Biotechnology:
Its Evolution, Application, and Environmental Implications

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INTRODUCTION

Biotechnology is a fascinating and rapidly evolving technology. In the Commonwealth of Massachusetts, the biotechnology sector has grown swiftly within the last decade. According to the Massachusetts Biotechnology Council, there were 215 biotechnology companies in Massachusetts in 1998, while there were only 100 companies operating in 1988.

The present paper, written for the Office of Technical Assistance for Toxics Use Reduction (OTA) to develop expertise in the area of biotechnology, is organized into three parts: historical evolution, applications and environmental issues.

In the first part, after defining the term "biotechnology" from different perspectives, the historical evolution of biotechnology is presented. This is followed by a description of the modern techniques of biotechnology. A review of the historical evolution of biotechnology reveals that it was the traditional microbial fermentation techniques, such as brewing, baking and cheese making, and the work of Louis Pasteur in the late 19th century which identified a number of bacteria and their functions, that laid the foundation for future advances in biotechnology.

The modern phase of biotechnology, which draws heavily on advances in Genetic Engineering, began with the discovery of the nature of DNA (deoxyribonucleic acid) by a group of scientists in the early 1950s. Modern biotechnology, using recombinant DNA (rDNA) technology, involves incorporating a gene from one organism into the DNA of another organism; and thereby managing the reproduction of living organisms or the ability of an organism to reproduce a desired product. Then, the replication process can produce large quantities of the desired product.

The second part of the paper is focused on the application of biotechnology to various industries. Advanced techniques of biotechnology have a wide range of applications, and have benefited many industrial sectors such as medical and pharmaceutical, food and agricultural, chemical, energy and mining industries.

In the fields of medicine and pharmacology, break-through advances have been achieved in the area of therapeutics and diagnostics. Using modern techniques of biotechnology, human insulin has already been produced to replace the insulin manufactured from animal sources. Notable successes have also been realized in the development of technologies such as protein therapy and gene therapy. Genzyme Corporation, a leading biotechnology company, has developed a biotherapeutic product and a protein replacement therapy for the treatment of Gaucher disease patients. Furthermore, this company in collaboration with the Howard Hughes Medical Institute has carried out the first successful human clinical experiment of gene therapy for cystic fibrosis.

The agricultural sector has also gained from advances in biotechnology. Using rDNA technology, agricultural products with slower ripening and extended shelf life properties have been developed. Considerable biological research is also being conducted to reduce the dependency of plants on chemical fertilizers, to replace dangerous chemical pesticides with biopesticides, and to develop genetically engineered plants having internal resistance to drought, frost, insect pests and infestation.

As far as chemical industries are concerned, biological research is geared toward developing advanced

technologies for the production of specialty chemicals such as amino acids, enzymes, polysaccharides, vitamins, food additives, and flavors. Optically pure amino acids have been produced using microbial and enzymatic synthesis. Also, an attempt has been made to improve the yield of an enzyme by transferring its gene encoding to a microorganism capable of producing the enzyme in larger quantities. Microbial production of polysaccharides is another area of interest for biological research. Some microbial polysaccharides, such as xanthin gum, have already found commercial use in food processing industries.

The application of biotechnology to the field of energy revolves around the idea that using available and abundant sources of biomass would generate a renewable and environmentally less hazardous source of energy. Furthermore, modern techniques of biotechnology are being tested to enhance oil recovery. This is due to biological research that has identified a fatty substance produced by bacterium that lowers the viscosity of crude oil. The injection of this substance into oil wells could help enhance oil recovery.

The mining industry constitutes another area of interest for the application of biotechnology. Utilizing microorganisms to leach metals from mines, it is hoped that the environmental and health hazards associated with traditional mining methods would be reduced.

The third part of the present paper treats environmental issues. Here, the dual potential impact of biotechnology on the environment is investigated. In addition to the potential benefits noted above for the industrial sectors, biotechnology is capable of conferring potential benefits on the environment through advances in the pollution control techniques of bioremediation and biotreatment. The biodegradation of a number of environmental contaminants and toxic substances has already been achieved. Utilizing appropriate enzymes or microbes capable of degrading specific compounds, biotechnology can detoxify waste effluents prior to their release to the environment, and clean up contaminated sites efficiently.

The third part of this paper also examines the risks to the environment potentially posed by biotechnology. The adverse potential impacts include accidental release of genetically altered microorganisms into the environment, and the use of toxic substances and the generation of toxic byproducts by biotechnology laboratories and industries.

A number of toxic chemicals including solvents, cytotoxic agents and acids are customarily used; and biotechnology laboratories and industries generate biological, radioactive, and solid byproducts.

PART I: DEFINITION, HISTORICAL EVOLUTION AND MODERN BIOTECHNOLOGY

I-1: DEFINITION

Various definitions are given for the term *biotechnology*. Biotechnology, an abbreviation of "biological technology", has been defined as "the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services." [1] The United States Office of Technology Assessment has defined biotechnology as "any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals or to develop microorganisms for specific uses."[2] The definition given by the American Chemical Society involves "the use of living organisms to carry out chemical processes or to manufacture products."[3] Biotechnology industries themselves view biotechnology as the commercialization of modern biology and define biotechnology "as the application of technology to biology, the latter being the study of living organisms and vital processes." [4]

What all these definitions relate is that biotechnology is the application of biological systems (animal, plant and microbial) to industries, agriculture and environment to produce better goods and services and enhance the well being of mankind.

It is important to note that prior to the popularity of the all-embracing term *biotechnology*, most biologically-based technology was defined by terms such as applied microbiology, applied biochemistry, enzyme technology, bioengineering, applied genetics and applied biology. In fact, biotechnology, by encompassing all biologically based technologies, and by relying on knowledge assembled and methods developed by many of these disciplines, can be considered as a group of inter-related technologies which are applicable to a broad range of industries – manufacturing as well as agriculture.

I-2: HISTORICAL EVOLUTION

A study of the historical evolution of biotechnology reveals that microbial fermentation for food and beverage production marked the beginnings of the field's evolution.

In the early periods, prior to 1800, by simply following rules of thumb, accidental discoveries by mankind were incorporated into daily life. These discoveries included biological processes such as the use of yeast in baking and brewing and enzymes in cheese making and leather tanning. However, these traditional fermentation industries were carried out under anaerobic conditions only and had little process control.

The nineteenth century saw some considerable advancement starting with the work of Louis Pasteur. It was Pasteur who, by identifying a number of bacteria and their functions, laid the foundation for the

future development of modern applied microbiology and, hence, much of biotechnology.

To identify some unwanted microbes that were spoiling the fermentation of beer, Pasteur put forward his "germ theory." He discovered that microbes were responsible for fermentation and different microbes gave different products. He also introduced some basic improvements in the approach to fermentation. The first improvement involved pasteurization. In this process, which bears his name, the liquid (wine, beer or milk) was heated and held at a temperature just below its boiling point. This resulted in killing off most of the bacteria contained in the liquid and thereby helped postpone the onset of unwanted fermentation that made milk or beer go sour. Pasteur's second improvement was to recognize the importance of oxygen to fermentation. He realized that careful aeration of the fermentation containers would lead to better product.[5] This realization brought about the change in industrial processes of fermentation from anaerobic (the conversion of plant carbohydrates to useful chemical products by microbes in the absence of oxygen) to aerobic (living organisms get energy from the controlled oxidation of organic compounds to carbon dioxide and water).

The latter years of the nineteenth century also saw advances in medical microbiology. The work of Robert Koch (1843-1910) greatly contributed to the recognition of the role of bacteria in human disease and the understanding of the immune system and the application of protective vaccines. In the 1860's Gregor Mendel discovered the genetic basis of heredity in his experimental work with peas.

Developments in the early years of the twentieth century included the production of industrial chemicals by fermentation such as the microbiological manufacture of acetone for explosives; the large-scale production of penicillin (the first antibiotic used for the treatment of infectious diseases) by aseptic fermentation systems; and the production of streptomycin as a consequence of Waksman's work. The work of Waksman was of special interest since he set out to screen microorganisms isolated from the soil for antimicrobial properties. Since then, many pharmaceutical companies have employed this strategy successfully in the isolation of numerous compounds, many from soil streptomycetes.[6]

Many products of fermentation became commercialized in this period. They included a number of amino acids, produced by aerobic microbiological processes, nucleotide, enzymes, vitamins and the gibberelins. Furthermore, microbial transformation of plant steroids was used to form mammalian hormones. This period also witnessed the development of an activated sludge process for mineralizing organic waste. The treatment of wastes by anaerobic digestion by mixed micro-organisms produced biogas (methane and CO2) which became an important source of energy generation.

In the 1960s, a number of chemical companies began research to develop processes for making single cell protein (SCP) from hydrocarbons, methane and methanol. Using methanol-utilizing bacterium, a foodstuff called Pruteen was manufactured in Europe.[7]

Today, still making use of traditional microbial techniques, the fermentation and cell culture industries employ highly advanced computers for control of temperature, aeration and stirring to create the optimum conditions for production. Controlled and large-scale continuous fermentation has assumed notable importance.

I-3: MODERN BIOTECHNOLOGY

The development of techniques of "gene splicing" or "genetic engineering" and the discovery of the nature of deoxyribonucleic acid (DNA) by Watson, Crick, Franklin and Wilkins in the early 1950's laid the foundation for the new and modern biotechnology.

Although genetic research (applied microbiology) had been in existence for many years, the methods used were rudimentary. Using soil samples, pieces of animal or plant tissues, yeast or mold, cultures were made by "seeding" on a surface of jellified broth containing agar. When microbes came into contact with the agar surface, "colonies" were developed. These colonies were then isolated and tested for useful properties. These techniques, in spite of being primitive, led to important discoveries. In fact, penicillin was discovered in this way. Also, people who were involved in the breeding of animals or cultivation of plants were aware of the "mutation" technique. This technique is used to breed an animal or cultivate a plant that has characteristics not passed on to it by its predacessors.[8]

The new genetic research makes use of recombinant DNA (rDNA) technology. Genetic engineering is involved in incorporating a gene from one organism into the genetic material of another organism and thereby modifying the traits of the latter organism.

In order to grasp how this specific modification and transfer of genetic materials from one organism to another may occur, an explanation of the cellular processes is necessary.[9]

All living organisms are made up of cells. While microbes are single cells, humans are composed of billions of cells. The outer layer of cells, called the membrane, has the function of regulating the passage of materials into and out of the cell.

The cell membrane encompasses a fluid, known as cytoplasm, which is composed of tiny structures called organelles. These tiny structures perform the most essential functions such as digestion, respiration and reproduction, thereby sustaining the life of the cell.

The largest cellular organelle is known as the "nucleus." The genetic material (DNA) or hereditary information is stored in the nucleus. It is this genetic material or DNA that constitutes the core in the study of genetic engineering and, hence, biotechnology. Within the nucleus, the genetic material (DNA) is organized into units called chromosomes. There are 46 chromosomes in each cell of the human body.

DNA (deoxyribonucleic acid): DNA (the genetic material) stores all genetic information about a living thing. All genetic messages are encoded within DNA molecules.

DNA takes the form of a long double-stranded helix. The DNA's genetic information determines the nature of its host organism and how that organism functions.

There are three chemical components that make up DNA: sugar molecules (the deoxyribose);

phosphate groups; and nitrogenous bases. The simplest DNA unit (with one sugar joined to a nitrogenous base and a phosphate group) is called a nucleotide. Each nucleotide is similar in structure with the exception that each may contain a different one of the four nitrogenous bases: adenine, thymine, cytosine, and guanine. They are abbreviated as A, T, C and G.

The structure of a DNA molecule is such that the sugar and phosphate components of the nucleotide alternately bond and form two strands. A nitrogenous base on one strand pairs up with another base on the opposite strand. The restriction imposed is that A only pairs with T and C only with G.

The DNA in one human cell contains about two billion base pairs, indicating the tremendous volume of information stored in the DNA. It is important to note that a nucleotide does not function alone. Rather, it is the entire ensemble of nucleotides and, in particular, the specific sequence in which they are linked that determines the structure and function of an organism.

Genes: A gene is a sequence of nucleotide in a DNA molecule. Scientists have discovered that a chromosome may contain 50,000 genes, each having a specific function to perform. Genes are responsible for determining hereditary traits.

DNA Replication: The structure of a DNA - a double helix - assists in the replication process. Replication occurs when the twin strands of DNA separate and form two new double helixes. Enzymes facilitate this separation by breaking the hydrogen bonds that hold the nitrogenous base together. Since the cytoplasm of the cell contains free-floating nucleotide, and due to the restriction that A can only pair up with T and C with G, each side of the separated DNA replicate or reproduces itself.

