COMPREHENSIVE BIOTECHNOLOGY

The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine

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The Principles of Biotechnology: Engineering Considerations

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United States and Canadian Governmental Regulations Concerning Biohazardous Effluents

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32.1 INTRODUCTION

32.1.1 Scope of the Chapter

The discovery of plasmid-mediated *in vitro* DNA recombination in 1972, resulted in a renaissance for commercial fermentation technology. The prospects of applying techniques in molecular biology to the development of a wide range of microbial products has given rise to several hundred new firms worldwide within a few years. While applications for genetically engineered microorganisms were being sought and commercial markets assessed, scientists and the public debated the safety of rearranging genetic material across disparate species. Some skeptics called for new laws, while others were satisfied to establish regulations under the authority of existing laws. Throughout a decade of intense controversy, primarily centered in the United States and Britain, many scientists emphasized that the risks associated with the manufacture of novel organisms were purely conjectural.

Much of the early discussion over biohazards was almost exclusively directed to gene-splicing experiments. As the debate over laboratory-scale recombinant DNA manipulations subsided, the focus shifted to industrial scale activities where environmental release and occupational health are highlighted. The question at the heart of the issue is: 'Will commercial biotechnology be clean in comparison to the chemical industry which is plagued with problems of treatment, transportation, storage and disposal of hazardous wastes?'

The decades of the 1960s and 1970s brought an unprecedented public response to the chemical hazards facing man and the environment. Throughout the industrial world, major pieces of legislation were enacted to protect land, water, air resources and the working environment from chemical contaminants. The commercial developments that were taking place to exploit the use of gene-splicing brought a new air of apprehension to some environmentally-minded individuals, government officials and public advocacy groups. Central to their concerns were the following possibilities: (1) a novel organism might be released into the environment with unpredictable and possibly irreversible effects on the environment; (2) a new microbial agent, infectious to humans or animals, might be released, or a conventional pathogen might have its host range broadened; (3) a rapid rise in large-scale biotechnology could result in bioeffluents that would place additional stresses on the quality of land and water resources; (4) organisms engineered to perform useful functions in the environment might produce adverse secondary effects of an unanticipated nature.

Initiated first by Britain (Working Party, 1976), followed soon after by the United States (National Institutes of Health, 1976), guidelines regulating experiments involving the production of recombinant DNA molecules were adopted by many countries actively pursuing research in this area. Scientists questioned the rationale that selects out genetically engineered organisms for regulation. The laboratory use of pathogens and infectious viruses has been an area of concern for the public health community. Infectious disease experts can point to studies of hospital-induced (nosocomial) infections traced in part to clinical laboratories and the lack of adequate attention to the disposal of infectious waste products. By 1980 many states had adopted regulations for the management of infectious wastes. Special treatment of infectious waste materials is required by some Canadian provinces. But in the laboratory uses of infectious agents, even in large clinical laboratories, the volumes have not been large relative to an industrial scale, and thus federal environmental initiatives which respond to the scale of pollution have been minimal.

In the food and pharmaceutical industries, the fermentation technology has utilized mainly non-pathogenic microorganisms cultured from natural environments, or their laboratory-induced hybrids. As a consequence, little attention has been given to regulating industrial effluent consisting of biological entities that are plentiful in nature. But as genetically engineered bacterial strains constructed in the laboratory are introduced into the industrial fermentation process, or released into the environment as pesticides or pollution degraders, new expressions of concern can be

heard among those who are inclined to draw connections between the developments in synthetic chemistry and those in synthetic biology.

The goals of this chapter are threefold. First, it examines six areas where the use of microorganisms in an industrial setting is expected to grow as a consequence of the revolution in applied molecular genetics that is currently taking place. Second, it reviews the laws in the United States and Canada which are or may be applicable to the regulation of biological agents released into the environment. Third, it summarizes statutory regulations and guidelines that have been issued in the United States and Canada pertaining to the release of biological agents. The inventory of environmental laws and regulations includes those in the area of occupational safety and health.

In contrast to the use of chemicals in industrial processes, the application of microbial agents in production or as pesticides has been small. This is reflected in the fact that few regulations have been established for contaminants from bioprocess sources. This study distinguishes between the authority to regulate and the actual regulations. For example, the United States Environmental Protection Agency may have a legal authority to regulate biological waste products, but it has not chosen to exercise this authority thus far.

