WALK-THROUGH SURVEY REPORT:

CONTROL TECHNOLOGY FOR FERMENTATION PROCESSES

AT

Genencor, Inc.
Bristol, Pennsylvania

REPORT WRITTEN BY:
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NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Physical Sciences and Engineering
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SIC CODE:	2869
SURVEY DATE:	May 11, 1983
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I. INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) is the primary Federal agency engaged in occupational safety and health research. Located in the Department of Health and Human Services (formerly DHEW), it was established by the Occupational Safety and Health Act of 1970. This legislation mandated NIOSH to conduct a number of research and education programs separate from the standard setting and enforcement functions carried out by the Occupational Safety and Health Administration (OSHA) in the Department of Labor. An important area of NIOSH research deals with methods for controlling occupational exposure to potential chemical and physical hazards. The Engineering Control Technology Branch (ECTB) of the Division of Physical Sciences and Engineering has been given the lead within NIOSH to study the engineering aspects of health hazard prevention and control.

Since 1976, ECTB has conducted a number of assessments of health hazard control technology on the basis of industry, common industrial process, or specific control techniques. Examples of these completed studies include the foundry industry; various chemical manufacturing or processing operations; spray painting; and the recirculation of exhaust air. The objective of each of these studies has been to document and evaluate effective control techniques for potential health hazards in the industry or process of interest, and to create a more general awareness of the need for or availability of an effective system of hazard control measures.

These studies involve a number of steps or phases. Initially, a series of walk-through surveys is conducted to select plants or processes with effective and potentially transferable control concepts or techniques. Next, in-depth surveys are conducted to determine both the control parameters and the effectiveness of these controls. The reports from these in-depth surveys are then used as a basis for preparing technical reports and journal articles on effective hazard control measures. Ultimately, the information from these research activities builds the data base of publicly available information on hazard control techniques for use by health professionals who are responsible for preventing occupational illness and injury.

The public debate over genetic engineering has focused on the possible hazards of genetically modified microorganisms, potential health hazards to workers involved with industrial applications of recombinant DNA (rDNA) techniques, and the potential uses of such technology. Several risk assessment experiments designed to investigate some of the characteristics of proposed host-vector systems which might affect hazard potential have been conducted. Likewise, the benefits of rDNA technology are being as vigorously promoted.

This particlular research effort was prompted by the anticipated surge of rDNA techniques in various industrial processes. Genetic engineering technology may be utilized in various manufacturing processes in the areas of agriculture, organic chemicals, energy, food processing, and pharmaceuticals. This potential growth and the possibility of uncharacterized occupational exposures indicate the necessity for careful evaluations of health risks. NIOSH is accustomed to examining new technologies for potential occupational hazards and developing recommendations for safeguarding the workers health.

Implementation of safeguards and protective engineering controls early in the growth of an industry can only minimize occupational health problems and avoid expensive retrofitting of production systems.

NIOSH is currently evaluating the potential occupational hazards involved with distinct applications of biotechnology and rDNA techniques. ECTB's involvement in this evaluation is the development of a study investigating the control technology being used to prevent occupational health hazards in the fermentation industry. This assessment will attempt to identify effective controls applicable to processes involving potentially hazardous microorganisms, innate as well as genetically modified, toxic processing chemicals, and biologically active products or intermediates. Documentation of effective controls and recommendations to minimize exposure in the fermentation industry will be accomplished through this assessment. The fermentation processes control technology assessment will impact imminent rDNA fermentation technology in addition to current fermentation technology.

This report contains results of this preliminary study, conclusions, and/or recommendations relevant to the operations at Genencor, Inc., a manufacturer of industrial enzymes. This survey was conducted as one of a series of initial preliminary surveys of firms involved in fermentation processes. Based on the information obtained during these walk-through studies, potential candidates for in-depth survey sites will be selected. The in-depth surveys will involve more detailed evaluations of the engineering controls, personal protective equipment, employee work practices, and industrial hygiene and medical monitoring.

II. PLANT AND PROCESS DESCRIPTION

Plant Description:
The Genencor, Inc. enzyme operation is contained within the Rohm and Haas Delaware Valley Inc. chemical plant complex located in Bristol, Pennsylvania. The enzyme operation was originally owned by Rohm and Haas.

Rohm and Haas initially began producing industrial enzymes, utilizing a flat-tray growth chamber operation, in 1919. This operation was superseded in the 1950's with the construction of a new building housing the equipment and facilities to perform batch, deep-tank reactor fermentation. Presently, the original building is used primarily for the final processing of the liquid enzyme concentrate produced in the new building.