Recent scientific achievements have enabled scientists to use restrictive enzymes (chemical scissors) to cut the DNA molecule at specific nucleotide sequences.

RNA (ribonucleic acid): DNA, however, does more than just replicate itself. As A. Crafts-Lighty has explained in his book, Formation Sources of Biotechnology:

> By a complex biochemical process, the sequence of bases allows DNA to synthesize other quite different biological substances. The first of these is ribonucleic acid (RNA). This is a chain of nucleotide (a "polynucleotide") with a structure very similar to DNA, with the exception that the nucleotide components of RNA all have an extra oxygen atom on their sugar (ribose) part making them ribonucleotide and the base thymine (T) is replaced by a base called uracil (abbreviated U). RNA polymers are made using DNA as a foundation. The double-stranded DNA separates over a short portion of its length, while a single-stranded RNA chain of a complementary base sequence is synthesized, in a process called "transcription." This "messenger" RNA (mRNA) is then used by the cell to direct other syntheses. Each group of three RNA bases (a "codon") is specifically recognized by another type of RNA, known as "transfer" RNA (tRNA), which allows the cell to translate the RNA and produce protein molecules. Proteins are another type of biological polymers but they are built up out of amino acids. There are about twenty different common naturally occurring kinds of amino acids.
>
> Proteins are very important to cells because they provide much of the structure of cells and some can catalyze chemical reactions. Proteins with such catalytic functions are called enzymes.[10]

Recombinant DNA Technology: As stated above, recombinant DNA (rDNA) technology simply involves the incorporation of a gene from one organism into the DNA of another organism. By manipulating the tiny pieces that make up the DNA molecule, biotechnologists are able to manage the reproduction of living organisms and enhance the ability of an organism to produce a desired substance. Furthermore, by the replication process, large quantities of substances in trace amounts can be produced.

In rDNA technology, four steps are involved:

1. Identifying the gene that provides instruction for the production of the desired substance;
2. Isolating the gene by the use of a restriction enzyme and placing it in another DNA;
3. Transferring the recombined DNA strands into proper host organisms such as bacteria; and
4. Cloning many copies of the host organism.

Scientists have used the amino acid sequence of the protein, which a gene produces, to identify an unknown gene. By examining this sequence, scientists have been able to guess the possible nucleotide sequence of the gene that codes for the protein. Then, a genetic probe is constructed to locate the desired nucleotide sequence or gene on the chromosome. This genetic probe, which is a sequence of nucleotide, is synthesized in the laboratory and labeled with a radioactive marker. When this synthetic genetic probe is mixed with cellular DNA that contains the desired gene, the probe will "hook on " to that part of the natural gene that matches the probe. The gene can then be identified by the probe's radioactive label.

The identified gene is spliced into a special kind of bacterial DNA called a plasmid. Plasmids are a circular form of DNA found in the cytoplasm of bacteria. The gene is spliced into the plasmid with the help of a group of enzymes called DNA ligase. These proteins seal together the ends of DNA segments. The resulting "recombined" DNA is then inserted into the host organism. The plasmid acts as a vector – that is, a means of transporting DNA into another cell.

PART II: INDUSTRIAL APPLICATIONS OF BIOTECHNOLOGY

Biotechnology industries are involved in research and development (R&D), and/or the production of medical and pharmaceutical, food and agricultural products, animal health care and chemical products, using advanced scientific methods and techniques of biotechnology.

II-1 ADVANCED TECHNIQUES OF BIOTECHNOLOGY

Advanced techniques of biotechnology may be summarized as:

Genetic Engineering: Involves methods for creating new trails at the cellular level in plants, animals, microorganisms and human. It includes recombinant DNA, plasmid transfer and protoplast fusion.
Hybridoma Technology: Consists of methods for producing very pure and specific antibodies in quantities not previously available, such as monoclonal antibodies.
Plant Tissue Culturing: Includes methods for manipulating and propagating plants and plant cell genetic traits.
Immobilized Whole Cells and Subcellular Components: Seeks practical ways to exploit the biochemistry and physics of cells and their components.
Bioreactors and Support Systems: Includes advanced methods for conducting fermentation and other bioconversions on a large scale such as continuous fermentation, advanced separation and purification methods, sensor and control technology systems.

According to the Massachusetts Biotechnology Directory, published in 1994 by the Massachusetts Biotechnology Council (MBC), there are 172 biotechnology companies in the New England area (second only to San Francisco, with 192 companies) of which 149 companies are located in the Commonwealth of Massachusetts. These companies employ an estimated 17,600 people.

Of the 149 companies located in Massachusetts, 37% are involved in medical therapeutics, 23% in clinical diagnostics, 21% in supporting equipment and/or services, 10% in specialty chemicals, 4% in veterinary, 3% in environmental production and 2% in agriculture.

II-2: MEDICAL AND PHARMACEUTICAL INDUSTRIES

The recent application of biotechnology to medicine and pharmacology has already revolutionized the diagnosis and treatment of human diseases.

The advanced techniques of biotechnology, which are applicable to medicine and pharmacology, include the use of rDNA technology for the production of insulin, vaccines, and interferon; DNA probes, monoclonal antibodies and gene therapy. Furthermore, modern techniques of biotechnology are used to develop new drugs, called "biologics," which are derived from biological sources such as glands or blood.

Insulin: In the fields of medicine and pharmacology, the initial application of advanced biotechnology has been in the production of human insulin for the treatment of diabetes. Prior to using modern biotechnology, animal insulin was extracted from beef and pork pancreas. Using human sequence insulin rather than that from animal sources does have an emotional appeal, and another consideration is the possibility that the supply of animal pancreases might act as a limiting factor on the availability of insulin. These two considerations led biotechnologists to use rDNA technology as the basis for human insulin.

Insulin in the body is produced in response to the presence of sugar in the blood stream. Although the gene that codes for the production of insulin is present in every cell of the body, it is only in pancreatic cells that it is "turned on." In other cells, suppressors regulate the insulin gene. In rDNA technology, only the gene from the pancreatic cells is inserted into E. coli (Escherichia coli – a common bacterium). The gene is identified based on the amino acid sequence of the protein.

The E. Coli, being a mono-cell and without the suppressor sequence, responds to the instructions of its new gene and produces insulin. Bacteria reproduce themselves very quickly, and within one day billions of bacteria can be cultured, each producing human insulin.

Vaccines: The idea behind vaccination as a means of immunization is that by injecting a weakened or dead pathogenic microbe into an individual, the body recognizes the antigen as foreign and stimulates antibodies to destroy the invading organisms. The antibodies are produced by white blood cells (B-cells). When exposed to a live pathogen in the future, these cells turn on antibody production and produce large amounts to protect against invading antigens.

It is not always possible for vaccines to be completely risk free. Since the entire genetic material of the pathogen is contained in vaccines, occasionally they cause the infectious disease that they intend to cure.

Using rDNA technology, scientists are trying to produce synthetic vaccines, which use only a part of a specific antigen of the pathogenic organism, thereby eliminating the disease causing capability of vaccines.[11]

Interferon: Interferons are a family of proteins that have two important biological effects – inhibition of cellular proliferation (potentially with anti-cancer action) and modulation of the immune system. Prior to using rDNA technology, interferon was obtained from human white blood cells. With the help of rDNA technology, "a human leucocyte interferon (gene 514) base pair long ago was synthesized, incorporated into a plasmid and subsequently cloned into E. coli." It is interesting to note that while natural interferon is a glycosylated protein, the interferon produced by rDNA technology is not.

DNA Probes: As described above, by using "restriction enzymes" scientists are able to cut DNA into fragments and devise DNA probes, and thereby identify defects and mutations that can cause diseases. DNA probes have been used in the diagnosis of the bacteria that cause periodontal (gum) disease, and a variety of genetic diseases such as Duchenne muscular dystrophy, cystic fibrosis and Huntington's disease.

Monoclonal Antibodies (Cell Fusion): Antibodies are proteins produced in our body as a defense mechanism against invasion of foreign substances (antigens) such as bacteria or viruses. The human body makes as many as 100 million different antibody molecules, each recognizing and binding to a specific antigen.

The aim of monoclonal antibody technology is to produce specific antibodies and to reproduce them in large quantities. Prior to the advent of this technology, antibodies were produced by injecting laboratory animals with an antigen, harvesting the antibodies from the animal's blood and then purifying them.

The monoclonal antibody technology, also called cell fusion, fuses two whole cells, combining their genetic material. Antigens are injected into a normal animal, thereby stimulating the production of antibodies. The antibodies carrying white blood cells are then removed and fused with rapidly growing tumor cells, taken from another animal, to form hybridomas. These hybridoma cells are cloned in order to produce large quantities of the desired monoclonal antibody.

Gene Therapy: The goal of gene therapies is to treat genetic disorders by replacing a specific defective gene with a healthy one, using rDNA technology. The healthy gene, it is hoped, will take over and produce the protein coded for by that gene.

Scientists are especially interested in using gene therapy to treat severe combined immunodeficiency disease (SCID). In this disease, the gene that codes for the enzyme adenosine deaminase (ADA) is defective. This causes the impairment of the immune system, meaning that the body's bone marrow cells become ineffective in producing functioning white blood cells to fight infection.

To treat SCID, a normal ADA gene is inserted into a virus, which is then combined with a patient's bone marrow cells. The virus, a vector, allows the normal ADA gene to enter into the bone marrow cells. The “repaired” cells is then inserted back into the bone marrow of the patient where they produce the missing enzyme ADA.[12]

II-3: FOOD AND AGRICULTURAL INDUSTRIES

The food and agricultural industries are another area that can greatly benefit from advances in biotechnology. The benefits that advanced techniques of biotechnology are capable of conferring on food and agricultural industries include the following:

- Development of genetically engineered plants which have internal resistance to drought, frost, insect pests and infestation;
- Reduction in dependency of plants on chemical fertilizers, and identification of alternatives to expensive fertilizers such as nitrogen fertilizers which require very large amounts of energy for their production;
- Replacement of dangerous chemical pesticides with biopesticides (microbial pesticides) to

manage and control the problem of pests;

- Reduction in the reliance on chemical treatments to control weeds by engineering herbicide tolerance into crops;
- Production of products that have high yield and enhanced nutritional value; and
- Development of novel biomass products as foodstuffs, using organisms such as algae, fungi, bacteria and yeast.

Prior to the use of rDNA technology, traditional methods such as crossbreeding were used to obtain hybrid species of plants or animals. This method, however, needed many plant or animal generations before the genes with desirable characteristics were brought together.

Today, using rDNA technology, the gene can be spliced into the DNA of the plant in a single generation. Also, with the help of rDNA technology, foreign genes can be introduced into plant genes, which could not be done by the cross breeding method.

A number of biotechnology companies using rDNA technology have been trying to develop food products with extended shelf life. Applying the modern techniques of biotechnology, Calgene Inc. in Davis, California has developed the FLAVR SAVR tomato. The FLAVR SAVR tomato has been genetically altered to delay the softening and decay that follows natural ripening, and to have an extended shelf life. This has been achieved by reversing one of the genes responsible for producing an enzyme that causes softening. The FLAVR SAVR tomato has been produced using vectors, which carry an antisense copy of the tomato polygalacturonase gene and a bacterial neomycin phosphotransferase gene with associated regulatory sequences.[13]

Calgene Inc. has also developed genetically engineered canola plants in southern Georgia. By inserting a thioesterase gene from the California Bay tree into the genome, Calgene Inc. has been able to produce canola oil seed crops with nearly 40% laurate. Lauric oils are an important raw material used in soaps and other personal care products. According to Calgene Inc., the plant will offer a reliable domestic supply of laurate for the U.S. manufacturers of soaps and detergents. At present, lauric oils, obtained primarily from coconut and palm kernel oils, are imported mostly from Southeast Asia.[14]

Also, DNA Plant Technology Corp. of Cinnaminson, New Jersey, in collaboration with ZENECA Group PLC of Great Britain, is in the process of developing genetically engineered slower ripening bananas. Like Calgene's FLAVR SAVR tomato, the gene that produces the hormone responsible for ripening will be suppressed. According to DNA Plant Technology Corp., this technique will allow the fruit to be left on the plant longer, improving flavor and nutritional value. It will also make possible the shipping of different varieties, such as a red banana that is crisper and starchier.[15]

In the field of agriculture, application of modern techniques of biotechnology has not been confined solely to plants. Transgenic techniques have been used to produce transgenic animals having improved disease resistance improved meat quality and quantity and useful proteins in milk.