32.1.2 Bioprocess Sources and Pathways for Environmental Release

The term bioprocess in this study is used in a general sense to mean any human activity that produces or transports microbial agents, disperses them into the environment, or uses them as part of a system of production. The laws and regulations reviewed were chosen with consideration of the following pathways through which biological agents are released into the environment: human to human, animal to animal, or plant to plant contact; transport of etiologic agents; laboratory and clinical wastes; manufacturing processes involving large cultures of bacteria or viruses; large-scale release of biological agents into the environment; release of biological agents from experimental laboratories, through drains, human, insect or rodent vectors.

In the pharmaceutical and food industries that employ fermentation technologies, biological agents may be released inadvertently during the venting of gases, through the wastewater effluent stream or by the disposal of the sludge resulting from the fermentation process. Some technologies, such as wastewater treatment, use the indigenous organisms within the effluent stream by enhancing their growth and thereby helping them break down organic contaminants. Some efforts are underway to improve the efficiency of secondary treatment plants by adding specially cultured or genetically engineered bacteria. In these uses, the microorganisms have a natural pathway into the environment unless these biodegraders of organic contaminants are themselves disinfected after their work is completed.

Relatively little legislation has been conceived with the release of biological agents in mind. It has been a small effort compared to the regulatory activities for chemical agents and radioactive materials. The legislation which has been enacted is concerned primarily with pathogenic agents infectious to humans, plants and animals. As an example, under the quarantine acts certain organisms or their hosts are prohibited from being imported into North America. Beyond direct infectivity, there are clearly other ways that biological agents can be ecologically troublesome. They may interfere with biochemical pathways, increase the coliform bacteria levels in drinking water or affect the biological oxygen demand in lakes, rivers and streams. The guidelines that were issued in the United States, Canada and elsewhere for genetically modified organisms are based upon a broader interpretation of biohazardous materials than what is understood by a pathogenic or infectious agent. That is also true with regulations for the use of biological pesticides, where studies are required that include host specificity, allergenic effects and toxicity prior to the granting of a permit for their use.

32.1.3 Overview of Environmental Laws

32.1.3.1 United States

Regulation has been the preferred method of dealing with environmental problems in the United States. American governments at all levels have chosen to regulate sources of environmental pollution, although alternative forms of governmental intervention have been suggested (Stewart and Krier, 1978).

Several major pieces of legislation have been enacted by Congress to deal with the problems of air pollution, water pollution and hazardous substances. The federal agency given primary responsibility for implementation and enforcement of government policy as directed by these laws is the Environmental Protection Agency (EPA). EPA's enforcement role covers a number of areas including stationary sources of air pollution, discharges into the nation's waters, mobile sources of air pollution, hazardous waste sites, toxic substances, solid wastes, drinking water and pesticides.

In many instances the federal and state governments share the burden of implementation and enforcement. State agencies analogous to the federal EPA regulate and enforce state environmental laws and state components of federal programs. Some federal programs are predicated on direct state regulation with varying degrees of federal overview, others on direct federal regulation only until states develop programs which are in compliance with federal guidelines. When compliance is achieved, the state directs the regulatory program. A third category of regulation involves direct federal regulation with little or no state involvement.

The Clean Air Act (33 U.S.C. § 7401 *et seq.*) established federal controls on air pollution. It was last amended in 1977 and it is that version of the Act which is now in effect. The requirements of the Act are expressed in terms of ambient air standards which are created by statute, federal or state regulations, or permits. It was intended by Congress that the states would be primarily responsible for administering the Clean Air Act requirements through state implementation plans (SIPs).

The Federal Water Pollution Control Act (FWPCA) (42 U.S.C. § 1251 *et seq.*) was shaped in its present form by the Amendments of 1972. The goals of the Act are: (1) the achievement of swimmable and fishable waters by 1983, and (2) elimination of pollution discharge by 1985. Under the law, effluent limitations were established for industrial and municipal sources of water pollution. The Clean Water Act went through certain revisions in 1982–83, including re-authorization of some programs and clarification of particular sections.

In response to the problems posed by hazardous waste and toxic substances which may enter the environment, Congress passed three major pieces of legislation. The Toxic Substances Control Act of 1976 (TSCA) (15 U.S.C. § 2601 *et seq.*) is a product control law in contrast to a pollution control law. TSCA regulates the manufacture, distribution and sale of chemicals which 'may present an unreasonable risk of injury to health or the environment'. The Resource Conservation and Recovery Act (RCRA) (42 U.S.C. § 6901 *et seq.*) gives authority to the federal government to provide technical and financial assistance to state and local governments and interstate agencies to promote improved solid waste management techniques, and to issue regulations for the treatment, storage, transportation and disposal of hazardous wastes. The Comprehensive Environmental Response, Compensation and Liability Act of 1980 (42 U.S.C. § 9601 *et seq.*) provides the EPA with authority to require generators, transporters, treaters and disposers of hazardous wastes to remedy actual or potentially endangering hazardous waste sites and associated damage to natural resources. The legislation established a 'Superfund' and authorizes use of the fund's resources to perform the remedy if the responsible party fails to do so.

