1878–1952), in 1919. He defined biotechnology as general processes of converting raw materials into useful products, such as on industrial farms, using living organisms. A previous term 'zymotechnology' was used in the nineteenth century for using microorganisms to produce products like bread, wine, tofu, and so on. In the early twentieth century zymotechnology also included biological chemistry and covered usage of biological molecules such as enzymes, amino acids, and proteins for industrial production.

Modern biotechnology took root after the discovery of genetic material and the central theme of the gene progression route, namely DNA → RNA → Proteins, during the 1930s to the 1950s. With better fundamental understanding of the macromolecular structure–function, recombinant technology, advancements in microbiology, and chemical engineering, with the use of instrumental tools for synthesis and analysis of molecular characteristics, the field of biotechnology has made

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giant strides, especially since the 1980s. Armed with advances in cell and tissue engineering, biotechnologists have developed several bold concepts about designer proteins, targeted drug delivery, novel bio-macromolecules, and a variety of bio-based pharmaceuticals, especially after the discovery of designer organisms (Table 1.1); and the detection and diagnostics of diseases, genetically modified plants and food, synthesis of a variety of pharmaceuticals, agriculture and crop protection drugs, and even organic rejuvenation of the Earth and its environment. Biotechnology has become a source of basic and applied research, employing and integrating with other major fields of study such as chemistry, biochemistry, physics, engineering, social sciences, humanities, business, medical care, and environmental protection.

Table 1.1 Events that shaped modern biotechnology.

In Ancient Times

8000–4000 BCE	Humans domesticate crops and livestock.
2000 BCE	Development of fermentation technology for beer, bread, production of cheese, and wine.
500 BCE	First antibiotic – Moldy soyabean curds used to treat boils (in China).
100 CE	First insecticide – powdered chrysanthemums (in China).

During Medieval Times (Prior to the 20th Century)

1663	Robert Hooke gave the first description of living cells.
1677	Detailed description of bacteria and protozoa by Antonie van Leeuwenhoek
1798	First viral vaccine (for smallpox) by Edward Jenner.
1802	The word “biology” first appears.
1830–1833	Proteins are discovered; first enzyme is discovered and isolated.
1857–1862	Bacterial origin of fermentation explained by Louis Pasteur. He later proposed the germ theory of disease. • Theory of evolution proposed by Charles Darwin.
1863–1870	The laws of inheritance was proposed by Gregor Mendal. Identification of DNA.
1871–1880	Discovery of an artificial sweetner. • Development of a staining technique for identification of bacteria. • Discovery of chromatin.
1881–1890	Vaccines developed against cholera, anthrax and rabies.
1893	Immunotherapy was used for the first time to treat a cancer patient.

During the First Half of the 20th Century

1915	Discovery of phages – viruses that only infect bacteria.
1917	Discovery of a beneficial strain of *E.coli*.
1919	First use of the term “biotechnology.”
1927	Herman Muller discovers that radiation causes defects in chromosomes.
1928	Discovery of the first antibiotic, penicillin, by Alexander Fleming.
1944	DNA is proven to carry genetic information.

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Table 1.1 *contd.*

Post 1950

1952	First continuous cell line (HeLa); developed by George Otto Gey
1953	Watson and Crick discover and explain the double-helix structure of DNA.
1954	First kidney transplant between identical twins conducted by Joseph Murray.
1955	Amino acid sequence of insulin discovered.
1957	Scientists reveal that sickle-cell anemia occurs due to an alteration in a single amino acid in hemoglobin cells.
1958	DNA created in a test tube by Arthur Kornberg for the first time. • Moore–Stein amino acid analyzer developed. • First X-ray diffraction depiction of protein crystal and myoglobin structure.

The 1960s

1960	Discovery of messenger RNA (mRNA).
1961	Concept of Operon developed by François Jacob and Jacques Monad.
1962	Discovery of green fluorescent protein (GFP) by Osamu Shimomura in the jellyfish *Aequorea victoria*. He went on to develop a cellular visualization technique from this.
1963	Measles vaccine developed by Samuel Katz and John F Enders. • Independent groups in the USA, Germany and China produce insulin, a pancreatic hormone.
1964	Discovery of reverse transcriptase.
1966	Genetic code for DNA cracked.
1967–71	First American vaccine for mumps developed by Maurice. • First vaccine for rubella developed • Measles/mumps/rubella (MMR) vaccine developed.
1968	Har Gobind Khorana synthesizes DNA in a test tube. Discovery of protein synthesis process in cells

The 1970s

1970	Discovery of restriction enzymes.
1972	Dicovery of DNA ligases and its uses. • The DNA composition of humans was found to be 99% similar to that of chimpanzees and gorillas. • First use of reverse transcriptase to prepare complementary DNA from mRNA in a test tube.
1973	rDNA techniques developed by Stanley Cohen and Herbert Boyer. • Blotting technique for DNA (Southern blot) developed by Sir Edwin Mellor Southern.
1974	The NIH formed a Recombinant DNA Advisory Committee to supervise recombinant genetic research. Chicken pox vaccine developed in Japan.
1975	Development of monoclonal antibodies technology.
1976	NIH publishes the first guidelines for rDNA research. • Employment of molecular hybridization for prenatal diagnosis of alpha thalassemia. • Yeast genes expressed in *E. coli* bacteria.