The transformation method, using retroviruses, has been employed to produce transgenic animals. Recently, this method was tried for transformation of cells in goat udders, by infusion with a retrovirus

carrying a gene for growth hormone. When lactation was induced in these goats, the resulting milk contained large quantities of growth hormone. Thus, this method is effective for producing proteins of interest, whether they be small quantities of highly valuable proteins or larger quantities of proteins of lower value.[16]

Monsanto Corporation has used BST (Bovine Somato tropin) to boost milk production. BST is a growth hormone produced naturally by cows in small quantities. Using rDNA technology, by inserting the gene for BST production into bacteria, Monsanto Corp. has been able to produce commercial scale amounts of BST. The injection of BST into cows can increase milk production by as much as 15%.[17]

For many years chemical pesticides were used as weapons in the battle against insects, infestations and weeds. Scientists are attempting to control these problems biologically using microbial pesticides. They have identified several strains of bacteria, fungi and viruses that produce toxins detrimental to insects. Using rDNA technology, the genes that code for the production of these toxins could be cloned and inserted into bacteria that are normally present on crops. For example, Monsanto Corp has taken from the microorganism Bacillus Thuringiensis (BT) the gene that codes for production of a protein that kills insects and has transferred it into plants such as tomatoes.[18]

Also, Upjohn Company of Kalamazoo, Michigan developed a squash line (called ZW 20) that contains the coat protein genes of watermelon mosaic virus 2 (WMV2) and zucchini yellow mosaic virus (ZYMV). The modifications have demonstrated remarkable field resistance against the two viruses. The ZW-20 squash has been developed with the use of vectors, promoters and terminators from plant pathogenic sources.

Another accomplishment of biotechnology in the area of agriculture has been the development of insect resistant rice by Japanese scientists at Plantech Research Institute of Yokohama. Plantech has introduced a truncated deltaendotoxin gene crylA (b) of BT into a japonica rice.

The Transgenic rice plants efficiently express the BT gene. Under tests, R2 generation plants were exposed to two major rice insect pests, striped stem borer and leaf folder; a subsequent bioassay revealed that the plants expressing the BT gene protein proved more resistant to the pests than untransformed plants.[19]

Furthermore, to combat grapevine fanleaf virus (GFLV), scientists at LVMH, Inc., in Paris, France have used a bacterium vector to introduce a GFLV coat protein gene into a chardonnay grape variety. According to LVMH, Inc., the gene has been effectively transferred and the engineered grapevines have exhibited resistance to infection by the virus.[20]

Also, applying modern techniques of biotechnology, agricultural scientists have tried to introduce herbicide and frost resistant genes in plants. Researchers discovered that certain proteins, found in the bacteria Pseudomononas syringe that grow on the leaves of plants, are "ice-nucleation proteins" responsible for frost damage to the plant. Using rDNA technology, they were able to identify the genes that code for these ice crystal-forming proteins and delete them from the bacterium.[21]

To control weeds, chemical herbicides have come to play a significant role in agriculture. However, there are risks associated with the use of chemical herbicides. In addition to causing serious environmental problems due to chemical contaminants, chemical herbicides themselves have undesirable effects on nontarget organisms.

With the help of rDNA technology, scientists are hoping to genetically modify plants tolerant to herbicides. Herbicide tolerance can occur when the phytotoxic compound fails to be taken up by living tissue or is rendered nonphytotoxic by conjugation, hydrolysis, or another metabolic event (detoxification).

By using rDNA technology, the genes that code for the protein involved can be identified, isolated and modified by directed mutagenesis and introduced into plant cultivar to confer the herbicide-tolerant phenotype.[22]

To reduce dependency on chemical fertilizers, agricultural biotechnologists are conducting considerable research using genetic manipulation to increase the range of plants that can fix atmospheric nitrogen.

II-4: CHEMICAL INDUSTRIES:

Chemical industries are involved in the production of specialty chemicals such as amino acids, enzymes, polysaccharides, vitamins, sweeteners, food additives, flavors, fragrances, etc. These industries are also interested in converting biomass to produce specialty chemicals from either plants or biological wastes, such as those generated from agriculture and food processing.

Although advanced techniques of biotechnology have not yet played a significant role in chemical production, there are areas of chemical industries, however, where this technology can have substantial impact, such as the production of amino acids, enzymes and polysaccharides.

Manufacturers are particularly interested in the potential for producing existing and new chemicals at lower cost with reduced energy requirements and waste disposal problems. Biocatalytic chemical production has the added advantage of specificity, controllability, low temperature operation, environmental acceptability and simplicity. For example, much of the present organic chemical industry is based upon petroleum and most of the chemical intermediates generated are partial oxidation products. Specific, controlled, partial oxidation is difficult to achieve by conventional catalysis. By using microorganisms, this type of reaction can be easily realized.[23]

Amino Acids: Amino acids are the building blocks of proteins in animals, plants and microorganisms. Twenty different amino acids have been identified in proteins. Amino acids are also important as nutrients, seasonings, flavorings and precursors for cosmetics and pharmaceuticals. As nutritional supplements in foods, lysine and tryptophan are used to enrich vegetable protein. As precursors, amino acids are used for the manufacture of detergents, polyamino acids (used in synthetic fibers and films), polyurethane and agricultural chemicals.

Amino acids can be produced either by isolation from natural materials – from hydrolysis of plant proteins – or by chemical, microbial or enzymatic synthesis. Whereas chemical synthesis produces a racemic (optically inactive) product that may require additional resolution, microbial and enzymatic syntheses give rise to optically pure amino acids.

Commercially, amino acid producing bacteria have been used since the 1950s. With the help of biotechnology, strains have been subsequently improved genetically by the generation of auxotroph or regulatory mutants.[24]

Enzymes: Enzymes are biochemical catalysts and, as a specific class of proteins, constitute the most useful tools of biotechnology. Enzymes increase the speed or efficiency at which a chemical reaction takes place without being altered itself. Common uses of enzymes include the production of starch, detergents, meat tenderizers, high fructose corn syrup and cheese. Enzymes have certain characteristics, which the most significant ones are:
1. High molecular weight proteins (> 10,000 molecular weight);
2. Extremely efficient – drive reactions 10^8 to 10^{20} times faster than normal;
3. Highly specific;
4. Can be extracellular or intracellular; and
5. Coded for DNA.

Using modern techniques of biotechnology, scientists are attempting to improve the yield of an enzyme by transferring the encoding gene to a microorganism capable of producing the enzyme in larger amounts. Because they are large and fragile molecules, enzymes tend to change their nature when exposed to heat, solvents and other extreme conditions that characterize most industrial processes. To remedy these problems, the current research seeks to modify the genetic information coding for an enzyme in order to introduce new chemical properties into the molecule, such as new chemical bonds that stabilize its structure. [25]

Recently, the Recombinant BioCatalysis Inc. of Sharon Hill, Pennsylvania began commercially offering kits of enzymes, called CloneZymes, which may be tailored to do biocatalysis in specific chemical processes. According to the manufacturer, the DNA of enzymes is screened in order to isolate those that have desirable qualities such as resistance to higher temperature and organic solvents. The DNA of a selected enzyme is copied, inserted into a host organism, such as E. coli, and then produced by fermentation. The kits of enzymes offered by Recombinant BioCatalysis Inc., include:
1. Aminotransferases, for making chiral intermediates;
2. Esterases-Lipases, for resolving racemic mixtures of alcohol and carboxylic acids;
3. Glycosidases, which work on hydrolysis and synthesis of sugars; and
4. Phosphatases, for hydrolysis of phosphates.[26]

Polysaccharide: Polysaccharide has important application in the food, cosmetics, chemical, medical and oil industries. Polysaccharides are produced by yeast, fungi and bacteria. They are also naturally available as cellulose, lignin and chitin. Polysaccharides are used as lubricants, viscosifiers, flocculating and gelling agents in food processing, and for stabilizing liquid suspensions.

At present, significant research is being conducted to apply modern techniques of biotechnology to the production of microbial polysaccharide.

Using advanced biotechnology, current research is also directed toward the conversion of biomass feedstocks to fermentable substrates, such as cellulose to glucose. While this generally requires relatively expensive microbial enzymes (cellulose) possessing desirable characteristics such as thermostability and high activity, manufacturers are also able to combine the desirable characteristics of several less optimal cellulose enzyme producers using recombinant DNA technology. Many laboratories are involved in detection, identification, isolation and gene transfer for strain improvement.[27]

II-5: ENERGY INDUSTRIES

Biotechnology can play an important role in the production of fuels from organic matter via biomass conversion. The most prominent fuel presently, petroleum, is a nonrenewable source that poses environmental risks in its extraction and use. Biotechnology, by using available and abundant sources of biomass, could generate a renewable and less environmentally hazardous source of energy called bio-energy.

The biological processes involved in producing bio-energy include harvesting of energy (photosynthesis), improving of feedstock (biomass) and conversion to fuel (by fermentation). Advanced techniques of biotechnology, including the use of tissue culture, protoplast fusion, single gene transfer, haploid, radiation-treated pollen transfer, chemical mutagen, transmission of chloroplast genome, etc.[28] offer improved varieties of plants for increased productivity and improved microorganisms for the conversion processes. Bio-energy, in the form of methane gas, may also be produced by bacteria consuming sewage sludge in anaerobic conditions.

Modern techniques of biotechnology can also be used to enhance oil recovery. It is believed that conventional oil-extracting technologies are capable of extracting only 50% of the world's oil supply, while the remaining 50% is trapped in rock or is too thick to pump. To enhance the recovery of trapped oil, scientists have isolated a fatty substance produced by a bacterium that reduces the viscosity of crude oil. The injection of this substance into oil wells makes the pumping of thick oil possible. [29]

II-6: MINING INDUSTRIES

The environmental and health hazards associated with traditional mining technologies have led many mining industries in the past few years to turn to a more efficient and environmentally risk-free method for extracting minerals from ores that use micro-organisms to leach metals from mine dumps.

Currently, more than 10% of the copper produced in the United States is leached from ores by microorganisms. Also, microbial leaching to a more limited extent has been applied to extract uranium

from pyritic ores. Microbial mining has improved recovery rates and reduced costs.

The iron-oxidizing bacterium Thiobacillus Ferooxidans has been used for bacterium-catalyzed leaching. It is naturally present in certain sulfur-containing materials and sequesters energy by oxidizing inorganic materials, such as copper sulfide minerals. This process releases acid and an oxidizing solution of ferric ions, which can wash out metals from crude ores. Sulfuric acid-ferrous iron solution is applied to piles of crushed copper ore or mine waste, which encourages the growth of Thiobacillus Ferooxidans. As the bacteria oxidize the ore, copper is released. The sulfuric acid-ferrous iron solution is recycled.

With the help of rDNA technology, genetic modification of the iron-oxidizing leaching bacteria can produce micro-organisms with desirable characteristics such as resistance to toxic metals and metalloids (which inhibit microbial activity), fixation of atmospheric nitrogen, resistance to chloride and the ability to withstand high temperature conditions in mines.

Using rDNA technology, it may be possible to genetically engineer bacterial strains that are able to leach heavy metals such as mercury, cadmium and arsenic that poison normal microbes and slow the bioprocessing. In addition, an iron-oxidizing leaching microbe with the ability to utilize atmospheric nitrogen may possibly be engineered.[30]

To resolve the problem of high temperature, researchers are turning to thermophilic bacteria found in hot springs and around oceanic vents. These bacteria could function in a high temperature oxidative environment.[31]

PART III: ENVIRONMENTAL IMPLICATIONS OF BIOTECHNOLOGY

The beneficial impact of biotechnology could be the use of modern biotechnology techniques to control pollution and reduce or eliminate toxic substances. The adverse effects of biotechnology on the environment, on the other hand, could involve the inadvertent release of genetically altered microorganisms into the environment, as well as the use of toxic materials and the generation of toxic by-products by the biotechnology industries. This section assesses the potential benefits and risks that biotechnology is capable of imparting to the environment.

III-1: ENVIRONMENTAL PROTECTION

The application of biotechnology in the area of environmental protection is not a new phenomenon – for example, microorganisms have long been used in sewage treatment to eliminate solid particles. It was in the early part of the 20th century that an activated sludge process for mineralizing organic waste was first developed. Since then, this process has become more complex and sophisticated, incorporating many modern technologies. Meanwhile, the use of anaerobic digestion processes for the treatment of wastes, and the coincidental production of biogas (mainly methane and CO2), has become an important source of energy generation.

It is believed that recent development in biotechnology could play an increasingly important role in pollution prevention and toxic waste treatment via bioremediation, biotreatment and biofiltration.

III-1-1: Bioremediation

Bioremediation may be defined as a process by which microorganisms degrade certain hazardous and toxic substances. Alternatively, bioremediation may be considered as a process by which microbes metabolically transform toxic organic compounds into harmless byproducts. In most cases, bioremediation is considered to be safer that incineration or other treatment technologies, and according to EPA, the popularity of bioremediation is the most rapidly growing of the cleanup technologies.