Further examination of these basic elements in the United States regulatory arsenal is necessary to understand their applicability to the industrial release of biological agents. While EPA has principal jurisidiction over industrial waste streams, other federal and state agencies, under public health statutes, can regulate the release of pathogenic agents in the environment from non-industrial sources.

32.1.3.2 Canada

In Canada, the demarcation between federal and provincial jurisdictions is framed in the British North America (B.N.A.) Act of 1867, a principal document of the Constitution of Canada along with its amendments in the Canada Act of 1982. Most of the federal powers are provided through Section 91 of the B.N.A. Act while Section 92 delineates the powers of the provinces. Amendments to Section 92 are found in Part VI of the Canada Act of 1982.

The B.N.A. Act does not address the environment specifically. Legal authority in this area has evolved through judicial interpretation. Some environmental problems lie within the jurisdiction of both the provincial and federal governments. Water pollution is a case in point. Whereas federal laws are designed to protect the fisheries, provincial laws are enacted to protect the public health.

The full impact of the new Canadian Constitution on the B.N.A. Act and its delineation of

powers between federal and provincial jurisdictions is not fully understood at this time. Therefore, this chapter emphasizes historically-developed environmental jurisdictions.

Both federal and provincial governments have powers with respect to agriculture. Each can pass laws regulating fertilizers, feed products and pesticides. In the case of pesticides, the provinces have promulgated additional regulations beyond those of the Parliament. Where there are areas of overlapping jurisdictions, the federal government will at times resort to measures involving negotiation and coordination with provincial authorities. And when federal and provincial statutes are in conflict, federal laws generally prevail.

Chemical contaminants in the environment are regulated by the federal government under four main acts: The Fisheries Act, the Clean Air Act, the Canada Water Act and the Environmental Contaminants Act. In most instances these Acts define an environmental contaminant broadly enough to include biological agents. The exception is the Environmental Contaminants Act which applies exclusively to 'inanimate matter'.

According to Section 92 of the B.N.A. Act, the provinces are given authority over the working environment and waste disposal. The federal government can enact occupational health and safety statutes for a select number of industries that fall under its jurisdiction, subject to Part IV of the Canada Labour Code. Generally, federal legislation has been upheld where problems have taken on 'national dimensions' or become a matter of 'national concern'.

Ince (1976) makes the following observations about the genesis of environmental law in Canada: 'Because provincial powers of legislation are framed in such general terms, it is very difficult to limit provincial powers. Over the past century, the courts have, quite understandably, interpreted these powers very broadly which has enabled the provincial legislatures to deal with a vast number of areas. On the basis of the provinces' powers to control property, civil rights and local matters, a great deal of environmental legislation is authorized. We can safely say that these powers allow the provinces to legislate on land, air, water and noise pollution, land use control, parks and industrial regulation'.

Indeed, many provinces have enacted laws and issued regulations for chemical contaminants, radiation, clean air and water, waste management, pesticides and the working environment. These statutes were not developed with biological agents in mind, although in some cases the language is sufficiently broad to justify their application to microbial forms, if certain conditions are satisfied. In contrast to the strictly formal and mandatory framework for regulatory policy in the United States, Canada is known for a more informal and discretionary system of lawmaking. Within this framework there is considerable federal–provincial negotiation, consultation and cooperation, as well as industry–government consultations.

Before U.S. and Canadian laws and regulations are examined more fully with respect to the release of biological agents, a brief review is given of the major applications of microorganisms in industry.

32.2 POTENTIAL HAZARDS OF BIOPROCESSES

32.2.1 Pharmaceutical Industries

The conventional organisms that are used to manufacture antibiotics consist of a relatively narrow taxonomic range. Nearly a thousand distinct antibiotics are derived from six genera of filamentous fungi including molds of the genus *Cephalosporium* and the genus *Penicillium*. By inducing mutations through radiation and chemical substances, these two molds were the main source of antibiotics for 30 years. Other drugs manufactured by microbial fermentation techniques are viral and bacterial antigens, antifungal agents, antitumor drugs, alkaloids and vitamins.