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1977	Procedures developed to swiftly sequence long sections of DNA. • Protein, somastatin, cloned using recombinant gene technology. *A landmark year: 1977 is held by many to have heralded the arrival of the* "*age of biotechnology.*" • First vaccine for pneumonia developed by R. Austrian *et al.* at the University of Pennsylvania.
1978	Boyer synthesizes a version of recombinant human insulin gene. • The first test-tube baby, Louise Brown, is born in the UK. • The first vaccine for meningococcal meningitis is developed.

The 1980s

1980	US Supreme Court rules that genetically altered life forms can be patented, creating vast possibilities for commercially exploiting genetic engineering. • First patent of this nature awarded to the Exxon oil company to patent an oil-eating micro-organism. *This would later be employed in the 1989 cleanup of the Exxon oil spill at Prince William Sound, Alaska.* • S. Cohen and D.H. Boyer receive US patent for gene cloning. • First automatic gene machine developed in California. • Establishment of Amgen, which would grow to become the world's largest biotechnology based medicine company.
1981	Frst vaccine for hepatitis B developed by Baruch Blumberg and Irving Millman. • First transgenic animals created.
1982	FDA supports the first recombinant protein.
1983	Isolation of AIDS virus by Luc Montagnier of the Pasteur Institute in Paris. • Demonstration of polymerase chain reaction (PCR) by Kary Mullis. • FDA sanctions monoclonal antibody-based diagnostic analysis to identify *Chlamydia trachomatis*. • Production of the first artificial chromosome. • Discovery of genetic basis for disease inheritance.
1984	Release of DNA fingerprinting technique. • Release of the first genetically engineered vaccine for hepatitis B. • Entire genome of the human immunodeficiency virus (HIV) virus cloned and sequenced.
1985	Genetic fingerprinting becomes court admissible. • Development of genetically engineered plants resistant to viruses, insects and bacteria. • Successful cloning of the gene encoding human lung surfactant protein. *This was a major step toward reducing premature birth problems*. • NIH releases guidelines for executing trials of gene therapy on humans.
1986	Use of antibodies and enzymes for therapeutics by Peter G Schultz from University of California, Berkeley. • FDA approval for first monoclonal antibody treatment to fight kidney transplant rejection. • FDA sanctions the first biotech-derived interferon drugs to treat cancer. • FDA sanctions the first genetically engineered human vaccine to prevent hepatitis B.

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1987 FDA approves genetically engineered tissue plasminogen activator to treat heart attack.
- Synthesis of yeast artificial chromosomes (YAC) by Maynard Olson and colleagues at Washington University. These are expression vectors for large proteins.
- Discovery of linkage between reverse transcription and PCR to augment messenger RNA sequences.
- DNA microarray technology developed.
- Indentification of genes whose expression is altered by interferon from pools of DNA.
- FDA approval for serum tumor marker test for ovarian cancer.

1988 Congress decides to fund the Human Genome Project.

1989 Amgen releases its first biologically derived human therapeutic.
- FDA approves oil-eating bacteria; which was employed later to clear up the Exxon Valdez oil spill.
- Discovery of the cystic fibrosis gene.

The 1990s

1990 The first gene therapy treatment is conducted.
- The Human Genome Project is launched.
- FDA approves first hepatitis C antibody test.
- FDA approves bioengineered form of the protein interferon gamma to treat chronic granulomatous disease.
- FDA sanctions a modified enzyme for enzyme replacement therapy to treat severe combined immunodeficiency disease. It is the first successful application of enzyme replacement therapy for an inherited disease.

1992 The US Army collects and collates blood and tissue tests from all new recruits as part of a genetic dog-tag plan to better identify bodies of soldiers killed in combat.
- FDA approves the first genetically engineered blood-clotting factor. It is a recombinant protein used to treat hemophilia A.
- FDA sanctions a recombinant protein to treat renal cell cancer.
- American and British researchers reveal technique for analyzing embryos in vitro for genetic abnormalities, e.g., cystic fibrosis and hemophilia.

1993 FDA approves a recombinant protein to treat multiple sclerosis.
- A rough map is created of all 23 pairs of human chromosomes by a research team led by Daniel Cohen from the Center for the Study of Human Polymorphisms, Paris.

1994 FDA approves a recombinant protein to treat growth hormone (GH) deficiency. .
- Discovery of the first breast cancer gene, BRCA1 by Mary-Claire King at University of California, Berkeley.
- FDA sanctions a modified enzyme to deal with Gaucher's disease.
- Identification of a number of genes, human and otherwise, with explanation of their functions. These comprise of: Ob, a gene connected to obesity; BCR, a breast cancer receptiveness gene; BCL-2, a gene linked to apoptosis (programmed cell death); Hedgehog genes (named because of their shape), which synthesize proteins that direct cell differentiation in complex organisms; and Vpr, a gene regulating the reproduction of the HIV virus.
- Genetic linkage studies recognize the role of genes in a variety of disorders, including bipolar disorder, cerulean cataracts, melanoma, dyslexia, prostate cancer, thyroid cancer, hearing loss, sudden infant death syndrome and dwarfism.
- FDA approvesa genetically engineered human DNase.