Bioremediation is mostly an in-situ process, meaning that the treatment of contaminated groundwater and other subsurface contaminants is done at its original place without excavating the overlying soil. In in-situ bioremediation, necessary process components such as microorganisms, electron acceptors, nutrients, etc. are delivered to the site of contamination. In-situ equipment and systems generally include injection wells, extraction wells, wastewater treatment systems, pumps and instruments or containment systems.

The inherent advantages of bioremediation include:

- It is an ecologically sound and environmentally friendly process;
- It often results in a complete decomposition of organic contaminants to harmless forms;
- It is generally an environmentally non-disruptive process or results in a minimum site

disruption;
- It eliminates liabilities pertinent to environmental regulatory requirements; and
- It offers the prospect of a cost-effective solution.

As indicated above, there are a variety of systems by which the bioremediation process may be implemented. There are also many types of microorganisms that could initiate microbial degradation of contaminants or be used in biological treatment. These microorganisms include bacteria (single cell organisms characterized by prokaryotic cell types), fungi (such as yeast and molds), algae (including aquatic microorganisms) and protozoa (group of unicellular microorganisms).

Bioremediation may be achieved through two primary mechanisms: aerobic respiration and anaerobic respiration. In aerobic respiration external oxygen is used as the electron acceptor. In anaerobic respiration, external alternative molecules such as nitrate, sulfate, ferric iron, manganese, carbon dioxide or other compounds replace oxygen as the electron acceptor.

In general, microorganisms can be considered as biocatalytic agents. For food sources, microorganisms depend on organic or inorganic nutrients. For microbial cells to grow and reproduce, they require energy and carbon. Energy includes light (photosynthesis) and chemical oxidation (chemosynthesis). Carbon could be organic (heterotrophic) or inorganic (autotrophic).

Bioremediation also relies on the following conditions:

1. Naturally occurring microorganisms must be present in sufficient number and capable of degrading contaminants.
2. The compound to be remediated must be biodegradable.
3. For aerobic environments, the subsurface oxygen level should be augmented.
4. Moisture levels should be appropriate. Too much moisture inhibits gas diffusion into the soil, rendering the site anaerobic (eliminating aerobic microorganisms). Too little moisture in the soil prevents microbial activity and growth.
5. An adequate presence of nutrients, such as nitrogen, iron potassium, phosphorous or other elements, in relation to the requirements of microorganisms, is important in order to ensure proper microbial growth.
6. Soil pH should be maintained between pH 6-7.5.
7. Temperature should be conducive to the normal growth of microorganisms. Each microorganism has an optimal temperature range for growth.

Sometimes, bioremediation does not result in the complete mineralization of the targeted organic compound. It may instead lead to the partial degradation of the compound to a point sufficient to render it environmentally acceptable.

Advances in Bioremediation Technology

The ultimate goal of the bioremediation field is to use existing and newly emerging technologies to treat and clean up contaminated sites efficiently and with relatively low cost. Contamination of the environment may occur as a result of accidental spillage, improper usage, or illegal disposal of chemicals. Thus far, bioremediation technology has been successfully applied in the clean up of oil spills, biodegradation of creosote and pentachlorophenol (PCP) and petroleum hydrocarbons.

- The most outstanding example of microbial degradation of oil was the clean up of the Exxon Valdez crude oil spills in March 1989.
- Coal-tar creosote and pentachlorophenol (PCP), commonly used as wood preservatives, are major environmental contaminants. Coal-tar creosote is composed of polycyclic aromatic hydrocarbons (PAHs) (85%), phenols and N-, S-, and O-heterocycles. PCP is always used in association with chlorinated dioxins. Using soil microorganisms indigenous to creosote sites, scientists discovered it was possible to degrade a majority of the organic contaminants present in PCP and creosote-contaminated groundwater.[32]
- The technology for bioremediation of petroleum hydrocarbon was developed in 1972 and since then has been used in the treatment of contaminated soils and groundwater. By supplying microbes with enhanced levels of oxygen and nutrients, this bioremediation technology has proved to be effective in reducing hydrocarbon contamination.

Over the years, bioremediation technology has evolved. Originally, the technology used in-well aeration along with the high concentration of ammonium salts and ortho phosphates. However, the effectiveness of this method was limited by the oxygenation system and, as a result, sites with high degree of contamination could not effectively be remedied. To improve the oxygenation system, hydrogen peroxide was used as an oxygen carrier. The hydrogen peroxide system shortened the time of treatment and extended the range of bioremediation to more contaminated sites, but was still limited in its effectiveness on highly contaminated sites. At present, advanced aeration technologies such as soil vapor extraction (SVE), bioventing and air sparging or oxygen release compound (ORC) technology are being used and have proved effective in cleaning up of contaminated zones.

Soil Vapor Extraction: At many sites the limiting operating parameter for bioremediation is the presence of oxygen. Through soil vapor extraction, the oxygen limitation is removed and the biomass activity increased. The process requires installation of vapor extraction wells, collection lines, and an extraction blower. Off-gas treatment equipment may be required at some locations. The extraction rates are managed to optimize biodegradation without excessive stripping or drying of the soil matrix.

The Environmental Protection Agency (EPA), the U.S. Department of Defense (DOD), and the U.S. Department of Energy (DOE) have jointly undertaken a number of site remediation projects for the purpose of testing various bioremediation technologies. The following table shows a few of the available case studies on soil vapor extraction technology.

Table 1: Summary of Remediation Case Studies: Soil Vapor Extraction (SVE)

Site Name, Technology, Media & Project duration	**Contaminants Treated & Source of Contamination (Principal Contaminants)**	**Highlights & Results**
Commencement Bay, South Tacoma Channel Well 12A Superfund Site, WA. Technology: SVE Media: Soil (98,203 yd^3) Project Duration: Operational Since 8/92	Contaminant: Chlorinated Aliphatics Source: Leaks from storage drums (PCA)	On-site solvent recovery for off-gasses. Pilot-scale results indicated that 3 to 4lbs/day per well of VOC could be removed from the upper 30 feet of soil
Fairchild Semiconductor Corp. Superfund Site, CA Technology: SVE Media: Soil (42,000 yd^3) Project Duration: 1/89 - 4/90	Contaminants: Chlorinated Aliphatics & Non-chlorinated Aliphatics Source: Underground Storage Tank (TCA, Xylenes)	Rapid reductions in contaminant concentrations occurred during the first two months of operation. After 8 months, the clean up goal of less than 10 lbs./day was achieved.
Hastings Groundwater Contamination Superfund Site, Well # 3 Subsite, NE. Technology:SVE w/GAC Media:Soil (185,000 yd^3) Project Duration: 6/92-7/93	Contaminants: Chlorinated Aliphatics Source: Spill of grain Fumigants (CCl_4)	Relatively large quantity of soil treated using SVE.
Hill Air Force Base, Site 914, UT Technology: SVE with catalytic oxidation followed by bioventing. Media: Soil (5,000 yd^3) Project Duration: 10/88 - 12/90	Contaminants: BTEX and/or TPH Source: Spill (JP-4 fuel)	SVE used to reduce concentration of hydrocarbons; system converted from SVE to bioventing after one year of operation.
Verona Well Field Superfund Site (Thomas Solvent Raymond Road-Operable Unit #1),MI Technology:SVE w/catalytic oxidation and GAC) Media: Soil (26,700 yd^3) Project Duration: 3/88-5/92.	Contaminants: Chlorinated Aliphatics & Non-Chlorinated Aliphatics Sources: Underground Storage Tank (PCE, TCA, acetone, Toluene)	First Superfund application of SVE; catalytic oxidation replaced activated carbon due to higher than expected recoveries.

GAC - Granular Activated Carbon

BTEX - Benzene, toluene, Ethylbenzene, and Xylene

TPH - Total Petroleum Hydrocarbons

Source: 'Abstracts of Remediation Case Studies', 'Federal Remediation Technologies Roundtable', EPA-542-R-95-001, March 1995, PP 7 & 61-80.

Bioventing: Bioventing has been used to remediate contaminants that are biodegradable under aerobic conditions. This technology introduces air into unsaturated subsurface soils to provide oxygen, stimulating biodegradation of organic pollutants by aerobic microorganisms. Equipment used in bioventing consists of vertical injection wells, lateral trenches, piping networks, a blower or vacuum pump for aeration or gas extraction.

Bioventing technology, although potentially applicable to any aerobically degraded compound, has two practical limitations:

1. Compounds with a high vapor pressure volatilize too rapidly to be degraded with bioventing technology. Bioventing is most effective for middle distillate fuels that have low volatility and are aerobically degradable.
2. Hydrocarbons with high molecular weights take far too long to degrade with bioventing. The slow rate results in longer clean up time – frequently on the order of ten years or more – to reach standards acceptable under relevant environmental regulations.

The following table illustrates a number of remediation case studies, jointly undertaken by the EPA, DOD, and DOE, using bioventing technology:

Table 2: Summary of Remediation Case Studies: Bioventing

Site Name, Technology, Media & Project duration	**Contaminants Treated & Source of Contamination (Principal Contaminants)**	**Highlights & Results**
Eielson Air Force Base, AK Technology: Bioventing Media: Soil Project Duration: Operational since 7/91	Contaminants: BTEX and/or TPH Source: Spills and leaks (JP-4 fuel)	Preliminary results indicate that bioventing with soil warming achieves biodegradation year around in a subarctic environment.
Hill Air Force Base, Site 280, UT. Technology: Biovening Media: Soil Project Duration: Operation since 12/90	Contaminants: BTEX and/or TPH Source: Spills and releases (JP-4 fuel)	Respiration rate tests (4/91 to 11/93) indicate that hydrocarbon degradation is occurring. As of 11/92, soil gas TPH concentrations had been reduced from 11,200 mg/kg to below 2,600 mg/kg.
Lowry Air Force Base, Co Technology: Bioventing Media: Soil Project Duration: Operational since 8/92	Contaminants: BTEX and/or TPH Source: Underground Storage Tank (heating oil)	Bioventing used to treat residual petroleum contamination in an excavated area.

Source: 'Abstracts of Remediation Case Studies', 'Federal Remediation Technologies Roundtable', EPA-542-R-95-001, March 1995, PP 5 & 18-34.

Biosparging: Biosparging or air sparging technology injects air or pressurized oxygen into the saturated zone (groundwater). The air moves in channels from the injection point to the unsaturated (vadose) zone. As it passes through the saturated zone, the oxygen is dissolved into the water and stimulates biodegradation. In biosparging system, it is possible to remove the highly volatile compounds from groundwater and transport them to the vadose zone. There, the contaminant may biodegrade or may be removed using soil vapor extraction or bioventing techniques.[33]

Oxygen Release Compound (ORC): As noted above, moisture and nutrients are generally present in sufficient quantities for aerobic bioremediation to occur, but oxygen is often the limiting factor. To stimulate aerobic microbial activity and growth, additional oxygen is required. The Oxygen Release Compound (ORC), developed by Regenesis Bioremediation Products Co., releases oxygen to enhance in-situ aerobic bioremediation of dissolved phase hydrocarbons. ORC is solid, insoluble magnesium peroxide that slowly releases oxygen when hydrated. The by-products of the magnesium peroxide-water reaction are oxygen and ordinary magnesium hydroxide.