Fermentation technology has been the principal process for manufacturing pharmaceuticals. Volumes produced in the fermentation vats may be as high as 100 000 l. A brief description of the process is given by Aharonowitz and Cohen (1981).

Recombinant DNA technology opens up opportunities for the biosynthesis of drugs, hormones and other biologically active substances by microorganisms containing the inserted relevant information. Somatostatin (a hormone made in the hypothalamus), insulin, growth hormone and interferons (antiviral agents) are currently being synthesized by *E. coli* K12 which have the requisite gene inserts. Gene-splicing techniques are also beginning to revolutionize vaccine production. Molecular cloning makes it possible to manufacture in large quantities non-virulent, nonselfreplicable segments of a virus that can be used to immunize a host.

32.2.2 Food Industries

The food processing industry uses microbial activity in two ways (Office of Technology Assessment, 1981): (1) inedible biomass is transformed by microorganisms into food for human consumption or animal feed; (2) organisms are used in food processing either by acting directly on food or by providing materials that can be added. Enzymes and vitamins are examples of the latter use. Food processing has utilized enzymes extracted from plants and animals. Microbial production of them has become economically competitive in some cases. Bacteria and molds also are used to make vitamins, such as riboflavin (*Ashbya gossypii*) and vitamin B12 (*Propionibacterium shermanii* and *Pseudomonas denitrificans*).

New developments in genetic engineering, *e.g.* protoplast fusion, are expected to broaden the use of fermentation in the food industry (Demain and Solomon, 1981). The genomes of two distinct species can be brought into a single cell. Recombinant DNA technology establishes genes as interchangeable elements capable of being transplanted between diverse organisms. Food processing need not depend exclusively on the enzymes found naturally in microorganisms; even human enzymes can be produced in large quantities by cloning their DNA in bacteria.

32.2.3 Energy Production

The production of liquid fuels through fermentation can be improved upon in two ways through genetic engineering (Office of Technology Assessment, 1981). First the genetic manipulation of plant seeds may yield better quality and greater quantity of biomass. Second, microbial mutants are being sought to improve the efficiency of converting agricultural and forest biomass into liquid fuel. Ethanol, among the most important organic substances in the chemical industry, has attracted significant attention in the biotechnology field. The genetic programming of conventional organisms used in the fermentation of ethanol, which include yeasts, *Zymomonas mobilis*, *Clostridium thermocellum* and *Trichoderma reesei*, has been proposed 'to increase the amount of certain enzymes in the cell or to replace one enzyme with another that has a higher specific activity' (Eveleigh, 1981, p. 168).

In a report entitled *Biotechnology: A Development Plan for Canada* (Task Force on Biotechnology, 1981), it was noted that the production of methane from the fermentation of agricultural, industrial and domestic wastes is another fuel prospect in addition to ethanol. Moreover, the report states, the bioproduction of substitute fuels to replace hydrocarbon-based conventional crude oil derivatives, 'could be of significance in determining alternate energy strategies for Canada'.

Some concern has been raised about the ecological impacts of genetically modified organisms released into the environment either purposefully or inadvertently through the waste stream. Potential hazards of disturbing biochemical pathways or altering ecological balances have been cited (Krimsky, 1982, p. 122; Wright, 1982). In Massachusetts, several communities have passed ordinances that regulate fermentation with recombinant organisms (Krimsky *et al.*, 1982) to insure that novel organisms are adequately contained or appropriately treated prior to being released in the waste stream.

32.2.4 Waste Treatment

Microbial activity is used in the detoxification and degradation of sewage and industrial wastes. Secondary sewage treatment facilities use some form of biological activity to degrade organic materials. The activated sludge process developed early in this century has depended upon the indigenous microorganisms in the waste stream. The activity of the microorganisms may be enhanced by additives or environmental controls, such as pH and temperature.

More recently, sludges have been inoculated with mixtures of microorganisms which are designed to accelerate the degrading process or broaden the types of chemicals broken down by bacteria. The next important breakthrough in this area will be made through genetic engineering. Microorganisms are being genetically constructed to degrade specific compounds found in industrial wastes. Organisms have been developed which are successful at the laboratory scale in degrading industrial organic compounds such as polychlorinated biphenyls (PCBs) and the herbicide 2,4,5-T. The Canadian Task Force on Biotechnology (Task Force on Biotechnology, 1981) cited two advantages offered by biological processes over other methods of detoxification of