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1995	First baboon-to-human bone marrow transplant conducted on an AIDS patient. • First vaccine for hepatitis A is developed. • Researchers at the Institute for Genomic Research complete the first full gene sequence of a living organism for the bacterium *Haemophilus influenzae*. • A European study group determines that a genetic defect is the most frequent cause of deafness.
1996	Researchers at the Department of Biochemistry at Stanford University and Affymetrix develop the gene chip, a small glass or silica microchip that contains thousands of individual genes that can be examined simultaneously. This is a major scientific breakthrough in gene expression and DNA sequencing technology. • Research groups sequenced the complete genome of a complex organism, *Saccharomyces cerevisiae*, otherwise known as baker's yeast. The accomplishment symbolizes the entire sequencing of the largest genome to date. • A novel, economic diagnostic biosensor test is developed to hasten the detection of a toxic strain of *E. coli*, the bacteria responsible for food-poisoning outbreaks.
1997	Discovery of the first human artificial chromosome. • Synthesis from a mixture of natural and synthetic DNA, of a genetic cassette that could possibly be adapted and employed in gene therapy. • FDA sanctions a recombinant follicle stimulating hormone to deal with infertility. • FDA permits the first bloodless HIV-antibody analysis, based on cells from patients' gums. • Researchers at the Institute for Genomic Research sequenced the entire genome of the Lyme disease pathogen, *Borrelia burgdorferi*, along with the genome for the organism associated with stomach ulcers, *Helicobacter pylori*. • Researchers at the University of Wisconsin–Madison sequence the *E. coli* genome. • FDA permits the first therapeutic antibody to treat cancer in the USA. It is employed for patients with non-Hodgkin's lymphoma. • Dolly, the first cloned animal is born.
1998	For the first time, Human skin is created in the laboratory. • Two research groups culture embryonic stem cells. Embryonic stem cells can be employed to regenerate tissue and produce disorders mimicking diseases. • Researchers at the Sanger Institute in the UK and at the Washington University School of Medicine in St Louis, USA, sequence the first whole animal genome for the *caenorhabditis elegans* worm. • Creation of a rough draft of the human genome map displaying the sites of more than 30 000 genes. • The first vaccine for Lyme disease is developed • FDA approves treatment of Crohn's disease by using a novel monoclonal antibody. • Employment of a monoclonal antibody therapy against breast cancer.

The 2000s

2000	Kary Mullis adds value to Har Gobind Khorana's findings by amplifying DNA in a test tube, to create a thousand times more DNA than the original amount. • First cloned sheep, Dolly, is displayed by Sir Ian Wilmut.

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Table 1.1 *contd.*

	• Craig Venter sequences the human genome; the first publicly accessible genomes would later be those of James Watson and Venter. • Successful completion of a rough draft of the human genome by researchers at Celera Genomics and the Human Genome Project.
2001	The human genome sequence is reported in journals *Science* and *Nature.*
2002	Conclusion of an era of very rapid shotgun sequencing of major genomes during which those of the mouse, chimpanzee, dog and hundreds of other species were all sequenced.
2003	Celera and the NIH successfully conclude the sequencing of the human genome.
2004	FDA approves the first monoclonal antibody with antiangiogenic properties (i.e., inhibiting blood vessel formation or angiogenesis) for cancer therapy. • FDA approves a DNA microarray analysis system.
2006	FDA approves a recombinant vaccine against the human papillomavirus. • Discovery of the three-dimensional (3D) structure of HIV.
2007	Researchers discover how to use human skin cells to produce embryonic stem cells.
2008	Venter replicates a bacterium's genetic structure completely from laboratory chemicals, taking a step nearer to generating the world's first living artificial organism.
2008	Development of first synthetic DNA for gene therapy by Japanese researchers.
2009	Discovery of three new genes connected with Alzheimer's disease. • FDA approves the first clinical trial by means of embryonic stem cells.
2010	FDA sanctions modified prostate cancer medicine for enhancing the capability of a patient's immune cells to distinguish and attack cancer cells. • FDA approves osteoporosis treatment based on genetic investigation. • Replication of a synthetic genome demonstrated by Craig Venter. He showed duplication can be done alone.
2011	First stem cells developed organ (trachea) is transplanted into a human recipient. • 3D printing technology is used for "skin-printing" for the first time. • FDA approves the first cord blood therapy.
2012	FDA issues Draft of Regulations for bio-similar drugs.
2013	CRISPR-Cas is used in human genome editing.
2014	Integrative Microbiome Project launched. • Embryonic stem cells for human skin are cloned for the first time.
2015	Oncology oriented gene therapy is approved in both US and Europe for the first time.
2017	Completion of the Human Microbiome Project.
2018	Human eggs are grown in a laboratory for the first time. • USFDA approves first-ever drug based on RNAi. • Chinese scientist announces existence of gene-edited babies for the first time.
2019	CRISPR technology used to edit human genes to treat cancer. • Prime editing technology for gene edition is introduced.
2020	RNA and DNA based vaccines against Corona virus approved by the United States, United Kingdom and other countries

(Table 1.1 is a modified version of that from Bhatia, 2018)

The scope of biotechnology ranges from tools and techniques for examination of the biological system itself for health, basic mechanism, refitting, and so on, to the creation of new molecules, drugs, cosmetics, and much more. Biomimicry is becoming an important source of technology for bio-engineering. DNA sequencing and other biomarker technologies have become part of the criminal justice system. One's individual genome is being used for devising medical treatment.