ORC is packaged in exchangeable filter socks. A string of ORC filter socks is laced together and lowered into a well through the length of the contaminated saturated zone where contact with groundwater will initiate the release of oxygen. ORC can also be used to form an oxygen barrier across a contaminated plume. A properly placed row of wells or a trench containing ORC will slowly release oxygen, enhance bioremediation, and cut off the plume in the oxygenated zone. ORC may be made into slurry to create permanent implants in the saturated zone, or mixed directly in soil to supply oxygen for the in-situ or ex-situ treatment of soil.[34]

Other Recent Development in Bioremediation Technology

Chlorinated hydrocarbons, used widely as solvents, are the most prevalent environmental contaminants. Generally, microbial degradation of these compounds has not been successful so far. In one instance, however, microorganisms that are able to utilize dichloromethane have been found and isolated; the pathway of degradation for these microorganisms is not yet fully understood. [35]

There are many chemical compounds, such as polychlorinated biphenyls (PCB) and other xenobiotic compounds, which are recalcitrant to biodegradation and inherently toxic to microbes. Unlike biogenic compounds, xenobiotic compounds are not produced through the normal metabolism of living organisms. To develop useful degradable microbial systems for these kinds of compounds, scientists are conducting research to locate naturally occurring communities of microorganisms that function cooperatively to destroy mixed-toxic contaminants.[36]

Intech One-Eighty Corporation of Utah has developed a technology for the degradation of organic contaminants based on white rot fungi (WRF). According to Intech Corporation, this represents a significant break-through in the bioremediation field. WRF species have the ability to

mineralize a number of recalcitrant environmental contaminants such as PCB and dioxins. The unique abilities of the WRF relate to their traditional role decomposing wood, which contains a durable polymer known as lignin. The lignin contains numerous substructures also found in common organic pollutants, phenolics, anisoles, aryl-o-ethers and biphenyls. Intech claims that in addition to posing no known health risks, WRF break downin treated soils once the substrate is exhausted, leaving a healthy soil and no long-term impact on the local ecology.[37]

Lynntech, Inc., of Texas has developed a new technology called Electrokinetic Remediation of Soil. This is an in-situ technology based on the electrically induced transport of contaminants in soil. According to Lynntech:

> An electric field applied between the electrode positioned in the soil induces the electrokinetic phenomena in soil which are utilized for:
>
> ?? Controlling the horizontal and/or vertical removal of contaminants from soils of variable hydraulic permeability and moisture content; and
>
> ?? Introduction and distribution of reagents into the soil which are utilized for in-situ contaminant degradation, in order to enhance the contaminant solubility, to immobilize them or to achieve an optimum in pH in soil during the treatment process.
>
> The method involves no excavation or off-site transportation of contaminated materials and can easily be combined with other remediation technologies. Soil decontamination is carried out using environmentally benign chemical additives. The electrokinetic transport can be applied in sandy as well as in clayey soil layers. Ions, including both contaminants and treatment agents present in the pore fluid, can be transported by electromigration only, i.e., with minimum liquid transport through the soil. This feature is especially important because it allows the electrokinetic contaminant transport to be efficiently implemented in vadose zone soils.[38]

Lynntech Inc., asserts that data collected from a clean up trial at the Army Munitions Plant in Redford, Virginia, where a lead contamination was treated in-situ, showed that approximately 60% of the total metal in the soil was removed after three months.[39]

The J.R. Simplot Company has developed an ex-situ bioremediation technology for biologically degrading nitroaromtic contaminants in soil such as dinoseb (2-sec-butyl-4, 6-dinitrophenol), an agricultural herbicide. In ex-situ bioremediation, the contaminants are usually transferred and treated in a reactor, which serves as a control system. The J.R. Simplot bioremediation technology uses an anaerobic consortium (a heterogeneous group of microorganisms that symbiotically exist without oxygen) to treat soils contaminated with nitroaromatic compounds. According to Simplot, the anaerobic degradation of nitroaromatics using Simplot technology does not result in the formation of potentially toxic polymerization products.

> Mixing a carbon source (a J.R. Simplot Company potato-processing by-product) with contaminated soil and then adding water and buffers to create a slurry. This prompts aerobic microorganisms to consume oxygen, thus creating anaerobic conditions in the treatment slurry. These conditions subsequently stimulate anaerobic microorganisms to consume toxins present in the slurry. The appropriate microorganisms are often indigenous to the treatment soil; however, treatment soil may also be inoculated with the necessary consortium to enhance degradation rates. For small soil volumes (less than 31 m^3), the treatment may take place in small, mobile

bioreactors. For larger soil volumes, treatment may take place in large modular bioreactors or line pits.[40]

According to J.R. Simplot Company, a full-scale demonstration of the technology was carried out at an airport where the soil was contaminated with dinoseb. Based on this demonstration, it was determined that "the J.R. Simplot ex-situ bioremediation technology can reduce levels of dinoseb in soil by >99.8% in less than 23 days at an average temperature of 18°C based on an average pre-treatment soil concentration of 23.3 mg/kg (dry basis) and a final post-treatment concentration below the detection limits of the analytical instrumentation."[41]

III-1-2: Biotreatment

Biotreatment involves the detoxification of waste effluents, particularly hazardous and toxic wastes, prior to their release to the environment by appropriate enzymes or microbes capable of degrading specific compounds.

Industrial wastes can be classified into two broad categories: those generated by biologically based industries such as food processing, and those generated by chemical industries.

Biological oxidation is used to treat wastes from biologically based industries, although the method is relatively expensive and does not assist in reducing the volume of dilute wastes from this source. To help minimize residue, enzymatic treatment has been suggested. For example, amylases have been used in the treatment of starch-containing food wastewater, and lactases have been used in the processing of dairy wastes. Whey, a by-product of cheese making, is a rich source of lactose. By enzymatic hydrolysis of lactose to glucose and galactose, commercial yeast has been produced. The system employed is the immobilized lactase system developed by **Corning Inc.** In this system, lactase enzyme is derived from Aspergillus niger.

Another group of hydrolases is proteases, which have been used successfully in fish waste processing and the processing of meat by-products. Utilizing bacterium-derived proteases developed by **Genencor Inc.**, nutritionally valued fish food is processed from fish wastes.

Oxido-reductases are another group of enzymes that catalyze oxidation and reduction reactions. Peroxidases are a kind of oxido-reductases, which have proven useful in the treatment of wastewater from food processing industries. It has been shown that using peroxidase and hydrogen peroxide can purify wastewater effluent; the enzyme causes the polymerization and the precipitation of phenolics out of the stream.

Furthermore, **Repligen Inc.**, has developed ligninases of peroxidase-type which have been demonstrated to effectively decolorize pulp mill effluent.[42]

Unlike the biologically based industries, the majority of wastes generated by the chemical industries require chemical or physical pretreatment prior to the application of conventional

biological effluent treatment.

Common chemical waste effluents include dyes and pigments released into the environment by the textile and dyestuffs industries. With the notable exception of cationic dyes and benzidine, most dyes and pigments are not considered toxic or carcinogenic to fish or mammals.

Microorganisms have been identified which are capable of degrading dyes of higher concentration. Azo-dye compounds can be degraded aerobically or anaerobically. A number of microorganisms have been found to possess non-specific enzymes that catalyze the reductive fission of the azo group.[43]

Envirogen Inc., of Lawrencevill, NJ has developed and introduced a number of biotreatment technologies since 1994. In November of that year, Envirogen successfully pilot tested a method for treating groundwater contaminated with fuel and trichlorethylene (TCE). This technology consists of a fluidized bed reactor (FBR) for the treatment of contaminants and an air stripper. The air stripper is designed to convert the TCE to a gaseous state for treatment in a gas base reactor (GBR). The FBR uses microorganisms growing on activated carbon to degrade contaminants as groundwater is pumped through the system. The carbon provides sites for microbial growth that result in high concentrations of biocatalyst in the reactor. Envirogen determined that the combination of benzene, toluene, xylene and ethylbenzene (BTEX) was acting as a co-substrate for the TCE degradation. The company claims that the FBR achieved removal rates greater than 97% for 1,2-dichlorobenzene (DCB), more than 95% for BTEX and 86% for TCE. The GBR managed to reduce TCE concentrations in contaminated air by an average of 75%.[44]

In 1995, Envirogen announced the development of a biological treatment technology that could be used to destroy gasoline oxygenate methyltert-butylether (MTBE). Since 1979, MTBE, a high-octane additive in gasoline, has become a significant groundwater contaminant due to leaking underground storage tanks, spills and improper disposal practices. Envirogen's technology is a membrane bioreactor (MBR) combining a suspended growth reactor and an ultrafiltration unit to achieve biomass separation from the bulk fluid. The MBR system can be operated either in an aerobic or anaerobic mode. Following biological oxidation and/or reduction, the contents of the reactors are pumped to the ultrafiltration membrane unit for solids/liquid separation.[45]

Another Envirogen biotreatment technology is designed to treat chloroform. Chloroform is mostly found in industrial waste gas streams and in groundwater due to its wide use as an industrial solvent. The technology utilizes a unique biocatalyst to convert chloroform into carbon dioxide and water. According to Envirogen, the biocatalyst can be maintained within a bioreactor to treat contaminated air or water and potentially may be added directly into contaminated groundwater. Envirogen asserts that this chloroform-degrading organism is the first microbe identified that can degrade chloroform without the need for methane gas to stimulate destruction of the pollutant.[46]

A biocatalytic process designed to reduce concentrations of alcohol contaminants in paper-treatment polymers by more than 90% to below detection levels has been developed and implemented by **Carbury Herne Ltd. (Cardiff, U.K.) and Hercules Inc. (Wilmington, Del)**. Halo-alcohols are byproducts of the reaction between the base enhancing chemicals (epichlorohydrin and polyaminoamide). The two most abundant halo-alcohols are 1,3-dichloro-2-propanol (DCP) and 3-chloro-1, 2-propanediol (CPD), whose detection limits are 1 ppm and 5 ppm respectively. The biocatalytic reaction takes place at near-ambient temperature and neutral pH. Selected strains of bacteria (agrobacterium tumefaciens and pseudomonas cepacia) use hydrolysis to convert the DCP to epichlorohydrin and then to CPD, which is converted to glycerol and finally broken down to chloride ions, carbon dioxide and biomass.[47]

III-1-3: Biofiltration

Biofiltration, developed fairly recently, is a biological air pollution control (APC) technology designed to treat off-gases containing biodegradable volatile organic compounds (VOCs) or inorganic air toxics. Although this APC technology has been used on a limited scale in the U.S. thus far [EPA established biofiltration as the best available control technology (BACT) for controlling air pollution], it has been successfully applied since the early 1980s in European countries, particularly in Germany and Holland. In these countries, biofilters are used to treat off-gases generated by industrial facilities (such as adhesive production, coating operations, chemical manufacturing, film coating, iron foundries and print shops), food processing industries (such as coffee roasting, coca roasting, fish frying, fish rendering, flavor and fragrance, pet food manufacturing, and slaughter houses), and waste treatment industries (such as industrial and residential waste water treatment plants, composting facilities, landfill gas extraction and waste oil recycling). The large volumes of off-gases emitted from these sources contain only low concentrations (typically less than 1,000 ppm methane) of the organic target pollutants. The primary application of an APC biofiltration system in food processing and waste treatment industries is to control odors, whereas for other industries the control of VOCs and air toxics are targeted.

According to Gero Leson (RMT, Inc., Santa Monica, CA) and Arthur M. Winter (Environmental Science and Engineering Program, University of California, Los Angeles, CA):

> A biofilter for control of air pollutants consists of one or more beds of biologically active materials, primarily mixtures based on compost, peat or soil. Filter beds are typically one meter in height. Contaminated off-gas is vented from the emitting source through the filter. Given sufficient residence time, the air contaminants will diffuse into a wet, biologically active layer (i.e., biofilm) that surrounds the filter particles. Aerobic degradation of the target pollutants will occur in the biofilm if microorganisms – mainly bacteria – are present that can metabolize them. End products from the complete biodegradation of air contaminants are CO2, water and microbial biomass. The oxidation of reduced sulfur compounds and chlorinated organic compounds also generate inorganic acids.
>
> Compost, usually produced from municipal wastes, wood chips, bark or leaves, has generally been the basis of filter material used in recent applications in Europe, although peat and heather-

mixtures have also been used. The biofilters originally built in the U.S. were mostly 'soil beds' for which biologically active mineral soils were used as filter materials.

The components needed for preconditioning of the off-gas, its transport to and distribution in the filter bed, account for the other main elements of a biofilter system. Heat exchangers to cool hot off-gases or filters for the removal of particulates may be required for certain types of emissions. Radial blowers are generally used to overcome the backpressure caused by the filter. The off-gas must also be saturated with water, since it would otherwise remove moisture from the filter material, resulting in drying of the bed, the death of most microorganisms and a total loss of control efficiency.[48]

Several groups of microorganisms are known to be involved in the degradation of air pollutants in biofilters, including bacteria, actinomycete, and fungi. Compost-based filter material typically shows significantly higher population densities of these organisms than soil and peat. Growth and metabolic activity of microorganisms in a filter depend primarily on the presence of dissolved oxygen in the biofilm, the absence of compounds that are toxic to microorganisms, the availability of nutrients, sufficient moisture, and suitable ranges for temperature and pH.[49]

It is recommended that off-gas temperatures be maintained between 20 and 40 degree centigrade (68 and 105 degrees Fahr.). As a guideline, a water use of 5-10 gallons per 100,000 sf, should be used, and the pH in compost filters needs to be between 7 and 8, a range preferred by bacteria and actinomycetes.[50]

III-2: TOXICS USE AND TOXIC BY-PRODUCTS GENERATION IN BIOTECHNOLOGY

As explained in the previous sections, advances in biotechnology offer enormous potential benefits. However, such practices could also affect society unfavorably if appropriate precautions are not taken.

III-2-1: Adverse Environmental Impacts

The potential adverse impacts of biotechnology may include risks to human health and environment.