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Plant extracts are being used to prepare nanomaterials from metals for medical, agriculture, and information technology applications. Biotechnology is limited not just to utility applications, but is also an integral part of basic research on biological systems. Its application ranges from dealing with subtle mental issues to whole bodies and on to environment concerns. Thorough understanding of the basic principles is thus vital to practical applications of such technologies and their future development.

Technology of every kind is basically the application of techniques and biotechnology (also being a field of technology) is all about techniques and their applications. However, given its source and use in biological science, it encompasses physics, chemistry, biology, engineering, computer, socio-economics, business, and the environment. Therefore, it is not sufficient to learn only techniques in a unidimensional way. Proficiency in biotechnology demands holistic learning. This book, therefore, presents not only practical technological protocols, but also fundamental principles. While it has not covered the entire spectrum of biotechnology, it still explores in depth, adequate aspects of the subject to enhance student learning and practicing.

1.2 Technology and Laboratory Practice

Biotechnology and associated courses like biochemistry, molecular biology, or cell biology include laboratory courses at the undergraduate and graduate levels. These are essential in developing proper understanding of chemical analysis and biological processes. Typical laboratory courses utilize "cookbook" experiments to introduce handling, analysis and interpretation of biological experiments using techniques borrowed from chemistry. Several variations have been introduced whereby more than one set of experiments have been integrated to teach protocol preparation, assay design, and interconnectedness of techniques (Hannan et al., 1999; Wolfsen et al., 1996). However, real research-based biotechnology laboratory courses have yet to fully develop. In the experience of the authors, this focus allows students to learn biotechnology skills in a context, and so it trains them for a better integration of chemical and biological techniques with research projects.

The biochemistry aspect of biotechnology encompasses a vast number of chemical processes involved in any biological phenomenon. Students generally need a high level of concentration and critical thinking to correlate several interconnected topics. While biological processes easily attract students' attention and enthusiasm out of, both, curiosity to learn about ourselves (as living beings) and a likely opportunity to improve the quality of life for society, the delicate nature of biological samples generally requires greater hands-on attention for students to learn and perform chemical analysis of biological samples. Generally, all chemical techniques are applicable to biological samples but they usually require substantial modifications and adaptations to be applied to, sufficiently, understand significant biological processes. Therefore, an advanced biochemistry laboratory course is necessary to introduce chemical techniques that are commonly used to analyze biological samples.

Biochemistry and/or biotechnology laboratory courses, especially at the undergraduate level, tend to be limited in their depth and are scattered in terms of their content, primarily because of the vast number of biochemical processes involving a wide range of substances (e.g., lipids, nucleic acids, carbohydrates, proteins) which are further complicated due to variations in organisms (e.g., bacteria, plants, animals). Biotechnology/biochemistry laboratory textbooks, currently available, describe

experiments such that a given "cookbook" experimental system is deemed enough for demonstrating a particular technique. For example, in a standard experimental biochemistry textbook, an enzyme kinetics experiment may involve egg lysozyme, hut for enzyme inhibition analysis it may be lactate dehydrogenase. In the same book, the gel filtration technique may consist of using immunoglobulin G as a biological sample. In such cases, while students may succeed in the "cookbook" exercise because a given biological system is the most suitable sample to demonstrate a technique, they lose the connection between different experimental exercises. Their ability to understand the design and execution of experiments remains limited, and their potential to utilize these techniques to solve any real-world problem remains minimal. An additional problem with biochemistry/biotechnology laboratory and biotechnology courses is that the lecture class material is not always easy to directly practice in the laboratory because the sophisticated instruments required are simply not there and neither is the substantial amount of time needed to complete experiments. One way to alleviate this is to teach a laboratory course based on a research topic that involves several commonly used cellular, molecular biological, immunochemical, and biochemical techniques. This book provides access to not only those techniques but also to the fundamental principles behind those techniques to enable a student to independently design research and learning projects. A brief summary of techniques employed in the various chapters, follows.

Chapter 2 on recombinant DNA and protein technology outlines the basic methods of genetic cloning, gene expression in various organisms, protein purification, and estimation. In addition to presenting the detailed scientific principles behind each of the technologies, advantages and pitfalls have also been pointed out to allow a student to assess their suitability for specific situations and/or examine the experimental data in view of those. Further strategies are pointed out to address any difficulties.

Chapter 3 on enzyme kinetics, proteomics, and mass spectrophotometry is primarily devoted to the characterization of proteins. The enzyme kinetics segment forms the bulk of this chapter, as this is one common functional technique that a biotechnologist must master, not simply to perform enzymatic activity assays but to understand various features of enzymes for practical applications, such as to devise inhibitor/activator use in bio-processing, conducting comparative evaluation of their function under different environmental conditions, and to understand the basic mechanics of operation for structure–function analysis. Adequate mathematical treatment and experimental precautions have been described in detail to provide students with the information they need for independent design and operation of a biochemical or biotechnological project. Proteomics is a field that examines proteins in space and time for their behavior and changes that may occur due to the biological processes. Mass spectrophotometry is a set of very highly sensitive methods for protein detection and identification, and thus has been detailed separately.

Chapter 4 covers analytical techniques that are readily available to examine biological molecules, both, small (pigments, hormones, metabolites, and such) as well as large (proteins, genetic material, and such). Such techniques can be used to either examine the characteristics of the molecules or their interactions in biologically relevant conditions. Numerous sedimentation, calorimetric, spectroscopic (UV/Vis, fluorescence, circular dichroism, and Fourier Transform Infrared or FT-IR) and electrophoresis techniques have been described in detail to understand the principles and applications for structure, stability, size, mobility, interactions, and biological activity. Examples of practical demonstrations are also provided for the instructors to engage students in the learning process.

Chapter 5 deals with molecular biology, which is perhaps a misnomer for molecular genetic biology, as biochemically speaking all the biological systems are based on molecules. However, recognizing that the genetic material is in fact the ultimate basis of all other molecules, biology of the genetic molecules is referred to as molecular biology. These molecules were also the first examples of molecular manipulation in the laboratory, and thus became a popular part of molecular biology. Techniques for molecular structures of DNA and RNA in isolated or complexed state with other biological molecules such as proteins: replication, transcription, mutations, recombinant gene expression, polymerase chain reaction (PCR), and reverse-transcription (RT) PCR for duplication and detection, genetic hybridization, electrophoretic mobility, blotting, nucleic acid sequencing, and such, have all been covered. Background information on genetic material, genes, and their discovery and role has been emphasized to enhance student ability to link the basic knowledge with the current experimental practices for developing projects.

Chapter 6 focuses on cell culture, which has emerged as a very useful technique for biotechnological exploration into microbial, plant, and animal systems. Cells provide an in situ environment to examine biological activities, for natural synthesis of biological molecules, cell–cell interactions, assays, organelle studies, metabolic experiments, developmental studies, and toxicity studies of therapeutics or poisons. Cell cultures of both prokaryotic and eukaryotic cells are possible now, including those of nerve cells. In eukaryotic cells, cancer cell lines are available as perpetual cultures convenient for most studies, but primary cell cultures are also possible to imitate natural cell growth and differentiation. Stem cell based development of cell culture allows preparation of cells and tissues of various kinds conveniently. Modern tools of light microscopy, electron microscopy, confocal microscopy, fluorescence microscopy, and atomic force microscopy allow detailed examination of cell structures, delivery of external molecules, and cell–cell tight junctions. Cell microchips with co-culturing options are becoming very useful for assays of drugs and for understanding cell–cell interactions, both physically and biochemically.

Chapter 7 on antibody technology primarily covers experimental uses of antibodies in immunochemical analysis and characterization encompassing topics ranging from immunoassays to immunotherapy. Basic information on antibodies, including monoclonal antibodies, is provided to let the reader grasp the variety of features special to these molecules which have been useful in unraveling the many facets of biological systems for detection, diagnostics, and therapy.

1.3 Pedagogical Strategy of Biochemical Technology Practice

There are numerous techniques in these six chapters which may be adopted for either a single experiment or as a series of related scientific questions. Such a series provides more realistic replication of real-life situations. For true imbibing of techniques, one has to attempt to use them to answer specific scientific questions, and each lab experiment needs to be part of that learning goal.

A Model

To illustrate, consider a research-based biochemical technology laboratory course that engages students in learning laboratory skills that are focused on solving a real science problem. A research

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problem can generally be chosen which is related to a significant biological question that can easily be understood by students with one semester of biochemistry and cell biology knowledge. And so, the project now being taken up relates to the analysis of glutathione-S-transferase (GST) of marine organisms from a local harbor which had been contaminated with polychlorinated biphenyls. In real-life, New Bedford harbor (in Massachusetts, USA) was a super-reservoir site for PCB pollution.

The enzyme exists in several isomeric forms which differ in isoelectric points that can be exploited for ion-exchange chromatography. Finally, it is inhibited by several polyaromaric hydrocarbons (PAHs), thus allowing varying kinetic analysis. We used the different biochemically relevant features of this enzyme to teach several biochemical techniques and to provide students with independent research projects for evaluating glutathione-S-transferases from different marine animals such as clams, oysters, quahogs, scallops, and other organisms. The availability of different organisms here, allows new projects to be formulated every year. By collaborating with a biology faculty to coordinate with one of their laboratories that ran a biochemistry laboratory course, we were able to conduct nucleic acid chemistry experiments on glutathione-S-transferases from local marine animals, and so, to understand the role of marine pollution in the genetic expression of this enzyme as well. Most students taking this course were quite enthusiastic about this approach. The approach can be applied in any part of the world because GST is ubiquitous in all organisms ranging from prokaryotes to mammals, and its gene expression is responsive to environmental conditions including pollution (Mannervik and Danielson, 1998; Prestera et al., 1993; Rushmore and Pickett, 1993; Vandkevaa et al., 1993). The great enthusiasm shown by students for this approach stemmed not just because they learnt laboratory techniques, but also because they felt they were contributing to solving a real local environmental problem.

Design

The course is designed to focus on the GST which is a detoxifying enzyme involved in the metabolism of PAHs, pesticides, herbicides, and other electrophilic xenobiotic compounds. The enzyme is known to catalyze the conjugation of glutathione to xenobiotics, which makes them water soluble so that they can be easily disposed of through further metabolism and excretion (Singh, 1999).