Direct health risks may occur as a consequence of acute or chronic exposure to biological agents, toxic chemicals, and radioactive materials used in biotechnology R&D laboratories or industrial processing. For example, industrial biotechnology practices such as the recovery of fermentation products[51] may generate aerosols containing microorganisms that may cause disease when inhaled.

Indirect risks include the potential upset of ecological balance by the introduction of new organisms. The extent of the risks to the environment depends on the likelihood of release and on the severity of the consequences. Once released into the environment, the following consequences must be assessed:[52]

- Disruption of ecosystem function;
- Displacement of existing plants and animals;
- Reduction of biological diversity;
- Alteration of species composition; and
- Changes in climatic patterns.

An assessment of the environmental impact of accidental or inadvertent release, as C. Hussey has put it, involves "consideration of the host microorganism and the genetically modified organisms (GMO)."

> Relevant characteristics of the host include reproductive cycles, survival, stability of genetic traits, pathogenicity, virulence, infectivity, toxicity, host range, natural habitat and geographical distribution, involvement in environmental processes and interactions with other organisms. Characteristics of the GMO which should be evaluated include the nature of the genetic modification, function of the cloned gene(s), the nature and source of the vector, the amount of non-native DNA in the final construct, genetic stability, frequency of plasmid mobilization or other forms of genetic exchange, the level of expression of inserted genes and the activity of expressed protein, survival and multiplication in the environment, and techniques for monitoring persistence and detecting gene transfer. Risk assessment should also take into consideration the site where accidental release might occur, and details of the ecosystem at risk, including geographical and climatic features, social factors and possible methods for decontamination of the area in the event of accident.[53]

III-2-2: Toxics Use in Biotechnology

To understand the sorts of chemical compounds used in biotechnology industries, it is necessary to review the processes involved. A number of toxic chemicals, radioactive materials and biological agents are used in various processes of biotechnology research and industries, including:[54]

1. Separation and Purification;
2. Identification and Characterization;
3. Identification of the gene; and
4. Insertion of the gene.

(1) Separation and Purification isolates the desired product, e.g., protein, from the mixture of biological materials such as cells, sugars, and smaller molecules and culture media.

There are four techniques used in the purification process. They include chromatography, liquid-liquid extractions, gradient centrifugation and gel electrophoresis. In column chromatography separation, the solvent mobile commonly used is an aqueous buffer solution with a pH between 6 and 8, made up of chemicals such as phosphates. To purify biological products such as protein and DNA, high-performance liquid chromatography (HPLC) is utilized with acetonitrile serving as the HPLC solvent. In liquid-liquid extraction technique, the common solvent used to extract DNA or RNA is a mixture of phenol, chloroform and an aqueous buffer solution. Other solvents used include ethanol and isopropanol. Gradient centrifugation commonly utilizes sodium

chloride, sucrose and cesium chlorides as concentration gradients.[55]

Finally, gel electrophoresis involves passing a substance to be purified through an electric field. In biological research, polyacrylamide gels are used to separate protein and oligonucleotides, while agarose gels are used to purify DNA. Polyacrylamide is made from powdered acrylamide, which is considered a carcinogen. When polymerized with N,N,N,N,tetramethyl- ethylenediamine (TEMED) and ammonium persulfate to an aqueous solution of acrylamide, polyacrylamide is no longer considered toxic. To denature proteins and nucleotides, detergents such as sodium dodecyl sulfate and reducing agents such as beta-mercaptoethanol are used to break disulfide bonds. Also agents such as guanidinium chloride and guanidinium isothiocyanate are used in the purification of RNA to dissolve contaminating proteins.[56] For denaturing of RNA prior to electrophoresis, chemicals used include glyoxal-dimethylsulfoxide (DMSO) mixture, formaldehyde, and volatile mercury compounds such as methylmercuric hydroxide.

(2) Identification and Characterization determines that the desired product has been isolated. In biotechnology, radioactive isotopes, e.g., 32P, are used to monitor, label and detect DNA. With the help of scintillation counters, geiger counters and X-ray film, experiments are monitored using radiation.[57] In biological research, a UV spectrophotometer is also used to monitor experiments. Photographic film is used to detect fluorescent compounds. With the help of dyes, compounds on gels are identified. Dyes such as coomassie blue, methylene blue and azine, as well as silver, are used to stain protein and bacteria.

To identify the amino acid sequences that make up a protein, purified protein is treated with phenyl-isothiocyanate (PITC). This chemical reacts with an amino acid at the end of the chain. Hydrochloric acid (HCl) is used to separate this amino acid from the rest. This process is repeated several times in order to find out the amino acid sequences in a protein molecule. Solvents such as heptane and ethyl acetate are used to dissolve the amino acid. The dissolved amino acid is passed through an HPLC column using a 10 percent acetonitrile aqueous solution. The retention time on the HPLC column then identifies the amino acid. Using enzymes or cyanogen bromide, proteins are sometimes cleaved prior to sequencing. To determine the relative concentration of amino acids present, proteins are hydrolyzed in 6N HCl.

Since the amino acid sequence of a protein is determined by the DNA sequence of the gene that encodes the protein with a cell, a substantial amount of knowledge concerning the gene may be obtained via protein sequencing.

(3) Identification of the Gene Process is possible through the use of several different technologies. They include Polymerase Chain Reaction Technology (PCR), screening libraries, and DNA sequencing.

PCR is an enzyme-based technology that is used to amplify small amounts of genetic material. This technology uses a water-based buffer system, but an oligonucleotide (a fragment of DNA) is required to start the reaction.

The screening library method is also used to identify the DNA responsible for formation of a particular protein. Two types of screening libraries have been developed. Genomic libraries that contain the entire DNA of an organism, and Coding DNA (cDNA) libraries that keep only the DNA that encodes proteins. Isolating RNA creates the cDNA libraries. Solvents, denaturing agents, and a transcriptase enzyme are used in this isolation process. A specific genetic library is initially transferred to a bacteriophage. The bacteriophage, which is a virus, in turn, transfers the library to a bacterium, such as E. coli.

Oligonucleotide probes, obtained from a DNA synthesizer, are used to screen genetic libraries. The probes are labeled with a radioactive marker and denatured into single strands by heating the DNA. To lower the temperature and to protect the molecules from extreme heating, formamide is added to the reaction mixture.

The DNA sequence can be identified after a gene has been isolated. An enzymatic technique, called Sanger Dideoxy Sequencing, is used to split the DNA and create sequencing templates.

(4) Insertion of a Gene Process splices the identified gene into a special kind of bacterial DNA known as plasmids, with the help of a group of enzymes known as DNA ligases. Plasmids are a circular form of DNA that act as a means of transporting DNA into another organism. This rDNA is then inserted into the host organism, which may be bacteria, yeast, or mammalian cell culture.[58]

The following table summarizes biotechnology processes and the chemicals used in these processes:

Table 3: Biotechnology Processes and Chemicals Used

Processes	Chemicals Used
(1): Purification & Separation Process: Chromatography HPLC	Phosphates Silica gel Acetonitrile Triethylamine Trifluoroacetic acid
Liquid-Liquid Extraction	Phenol Chloroform Ethanol Isopropanol
Gradient Centrifugation	Sodium Chloride Sucrose Cesium Chloride

Gel Electrophoresis	Acetic acid Acrylimide Agarose gels Ammonium persulfate Betamercaptoethanol Dithiothreitol (DTT) Formaldehyde Guanidinium isothioscyanate Glyoxal/dimethylsulfoxide Methylthianine chloride Methylmercuric hydroxide Polyacrylamide gels Sodium dodecyl sulfate
(2): Identification Process: Protein Sequencing	Coommassie blue Methylene blue Butyl chloride Cyanogen bromide Ethyl acetate Heptane Hydrochloric acid Silver Phenyl isothiocyanat
(3): Identification of the Gene Process Screening Libraries DNA Sequencing	 Acetic anhydride Ammonia Iodine Methyl imidazole (dye) Tetrazole Thymidine Trichloroacetic acid Acetic acid Dimethyl sultate Dithiothreitol (DTT) Ethylene diamine-tetracetate Formamide Formic acid Hydrazine

Source: G. Frantz, et. al., and others, 'Toxics Use in Biotechnology', Capstone III Project, Tufts University 1992, and The EPA Computer Database, EPA Region I Library, Boston, MA

The following tables illustrate the chemicals used in biotechnology laboratories and industries and their toxicity status under US EPA SARA 313 (Superfund Amendments and Re-authorization Act) and/or CERCLA (Comprehensive Environmental Response, Compensation and Liability Act):

Table 4: Chemicals Used in Biotechnology and Listed Reportable under US EPA SARA 313 and/or CERCLA

Chemicals Used	Regulations Applied
Acetic acid	(C) (Concentration of 12% or less not reportable)
Acetic anhydride	(C)
Acetone	(C) & (313)
Acetonitrile	(C) & (313)
Acrylamide	(C) & (313)
Ammonia	(C) & (313)
Ammonium sulfate (solution)	(313)
Arsenic Compounds	(C)
Chloroform	(C) & (313)
Cumene	(C) & (313)
Cyanogen bromide	(C)
Dichloromethane	(C) & (313)
Dichlorodifluoromethane	(C) & (313)
Di(2-Ethylhexyl) Phthalate	(C) & (313)
Dimethylformamide	(C) & (313)
Dimethyl sulfate	(C) & (313)
Ethylene Glycol	(C) & (313)
Ethylenediamine-tetraacetate	(C) & (313)
Ethyl acetate	(C) & (313)
Formaldehyde	(C) & (313)
Formic acid	(C) & (313)
Hydrazine	(C) & (313)

Hydrochloric acid	(C) & (313)
Methanol	(C) & (313)
Methylene chloride	(C) & (313)
Methyl isobutyl ketone	(C) & (313)
2-Methoxyethanol	(313)
Naphthalene	(C) & (313)
n-Butyl alcohol	(C) & (313)
N-Methyl-2-pyrrolidone	(313)
Phenol	(C) & (313)
Piperidine	(C)
Phosphoric acid	(C) & (313)
Silver	(C) & (313)
Sodium hydroxide	(C)
Sulfuric acid	(C) & (313)
Triethylamine	(C) & (313)
Toluene	(C) & (313)
Trichlorfon	(C) & (313)
Trimethylamine	(C)
Xylene	(C) & (313)

(C): CERCLA Chemicals

(313): US EPA SARA 313 Reportable Chemicals

Source: Toxics Use Reduction, 1996 Form S Reporting Package, Appendix B-ALP-1-26. EPA Computer Database, EPA Region I, Boston, MA, and Capstone III Project 1992.

Table 5: Chemicals Used in Biotechnology and not listed Reportable Under SARA31and/or CERCLA :

Chemicals Used:
Actinomycine
Adenine
Agarose
Alkaline phosphates
Butyl chloride
Cesium chloride
Coommassie blue
Cytosine
Diethyl ether
Dimethylsulfoxide
Dithiothreitol
Ethanol
Ethidium bromide
Formamide
Gluteraldehyde
Glyoxal
Guanidinium chloride
Guanidinium isothioscyanate
Guanine
Heptane
Iodine
Isopropanol
Beta-Mercaptoethanol
Methylthianine chloride (Methylene blue)
Methyl imidazole (dye)
Methylmercuric hyroroxide
Monomer
N-Methylmorpholine

N,N,N',N'-tetramethyl ethylenediamine
Piperazine
Phenyl isothiocyanate
Polyethylene glycol
Sodium acetate
Sodium chloride
Sodium dodecylsulfate
Sucrose
Tetrahydrofuran
Tetrazole
Thiols
Thymidine
Trichloroacetic acid
Trifluoroacetic acid

Source: Toxic Use Reduction, 1996 Form S Reporting Packages, Appendix B-ALP-1-26, The EPA Computer Database, EPA Region I, Boston, MA and Capstone III Project, 1992.

Data on the actual usage of toxic chemicals in biotechnology research and industrial applications is limited. Sources indicate that most biotechnology companies and laboratories are small users of toxic chemicals, [59] and the data compiled by U.S. EPA support this notion. Likely, these companies fall under the category of SQTUs (Small Quantity Toxics Users). The SQTUs, defined by TURA (Toxics Use Reduction Act), are those facilities which employ less than 10 full-time employees; are classified within certain SIC codes (Standard Industrial Classification: 10-14, 20-39, 40, 44-51, 72, 73, 75, 76); and use less than 10,000 pounds of substances regulated under Title III of the U.S. EPA SARA and CERCLA.

A survey of the industry is necessary to acquire accurate and reliable information and data on the volume of specific toxic chemicals used by biotechnology companies. The accuracy and the scope of the survey would, of course, depend on the willingness and cooperation of the relevant companies to respond, since many biotechnology companies and laboratories are highly protective of their scientific processes. No data summarizing chemical usage in biotechnology were available for this report.