About two-thirds of the laboratory course incorporated ten advanced biochemical techniques. Since this project was connected to the PCB pollution that had drawn great publicity from local media, it caught the absolute attention of the students involved.

Strategy

The enzyme (GST) has several desirable features that make it particularly suitable to be used for an undergraduate/graduate project in a biochemical laboratory set-up. It is a relatively small protein (25 kDa) that exists as a dimer in solution. It is an enzyme whose genetic expression is induced by organic pollutants, including PCBs. It specifically binds to glutathione so that affinity chromatography (focused to analyze various chemical characteristics of the glutathione-S-transferase (GST)) may be used for its purification. The remaining third of the semester time, students could work on a project that involved application of all the newly acquired techniques to solve a biochemical problem that encompassed the same detoxifying enzyme. One particular year, students decided to analyze the enzyme from a local shellfish species, quahog, obtained from a superfund site for

PCB (polychlorinated biphenyls) pollution. Because of the link of the course to the local superfund environmental site-related problems, students found the whole course exciting and relevant.

In deciding such a project one has to keep several points in mind. Glutathione-S-transferases are well known to be important for biomedical reasons because of their relationship to cancer in animals and humans; and for environmental reasons, as GSTs are responsible for a large part of xenobiotic metabolism. The enzyme being a protein allows application of all protein techniques to study its characteristics. In literature, GSTs are classified based on their isoelectric points, thus providing students with further interesting reasons to determine the enzyme's isoelectric point (pI) so that they can classify it. Of course, pI is also used to strategize ion-exchange chromatography. The enzyme exists as an oligomer which allows students to observe differences in the molecular size determined with the use of sodium dodecyl sulfate polyacrylamide gel electrophoresis and size-exclusion chromatography. GSTs are two-substrate enzymes, and are inhibited by a variety of biological metabolites such as bilirubin, hemin, anthocyanin, and suchlike providing ample opportunity to explore various enzyme kinetic issues.

To integrate it further with student learning, a biology professor (Professor Robert Leamnson) who taught cell biology laboratory courses was roped in to introduce GST activity, gene cloning, and its expression. This exposed students to the genetic aspect of the protein, leading to greater familiarity with the subject, and to develop curiosity about the biochemical techniques regarding the enzyme.

Students need exposure to various techniques with different systems, and an integrated approach that includes analysis of systems provides contextual learning of the techniques. In the process, not only do students master the techniques that may be employed, but also learn how to interpret data for a biological system.

Table 1.2 Course outline and tentative schedule of a biochemical technology course

Week 1: *Introduction to the laboratory and literature search and buffer preparation*
Objective: Library literature search to obtain biochemically relevant information on glutathione-S-transferases. **Examples:** Group 1-Metabolic substrates of glutathione-S-transferases; Group 2-Effect of environmental factors on glutathione-S-transferases; Group 3-Role of glutathione-S-transferases in human or animal ageing; Group 4-Plant senescence and glutathione-S-transferases.
Week 2: *Spectroscopic determination of protein pK*
Objective: Demonstrate the use of absorption spectroscopy for pKa determination. Observe variation in the p.Ka of Tyr side chains in a protein (glutathione S-transferases) compared with those in free aqueous solution. Calculate the number of ionized Tyr residues in glutathione-S-transferases.
Week 3: *Protein estimation*
Objective: Use three different common methods of protein assay (Bradford, Lowry and spectroscopic) to determine absorption extinction coefficient of a protein, glutathione-S-transferases.
Week 4: *Discussion and catch-up*

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Table 1.2 *contd.*

Week 5: *Polyacrylamide gel electrophoresis*

Objective: Use sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SOS-PAGE) to estimate molecular weight of a protein, glutathione-S-transferases.

Week 6: *Isoelectric focusing of protein(s)*

Objective: Use isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE) to estimate p/ of a protein, and classify the given glutathione S-transferases.

Week 7: *Size exclusion chromatography*

Objective: Determine the native molecular weight of a protein, glutathione-S-transferases, using size exclusion column chromatography, and compare your results with the molecular weight obtained from SOS-PAGE.

Week 8: *Affinity chromatography*

Objective: Purification of glutathione-S-transferases using glutathione agarose affinity column chromatography. Obtain pure homogeneous glutathione S-transferases (GST) from oat plants or Tetrahymena (examples).

Week 9: *Immunochemical technique ELISA*

Objective: Examine the relationship between glutathione-S-transferases from two sources with a polyclonal antibody using enzyme linked immunosorbent assay (ELISA).

Week 10: *Enzyme kinetics*

***Objectives*:** Estimate Km and V"""" of glutathione S-transferases using glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates.

Weeks 11–15: *Isolation and biochemical characterization of glutathione-S-transferases from scallops, oats, quahogs or cranberries (examples)*

Objectives: Utilizing all the techniques learnt in the course, isolate, purify and characterize glutathione S-transferases for its absorption extinction coefficient, subunit structure, pI, enzyme kinetic parameters, and relationship to glutathione-S-transferases from other sources. Write your results in the form of a manuscript.