III-2-3: Toxic Wastes and Byproduct Generation in Biotechnology

As indicated above, an array of toxic chemicals, radioactive materials and biological agents are used by biotechnology facilities. The wastes or byproducts generated by these facilities likely to include these chemical substances, materials and agents. Wastes might be in the form of discarded chemicals (container residuals), chemical mixtures and products of chemical reactives, waste effluent and solid waste.

In general, taking into account the various processes involved in biotechnology laboratories and industries, one can classify the hazardous wastes generated by these facilities into four broad categories:

1. Chemical Wastes: including solvents, reagents, buffers and other chemical compounds.
2. Biological Wastes: consisting of genetically engineered microorganism wastes, cell cultures, agar, animal wastes, and blood wastes.
3. Radioactive Wastes: including radioactive isotopes, and other low-level radioactive wastes.
4. Metallic Wastes: mostly silver, used in film processing and developing.

III-3: ENVIRONMENTAL REGULATIONS AND GUIDELINES APPLICABLE TO BIOTECHNOLOGY

Concern over the release to the environment of genetically engineered organisms (GEO) such as products of cell fusion, induced mutation and recombinant DNA molecules was expressed as early as 1973 by a group of scientists attending a Gordon Conference on nucleic acids. As a consequence of this concern, a National Academy of Science committee was appointed which in July 1974 published a letter in Nature and Science inviting the National Institutes of Health (NIH) to establish a Recombinant DNA Molecule Program Advisory Committee (RAC). The goal of the RAC was "to oversee a program to evaluate the potential biological and ecological hazards of such plasmids, to develop procedures to minimize their spread within human and other populations, and to devise guidelines for working with potentially hazardous DNA molecules."[60]

The RAC was set up in 1975 and began to draft the first NIH Guidelines for Recombinant DNA Research, which was published on July 7, 1976. The first Guidelines outlined levels of biological and physical containment presumed to be appropriate for a range of permissible experiments. Until 1979, the RAC was mostly concerned with in laboratory experiments under conditions of physical containment and devoted little attention to evaluating potential biological and ecological risks outside the laboratory.

The Revised Version of the Guidelines, which appeared on November 23, 1984, did provide "for both deliberate release and field testing. One section (III-A-2) specified the conditions for the deliberate release into the environment of an organism containing recombinant DNA. These included submission of relevant information on the proposed experiment to the NIH, the publication of the proposal in the Federal Register for 30 days of comments, review by the RAC,

approval by NIH, and approval of the local bio-safety committee."[61]

Furthermore, in 1984, in response to concern regarding the growing rate of research on genetically engineered organisms, the Biotechnology Science Coordinating Committee (BSCC), an advisory committee to the Office of Science and Technology Policy (OSTP), was established. On December 31, 1984, the BSCC published a Proposal for a Coordinated Framework for Regulation of Biotechnology in which laws, regulations and guidelines applicable to products of biotechnology were detailed. In 1986, the final version of the Coordinated Framework for Regulation of Biotechnology appeared. The BSCC also tried to provide a forum for coordinating issues in biotechnology and resolving disagreements among various federal agencies. Five agencies [EPA, FDA, USDA, NIH, and the National Science Foundation (NSF)] were each requested to establish a scientific advisory committee to review applications or related-biotechnology issues submitted to them.[62]

At present, there are many laws and regulations, requirements and guidelines designed to protect the health and safety of human beings and environment that are also applicable to biotechnology laboratories and industries and provide many safeguards. The specific application or relevancy of the regulations and guidelines depends on the type of materials such as chemical, biological; radioactive and metallic used by these industries. Several of the laws and regulations relevant to biotechnology are discussed in the following sections.

III-3-1: Toxic Substances Control Act (TSCA)

The Toxic Substances Control Act of 1976 authorizes the EPA to control the manufacturing, import, processing, use, and disposal of any chemical substance that is dangerous to human health or the environment. To identify and control toxic chemical hazards, the TSCA (40 CFR) entails a Chemical Substances Inventory list and has established threshold and disposal guidelines as well as cleanup procedures, based upon the volume and concentration of the spilled material, for many toxic and hazardous chemicals.

Section 5 of TSCA contains the pre-manufacture notification (PMN) provisions requiring any manufacturer or importer of a new chemical substance to submit a notification to EPA at least ninety days in advance of a chemical's production or importation. The main purpose of the PMN is to enable EPA to identify and list the substance on the TSCA Chemical Substances Inventory, and to assess its potential risks to human health and the environment.[63] Recently, however, the EPA has modified its biotechnology policy and asserted that "living microorganisms are sufficiently different from the traditional chemical substances reviewed under Section 5 of the Toxic Substances Control Act (TSCA) to warrant development of separate regulations and guidance." The new regulations under TSCA applicable to biotechnology submissions were published in the Federal Register on April 11, 1997 (62 FR 17910-17958). [65]

The regulation under which the TSCA Biotechnology Program functions is titled "Microbial Products of Biotechnology; Final Regulation Under the Toxic Substances Control Act." In accordance with this regulation, manufacturers are required to report certain information to EPA

before commencing the manufacture of new microorganisms. "New microorganisms" are intergeneric microorganisms that are not listed on the TSCA Inventory. Intergeneric microorganisms are those that have been formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera.

The term "intergeneric microorganism" refers to "a microorganism which contains a mobile genetic element which was first identified in a microorganism in a genus different from the recipient microorganism."[66] The TSCA Section 5 also applies to microorganisms that are imported or processed for commercial purposes.

Subpart D of part 725 established the reporting program for new microorganisms manufactured or imported for distribution into commerce and requires submission of a Microbial Commercial Activity Notice (MCAN) 90 days prior to initiating manufacture or import of the new microorganism. This subpart codifies the requirements for information to be included in the MCAN at S725.155 and S725.160. Any manufacturer, importer, or processor of a living microorganisms required to report under Section 5 of TSCA must file a MCAN with EPA, unless the activity is eligible for one of the specific exemptions.[67]

Subpart E of part 725 establishes the exemptions from full MCAN reporting for R&D activities. At S725.205 (b), EPA defines "commercial purposes" for R&D activities to include all R&D directly funded in whole or in part by a commercial entity, and all R&D activities, regardless of funding source, for which the researcher intends to obtain immediate or eventual commercial advantage. Subpart E establishes, at S725.232, a complete exemption from TSCA Section 5 for certain R&D activities that are conducted in contained structures and are subject to regulation by another Federal agency. Under S725.232, EPA has established a complete exemption from review, reporting and record keeping requirements for contained research conducted in compliance with the NIH guidelines. All other manufacturers conducting contained TSCA research and development activities may qualify for a more limited exemption under S725.234 and S725.235.[68]

Subpart E also establishes at S725.238 and S725.239 the TSCA Experimental Release Application (TERA) exemption process for R&D activities, primarily those involving intentional environmental release. EPA has revised requirements in S725.239 to limit the antibiotic resistance markers that may be used in the microorganisms eligible for the TERA exemption. For researchers conducting small-scale field tests with the two eligible microorganisms, *Bradyrhizobium japonicum* and *Rhizobium meliloti*, the final rule creates an exemption from EPA review, providing certain conditions are met. The field testing must occur on no more than 10 terrestrial acres; the introduced genetic material must comply with certain restrictions, and appropriate containment measures must be selected to limit dissemination.[69]

Subpart G establishes two exemptions from MCAN reporting for certain microorganisms used in closed systems. To quality for the Tier I exemption, a manufacturer must use one of the recipient organisms listed at S725.420, and implement specific physical containment and control technologies as noted in S725.422. In addition, the DNA introduced into the recipient

microorganisms must be well characterized, limited in size, poorly mobilizable, and free of certain sequences (see S725.421). In the Tier I exemption, if these three requirements are met, manufacturers only need to notify EPA that they are manufacturing a new microorganisms that qualifies for this exemption 10 days before commencing manufacture, and to keep certain records. A manufacturer is not required to wait for EPA approval before commencing manufacture.

A manufacturer who otherwise meets the conditions of the Tier I exemption may modify the specified containment restriction, but must submit a Tier II exemption notice under subpart G. The Tier II exemption requires manufacturers to submit an abbreviated notice describing the modified containment, and provides for a 45 day period during which EPA may review the proposed containment. The manufacturer may not proceed under this exemption until EPA approves the exemption.[70]

III-3-2: Food and Drug Administration (FDA) Regulations

The U.S. Food and Drug Administration promulgated stringent regulations to insure safety of new drugs. Drug producers are required to file applications and obtain approval of an Investigation New Drug (IND) Application by the FDA prior to clinical trials. Furthermore, after clinical trials and before manufacturing and marketing a new drug, the drug manufacturers must submit and receive FDA approval for a New Drug Application (NDA).

The FDA also emphasizes Good Manufacturing Practices (GMP), and has incorporated these practices in the Federal Food, Drug, and Cosmetic Act (FDCA). In accordance with GMP, "written production and process control procedures shall be followed in the execution of the various production and process control functions and shall be documented at the time of performance." Also, manufacturers must comply with record-keeping requirements and ensure that relevant records are readily available for examination by authorized FDA personnel during an inspection.

In July 1997, the FDA established a new guideline that has profound bearing on biotechnology companies. This applies only to specified biotechnology and specified synthetic biological products, including recombinant DNA-derived protein/polypeptide products approved under the Federal Food, Drug, and Cosmetic Act (FDCA) and complexes or conjugates of a drug with a monoclonal antibody approved under the FDCA, or biological products licensed under the Public Health Service (PHS) Act and outlined in 21 CFR 601.2(c).[71]

In accordance with this guidance:

> Section 314.70 and 601.12 of Title 21 of the Code of Federal Regulations (21 CFR 314.70 and 601.12) prescribe the requirements for the reporting to FDA of changes to the approved applications for licensed biological products and approved drug products.
>
> Under 601.12 and 314.70(g), a change to a product, production process, quality controls, equipment, or facilities is required to be reported to FDA in:
> 1. A supplement requiring approval prior to distribution;
> 2. A supplement at least 30 days prior to distribution of the product made using the change; or

3. In an annual report, depending on its potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product.[72]

For licensed biologics subject to 601.12, changes to a product package label, container label, and package insert require either:

1. Submission of a supplement with FDA approval needed prior to product distribution;
2. Submission of a supplement with product distribution allowed at the time of submission of the supplement; or
3. Submission of the final printed label in an annual report.[73]

In addition to the requirements in 21 CFR 601.12 and 314.70(g), an applicant making a change to an approved application must conform to other applicable law and regulations, including the current good manufacturing practice (CGMP) requirements of the FDCA (21 U.S.C. 351(a)(2)(B) and applicable regulations in 21 CFR parts 210, 211, 600 through 680 and 820.[74]

III-3-3: Occupational Safety and Health Administration (OSHA)

OSHA was established by the Occupational Safety and Health Act of 1970 as an agency within the Department of Labor. OSHA regulations intend to ensure the health and safety of employees and workers in industries and to eliminate or minimize exposure to toxic materials. Since various processes of biotechnology make use of biological agents, radioactive materials and toxic chemicals, biotechnology companies and research laboratories are also affected by OSHA regulations.

The OSHA regulations that are most relevant to biotechnology companies include Bloodborne Pathogens Standard (29 CFR 1910.1030), Hazard Communication Standard (29 CFR 1910.1200) and Laboratory Safety Procedures. The safeguards provided under these standards and procedures encompass proper labeling, handling, storing, packaging, and transporting of hazardous and pathogenic materials and waste disposal. They also require appropriate training programs for workers, and the communication of information concerning the potential hazards involved to workers.[75]

To enforce its standards, OSHA has been granted authorization to conduct workplace inspections and investigations, and to issue a citation if violations are found. OSHA is also empowered to impose penalties. "The maximum allowable penalty is $70,000 for each willful or repeated violation; and $7,000 for each serious or other-than-serious violation as well as $7,000 for each violation of the posting requirements and $7,000 for each day beyond a stated abatement date for failure to correct a violation."[76]

III-3-4: The Resource Conservation and Recovery Act (RCRA)

RCRA, legislated in 1976, and amended in 1984 to add Subtitle I governing underground storage tanks, addresses solid (Subtitle D) and hazardous (Subtitle C) waste management activities. Regulations promulgated under Subtitle C govern hazardous waste from the point of generation

to disposal ("from cradle to grave").

The bulk of wastes generated by biotechnology companies include solvents, acids, and bases. Solvents may be ignitable, or toxic, and corrosives. Therefore, the important regulatory standards under RCRA that have bearing on biotechnology companies consist of:

?? *Identification of Solid and Hazardous Wastes (ISHWs)* (40 CFR Part 261) sets out the procedures for determining whether the waste generated are considered hazardous waste, solid waste, or is exempted from regulation.