Problems and Solutions

While students have enjoyed the "integrated research approach" to the teaching of the biochemistry laboratory course in the past two years, they have repeatedly suggested that more time was required to complete the research project portion of the course. I have observed myself that while the "cookbook" set of course experiments works very well, application of the same techniques in the

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research project (where students have to design all the details themselves with only minimum input from the instructor) does not produce successful results, at least from students' first attempts. With limited time available in the semester, students in the past have been able to succeed in applying their techniques only when they continued their experiments over part of the summer months. Therefore, a second semester of the course not only allows students to conclude their incomplete projects but also allows for additional techniques to be taught to expand their learning. Several critical aspects of modern biochemistry, nucleic acid analysis and manipulation through molecular biology techniques that are not covered in a single semester biochemistry laboratory course due to the lack of time can be included with an additional semester of the course. To elaborate, techniques such as DNA isolation, cloning, polymerase chain reaction, and recombinant gene expression have become common in workplaces as well as in graduate research. The monomeric subunit of the GST has a size of 25–30 kDa, which is small enough to present advantages for genetic manipulation. Additionally, since the GST gene expression is responsive to environmental conditions, experiments related to gene expression can be designed using this system.

1.4 Laboratory Safety

General Laboratory Practices and Procedures

Due to the wide variety of experiments listed in this text it would be cumbersome to roster each specific chemical danger here. Therefore, dangers inherent to each exercise will be listed in the experiments in each chapter. The following safety precautions are a generalized list that will be encountered in each of the exercises. Individuals involved in biochemical experimentation should be intimately familiar with the below mentioned precautions.

General Precautions

1. Wear safety goggles at all times in the laboratory. For biochemical experimentation regular eyeglasses do not provide sufficient protection from either chemical hazards or broken glassware. Recently, the American Chemical Society has approved the use of contact lenses but only when worn in combination with safety goggles. The contact/goggle combination is important because most modern eyewash stations are not able to remove chemicals trapped behind contacts.
2. Proper clothing should be worn in the laboratory at all times. Long sleeve shirts, long pants and full shoes are the best choice. Skin protection will be at a maximum if such garments are covered with a full lab coat/apron.
3. Make use of the fume hoods for handling volatile and/or hazardous chemicals. When handling the chemicals, gloves should be worn to protect the hands.
4. Dispose of solid and liquid waste in containers, which are properly labeled. Notify the instructor if any of the waste containers are full or damaged. If you are not sure where to dispose of the waste ask your instructor for help.
5. Get acquainted with the layout of the lab, paying special attention to the fire extinguishers, emergency eye wash stations, first aid kits and the nearest emergency phone. Knowledge of the location of this apparatus may help save your life and prevent injury to others.

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6. Never eat or drink in the laboratory area. Contamination of food or drink can take place without your knowledge. Dropping a reagent bottle on the lab floor may cause a toxic substance to spatter into your food or drink. Because the biochemistry lab usually involves long hours of analysis it is sometimes unavoidable for lab time to overlap with meal-time. If you must eat or drink something during the laboratory period leave the food or drink outside the laboratory. A short break to eat or drink will not be detrimental to the outcome of your experiments.

General Laboratory Courtesy

Like most teaching laboratories, the biochemistry laboratory can be a confusing crowded place for the new student. A lack of experience with the chemicals involved in the biochemistry laboratory can cause time consuming mishaps. Therefore, all biochemistry laboratory students, experienced or inexperienced, should observe the following laboratory courtesies:

1. Prior to the laboratory period the student should read the experimental protocol thoroughly, noting any questions for the instructor. As confusing as the biochemistry laboratory is to the new student it becomes many fold more confusing when the student has no idea what comes next.
2. When preparing reagents for your use from a common stock solution, be sure to return the stock solution to its proper storage place. Many chemicals used in biochemistry experiments degrade rapidly below 4°C. If you are preparing a mixture from a purchased chemical stock, check the label on the reagent bottle for the proper storage procedures.
3. After using common laboratory equipment, micropipettes, distilled water bottles, timers, etc., return them to their proper place immediately after cleaning. Many biochemistry laboratory courses do not have enough funding to provide equipment such as micropipettes to each student. Therefore, it is imperative for events to flow smoothly during the experiments; that common equipment be cleaned and returned as soon as possible.
4. Use only as much of the chemicals supplied to you as you need. In order to cut costs and produce less environmental waste many instructors prepare only required quantity of the common reagents for the experiment at hand. Use of excessive amounts of common reagents may cause a delay in the experiment for the others in the course, while they wait for more reagents to be prepared by the instructor.
5. Never insert the tip of your pipet/micropipetter into a common reagent bottle. This practice can easily spread contamination throughout laboratory, thus ruining many hours of work by yourself and other students.
6. After each lab period, clean your work area and any glassware that you used. After cleaning used glassware and rinsing with tap water rinse the glassware with distilled water and place it in a strainer to dry overnight.

1.5 Biosafety and Biosafety Levels

Biotechnology practices necessarily involve biological samples, which could be hazardous to health. Therefore, biosafety protocols have been developed by various organizations, including the US Centers for Disease Control and Prevention (CDC). The biosafety information provided here is largely based on CDC protocols.

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Biosafety

The definition of biosafety is as follows: It includes the set of safety precautions which reduce a laboratorian's risk of exposure to potentially hazardous chemicals and infectious microbes. Additionally, it limits the contamination of the work environment and, ultimately, the community.

Biosafety Levels (BSLs)

There are four biosafety levels consisting of measures for containment of microbes and biological agents. Levels of biosafety are determined by infectivity, severity of disease, and transmissibility of microbes and biological samples. This also include: the nature of the work conducted, origin of the microbe, the agent and route of exposure. Based on these criteria BSL-1 to BSL-4 are defined (Figure 1.1). Each biosafety level builds on the controls of the level below it.

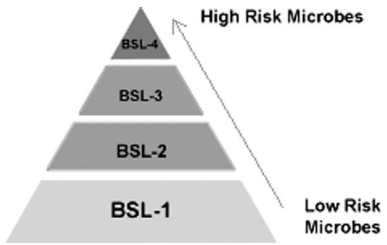

Fig. 1.1 Biosafety levels.

Every microbiology laboratory, regardless of biosafety level, follows standard microbiological practices, such as no mouth pipetting, labeling all the reagents, washing hands, sterilizing equipment, disinfecting the work areas, and so on.

Each biosafety level has its own specific containment controls that are required for the following:

BSL-1 Laboratory

A lab is designated a BSL-1 when the microbes there are not known to consistently cause disease in healthy adults and present minimal potential hazard to laboratorians and the environment. An example of a microbe that is typically worked with at a BSL-1 is a nonpathogenic strain of *E. coli*.

Specific considerations for a BSL-1 laboratory include the following (Figure 1.2):

Fig. 1.2 BSL-1 type open workplace and personal protective equipment (PPE).

Laboratory Practices

- Standard microbiological practices are followed.
- Work can be performed on an open lab bench or table (A).

Safety Equipment

- Personal protective equipment (B), (lab coats, gloves, eye protection) are worn as needed.

Facility Construction

- A sink must be available for hand washing.
- The lab should have doors to separate the working space from the rest of the facility.

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BSL-2

BSL-2 builds upon BSL-1 for a lab that works with microbes that pose moderate hazards to laboratorians and the environment. The microbes are typically *indigenous* and associated with diseases of varying severity. An example of a microbe that is typically worked with at a BSL-2 laboratory is *Staphylococcus aureus*.

In addition to BSL-1 practices, BSL-2 laboratories have the following containment requirements (Figure 1.3):

Laboratory Practices

- Access to the laboratory is restricted when work is being conducted.

Safety Equipment

- Appropriate personal protective equipment (PPE) (A) is worn, including lab coats and gloves. Eye protection and face shields can also be worn, as needed.
- All procedures that can cause infection from aerosols or splashes are performed within a biological safety cabinet (BSC) (B).
- An autoclave or an alternative method of decontamination is available for proper disposals.

Fig. 1.3 BSL-2; working with personal protective equipment (PPE) in a safety cabinet.

Facility Construction

- The laboratory has self-closing doors.
- A sink and eyewash are readily available.

BSL-3

BSL-3 builds upon the containment requirements of BSL-2 for working with the microbes that can be either indigenous or exotic, and that can cause serious or potentially lethal disease through respiratory transmission. Respiratory transmission is the inhalation route of exposure. One example of a microbe that is typically worked with in a BSL-3 laboratory is *Mycobacterium tuberculosis*, the bacteria that causes tuberculosis.

In addition to BSL-2 requirements, BSL-3 laboratories have the following containment requirements (Figure 1.4):

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Laboratory Practices

- Laboratorians are under medical surveillance and might receive immunizations for microbes they work with.
- Access to the laboratory is restricted and controlled at all times.

Fig. 1.4 BSL-3 type workplace - note extra PPE and contained safety cabinet.

Safety Equipment

- Appropriate PPE must be worn, and respirators might be required (A).
- All work with microbes must be performed within an appropriate biological safety cabinet or BSC (B).

Facility Construction

- A hands-free sink and eyewash are available near the exit.
- Exhaust air cannot be recirculated, and the laboratory must have sustained directional airflow by drawing air into the laboratory from clean areas towards potentially contaminated areas.
- Entrance to the lab is through two sets of self-closing and locking doors (C).

BSL-4

BSL-4 builds upon the containment requirements of BSL-3 and is the highest level of biological safety. There are a small number of BSL-4 labs in the United States and around the world. The microbes in a BSL-4 lab are dangerous and exotic, posing a high risk of aerosol-transmitted infections. Infections caused by these microbes are frequently fatal and without treatment or vaccines. Two examples of microbes worked within a BSL-4 laboratory include Ebola and Marburg viruses.

In addition to BSL-3 considerations, BSL-4 laboratories have the following containment requirements (Figure 1.5):

Fig. 1.5 BSL-4 workplace - note positive pressure body suit (PPE) in a Class III BSC.

Laboratory Practices

- Change clothing before entering.
- Shower upon exiting.
- Decontaminate all materials before exiting.

Safety Equipment

- All work with the microbe must be performed within an appropriate Class III BSC, or by wearing a full body, air-supplied, positive pressure (A) suit.

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Facility Construction

- The laboratory is in a separate building or in an isolated and restricted zone of the building. The laboratory has dedicated supply and exhaust air, as well as vacuum lines and decontamination systems.

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