?? *Standard for Generators of Hazardous Waste (SGHW)* (40 CFR Part 262) specifies the obligations of hazardous waste generators, including acquiring an ID number, preparing a manifest, ensuring proper packaging and labeling, meeting standards for waste accumulation units, and record keeping and reporting.

?? *Land Disposal Restrictions (LDRs)* (40 CFR 268) deal with the disposal of hazardous waste on land. According to these regulations, hazardous wastes must meet LDR treatment standards prior to being disposed of at a RCRA land disposal unit such as a landfill. Biotechnology wastes including solvents, radioactive isotopes and acids are subject to the LDRs regulations.[77]

III-3-5: Clean Water Act

The primary objective of the Clean Water Act is to regulate the amount of chemicals and hazardous substances released by effluent discharges. EPA has categorized the effluent discharges into direct and indirect discharges, and developed the following guidelines to regulate them:

?? *National Pollutant Discharge Elimination System (NPDES):* The NPDES guideline (CWA S402) regulates direct discharges into a body of water. Direct discharges are from sources such as pipes and sewers. A facility that intends to discharge directly into the nation's water is required to obtain a NPDES permit. The permit contains industry-specific and technology-based limits. It also specifies what type of pollutants can be discharged, and their monitoring and reporting requirements.

In 1987 the CWA was amended to allow EPA to establish permit application regulations pertaining to the NPDES Storm Water Discharges. Under these regulations, a storm water discharge associated with an industrial activity requires a NPDES permit.

NPDES Storm Water Discharges cover eleven categories of industrial activity, defined by 40 CFR 122.26. "Six of the categories are defined by SIC codes while the other five are identified through narrative descriptions of the regulated industrial activity. If the primary SIC code of the facility is one of those identified in the regulations, the facility is subject to the storm-water permit application requirements. If any activity at a facility is covered by one of the five narrative categories, storm water discharges from those areas where the activities occur are subject to storm water discharge permit application requirements."[78]

?? *CWA S 307(b) - Publicly-Owned Treatment Works (POTWs)* The Clean Water Act Section 307(b) regulates the indirect discharge of pollutants to POTWs by "industrial users." Under this Section, the regulated facilities are required to meet certain pretreatment standards.

The goal of the pretreatment program is to protect municipal wastewater treatment plants from damage that may occur when hazardous, toxic, or other wastes are discharged into a sewer system and to protect the quality of sludge generated by these plants. Discharges to a POTW are regulated primarily by the POTW itself, rather than the State or EPA.

EPA has developed technology-based standards for industrial users of POTWs. Different standards apply to existing and new sources within each category. "Categorical" pretreatment standards applicable to an industry on a nationwide basis are developed by EPA. In addition, another kind of pretreatment standard, "local limits," are developed by the POTW in order to assist the POTW in achieving the effluent limitations in its NPDES permit.[79]

Since most biotechnology companies in Massachusetts discharge waste effluents into POTWs, the Massachusetts Water Resource Authority (MWRA) has regulated these discharges, like other industrial discharges, under 360 Code of Massachusetts Regulations (CMR) 2.00 and Sewer Use Regulations (360 CMR 10.00), and established permit requirements, pH neutralization, discharge limitation and pretreatment standards.

Furthermore, in accordance with "Specific Prohibition" (Section 10.023 of 360 CMR), discharge of the following biotechnology wastes to POTWs are prohibited by the MWRA:

> (1) Any radioactive waste or isotope with a half-life or concentration in excess of any limit established by federal or state law;
> (2) Any substance containing pathogenic organisms in such quantities as determined by local, state and/or federal law as hazardous to the public health or the environment, including but not limited to any 'Infectious or Physically Dangerous Medical or Biological Waste' as defined and identified by the Massachusetts Department of Public Health in its regulations.[80]

III-3-6: Massachusetts' Medical Wastes Regulations (105 CMR 480.00)

In the Commonwealth of Massachusetts, medical wastes, including biological wastes from biotechnology companies, are regulated by Section 105 of CMR 480.00. Based on these regulations, medical and biological wastes that are not sterilized must be labeled with the international biohazard symbol and red color. Sharp wastes must be segregated and labeled clearly so that the potential for punctures could be avoided. Records of temperature and dwell times for medical and biological wastes that are sterilized by generators prior to disposal must be kept for a period of three years. Section 105 of CMR 480.00 also requires that manifests should be used for shipping and trucking of wastes disposed off-site.[81]

III-3-7: Emergency Planning and Community Right-to-Know Act (EPCRA)

The Superfund Amendments and Re-authorization Act (SARA) of 1986 established EPCRA, also known as SARA Title III. The main objective of this regulation is "to improve community access to information about chemical hazards and to facilitate the development of chemical emergency response plans by State and local governments."

Section 313 of EPCRA "requires manufacturing facilities included in SIC codes 20 through 39 which have ten or more employees, and which manufacture, process, or otherwise use specified chemicals in amounts greater than threshold quantities, to submit an annual toxic chemical release report. This report, commonly known as the Form R, covers release and transfers of toxic chemicals to various facilities and environmental media, and allows EPA to compile the national Toxic Release Inventory (TRI) database."[82]

The threshold amount for manufacturing or processing a specified chemical is set at 25,000 pounds each year, and for a chemical "otherwise used," the threshold amount is 10,000 pounds per year. The releases and transfers reported on a Form R include:

- Emission of gases or particles to the air;
- Wastewater discharge into rivers, streams, and other bodies of water;
- Release to land on site including landfill, surface impoundment, land treatment, or other methods of land disposal;
- Disposal of wastes in underground injection wells;
- Transfers of wastewater to POTWs;
- Transfers of wastes to other off-site facilities for treatment, storage and disposal.

As stated above, most biotechnology companies and laboratories are considered to be small users of toxic chemicals, and as such they use chemicals in amounts smaller than threshold quantities

IV: CONCLUSIONS AND RECOMMENDATIONS

There is no doubt that progress in biotechnology can lead to break-through achievements in various fields such as human health care, animal heath care, food production and processing, generation of renewable and environmentally sound sources of energy, and less hazardous methods of mining. Furthermore, via bioremediation and biotreatment technologies, advances in biotechnology could play a remarkable role in pollution prevention and the reduction or elimination of hazardous and toxic substances.

However, without the application of stringent laws and regulations and the adoption of rational containment procedures and appropriate precautions, progress in biotechnology also has the potential to adversely affect human health and the environment. Hazards are posed by the use of genetically engineered microorganisms, cytotoxic agents, biological agents, hazardous chemicals and radioactive materials in biotechnology.

Since the primary objective of the Office of Technical Assistance (OTA) is to help manufacturing companies and other facilities develop and sustain Toxic Use Reduction (TUR) programs, it is logical that OTA believes biotechnology industries and facilities could also benefit from such preventive practices.

Keeping in mind the sensitivity of biological systems and laboratory procedures, and the technical complexity of the processes that might create barriers to an efficient application of TUR, the same strategies and procedures designed to assist other manufacturing facilities prevent pollution are applicable to biotechnology facilities as well. These strategies and procedures are discussed briefly in the following sections.

IV-1: GENERAL POLLUTION PREVENTION AND TOXIC USE REDUCTION PRACTICES

These general source reduction practices are not industry-specific, and are applicable to all manufacturing industries including biotechnology companies and laboratories. They are simple, inexpensive, and easy to execute. Their effective implementation could result in reducing waste generation, improving regulatory compliance, and more cost-effective operations.

IV-1-1: Establishing A Pollution Prevention Program

The first step toward ensuring compliance and minimizing or eliminating environmental and workers health liability is to establish an effective pollution prevention program within the facility.

The components of a successful pollution prevention program should include:

- Well-defined goals, objectives and priorities;
- Clearly-identified methods and options for meeting program goals;

- Management support and commitment;
- Creation of a task force to plan, coordinate, implement, monitor, and assess the program accomplishments;
- Involvement and cooperation of all employees and particularly those with direct contact with waste generation;
- Financial and non-financial incentives to attract effective involvement of employees.[83]

IV-1-2: Employee Awareness and Training

Employee awareness and training can play a significant role in reducing waste generation meeting the goals of a pollution prevention program. Specifically, facilities should ensure that employees are familiar with the following points:

- How and where waste is generated and how it can be minimized;
- How to perform pollution prevention techniques in the working area;
- How to handle hazardous process chemicals, and the health and safety advantages connected with proper handling;
- How to seek assistance in case a non-routine problem arises;
- How to execute preventive maintenance techniques that result in waste generation reduction.

Furthermore, employees should be made aware of environmental and health and safety rules and regulations, and the high cost of liability associated with non-compliance. The facility's measures and procedures for dealing with pollution prevention must be conveyed to the employees.[84]

IV-1-3: Good Housekeeping

As a performance oriented method of pollution prevention, good housekeeping practices can provide inexpensive and practical opportunities for minimizing waste generation. Proper storage of materials, routine inspection, and regular preventive maintenance are all effective measures of good housekeeping.

IV-1-3-1: Proper Storage of Materials

Proper storage of materials can reduce the potential for waste generation. Adoption of the following procedures may assure appropriate storage of materials:

- Store materials in a protected and locked area;
- Limit access to storage area to select, authorized employees;
- Space containers in storage area to allow easy inspection;
- Label all containers with storage and shelf-life date;
- Adopt a first-in, first-out policy for material usage to avoid product expiration;
- Use the instructions of manufacturers for stacking containers to avert breaking and tearing for improper weight distribution.

IV-1-3-2: Routine Maintenance

Adoption of a routine inspection and replacement program, as well as regular preventive maintenance may result in minimizing leaks, spills, evaporative losses and other releases of hazardous chemicals to the environment, and, consequently, reducing pollution generation.

IV-1-3-3: Operating Practices and Procedures Applicable to Biotechnology Facilities

Significant toxics waste reduction in biotechnology companies could be realized and the safety of workers ensured if the following industry-specific operating practices and procedures are implemented:

- Efficient handling of chemicals and radioactive materials.
- Proper storage of radioactive materials and chemicals.
- Labeling of reagents in accordance with OSHA regulations.
- Explaining MSDSs to workers.
- Use of most chemicals inside a fume hood.
- Avoidance of an overloaded chemical fume hood.
- Maintaining up to date certification on biological safety cabinets.
- Storage of flammables in explosion-proof cabinets or refrigerators.
- Segregating wastes whenever possible, and restricting hazardous wastes from the general waste stream.

The protection of laboratory's workers and personnel could further be assured if the following basic laboratory safety rules are observed:

- Never mouth pipette solvents or solution.
- Avoid eating, drinking, smoking or applying cosmetics in the lab.
- Hands should be washed after handling chemicals.
- Wear lab coat, gloves and safety glasses, and protect feet and clothing while performing activity in the lab.
- Use a safety cabinet for handling infectious materials.
- Put biological safety cabinets in non- or low-traffic areas, minimize activities that disrupt airflow in or around the cabinet.
- Label and place contaminated and toxic materials in covered and leak-proof containers.
- Decontaminate (by autoclaving for chemical disinfecting) all contaminated apparatuses and equipment before reuse or disposal.
- Dispose of broken glass, disposable glassware, and jagged metal in specially designated containers.[85]

IV-2: PROCESS MODIFICATION

Process modification involves implementing those processes, which increase efficiency in material use. In biotechnology, a shift from manual to automation of processes would help reduce the

chance of spillage and avoid overuse of chemicals in experimental procedures.

IV-3: INPUT SUBSTITUTION

Input substitution involves the replacement of a toxic or hazardous chemical substance or raw material used in the production unit with a nontoxic or less toxic substance. Due to the sensitivity of biological systems and laboratory procedures, input and chemical substitution may be limited, but there might be opportunities in the substitution of organic solvents with aqueous buffers in equipment cleaning and in other standard operations.

IV-4: MATERIAL MAMAGEMENT AND INVENTORY CONTROL

Appropriate materials management and proper inventory control procedures involve efficient monitoring and maintenance of records of process chemicals purchasing, storage, and usage. Tracking and proper handling of process chemicals is also crucial, since misuse and spills can substantially contribute to pollution and waste generation. By tracing of the usage of chemicals, sources of waste may be identified.

Stockpiling of sample chemicals should be avoided. It is important that manufacturers stipulate that test samples will be accepted only if the vendors agree to take back leftover samples.

By record keeping and controlling the purchasing of materials, over-purchasing and stockpiling may be avoided. Materials remaining after the expiration date will require disposal, and result in incurring unnecessary expenses.

IV-5: RECYCLING

Recycling is considered to be a cost-effective alternative for reducing toxic waste effluents. In biotechnology, on-site reuse and recycling of solvents might be limited due to the small amounts being used and the concern over purity. There may be more opportunities for off-site recycling.

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