Rohm and Haas continued manufacturing industrial enzymes, specifically α-amylase, pectinase, and acid, neutral, and alkaline proteases, until July 1981. At that time, Corning purchased the enzyme manufacturing equipment and product name from Rohm and Haas. In June 1982 an agreement was signed between Corning and Genentech, Inc. to form and incorporate the company Genencor, Inc. as a joint effort between the two companies to continue the Rohm and Haas enzyme operation. Coordination and supervision of the enzyme production process is handled by Genencor, Inc. personnel, but the actual operation is maintained by Rohm and Haas hourly employees. Enzyme production is conducted 7 days per week in 4 work shifts.

Process Description:
Genencor, Inc. manufactures three major industrial enzymes at the Bristol, Pennsylvania plant; α-amylase, pectinase, and acid, neutral, and alkaline proteases. Aspergillus niger and oryzae microoganisms, classified as fungi, are used to produce pectinase and acid protease, respectively, whereas Bacillus subtilis microorganisms, classified as bacteria, are used in the fermentation of α-amylase and neutral and alkaline proteolytic enzymes. All three strains of microorganisms are non-pathogens.

The manufacture of the industrial enzymes is accomplished in four different phases of production; a laboratory phase, a plant inoculum phase, a plant growing phase, and a processing phase. Each phase of the enzyme operation is executed in the new building and only the final processing of the liquid enzyme concentrate is done in the original building.

The laboratory phase is where initial preparation and development of the microorganisms are maintained. All operations within the laboratory phase are conducted using sterile equipment with aseptic transfer. First, stock cultures of bacteria or fungi are transferred from cold storage to a 4 liter shaker flask to be propagated to 1 liter of viable liquid. This 1 liter of culture fluid is subsequently increased in microbial population to be used as an inoculant in the germinator tank. The culture fluid inoculant is manually transfered from the laboratory to the germinator tank.

The next phase in the enzyme manufacturing process, the plant inoculum phase, is designed to promote the growth of the microbial population to the levels needed for proper fermentation in the deep-tank reactor vessels. Nutrient

media is added to the germinator tank where it is sterilized at 120°C and 15 psig for 30 to 60 minutes. City water is used for nutrient make-up. The nutrient media is then cooled to 30° to 35°C. Next, the germinator tank is inoculated with the culture fluid prepared in the laboratory phase. The batch mixture is aerated and mechanically agitated so that the number of bacterial or fungal cells increase at a constant rate of growth.

The plant growth phase is where fermentation essentially occurs and the product of interest is biologically synthesized. A batch fermentation process is employed in two different types of deep-tank reactor vessels. The fermentor tanks have been in service for approximately 20 to 30 years. These systems are standard fermentors utilizing top-mounted mechanical agitators with circular air spargers. The mechanical agitators are variable-speed which allows for experimentation with the aeration potential of a given batch process. Temperature control is maintained with a heating jacket surrounding the base of the fermentor tank.

Nutrient media, a composite mixture of various agricultural commodities and other nutrients, is charged into the fermentors from a batching tank. City water is used for nutrient make-up. The nutrient is then sterilized for 15 minutes utilizing steam or by increasing the temperature of the heating jacket to the appropriate level. The fermentor is cooled and inoculated with the broth from the germinator tank. This new broth mixture is aerated, mechanically agitated, and allowed to ferment. Foaming during the fermenting cycle is detected visually and if judged necessary an anti-foaming agent is added to the broth. Analytical broth samples, routinely extracted during the cycle, are obtained manually from a port valve directly connected to the fermentor tank.

Air that is used to entrain oxygen in the broth mixtures of the germinator and fermentor tanks must be free from contaminants and sterilized. To accomplish this, atmospheric air is compressed and perfused through a mist eliminator, prefilter, and five air filters (glass wool or carbon based). Sterilization of the air is accomplished with steam.

The final phase of enzyme production, the processing phase, will concentrate the enzyme liquid and remove unwanted contaminants from the harvested broth. This broth or slurry is transfered from the fermentor tank to a crude tank via pipe where it is cooled. Agitation is maintained in the crude tank while process aids are subsequently added. The enzyme slurry is pumped to a rotary vacuum drum filter (diatomaceous earth is used for a precoat) where a major portion of the suspended solids will be separated from the enzyme liquid. A stellite doctor blade shaves off the filter cake and a fraction of the diatomaceous earth precoat. The cuttings are deposited into a dumpster which is transported by truck to a landfill. The filtrate liquid is then transfered to a filtrate tank. Agitation is again maintained. The ultrafiltrator will concentrate the enzyme liquid and remove salts and low molecular weight organics. The permeate is released into the plant sewer network and the high molecular weight retentate is returned to the filtrate tank.

Up to this point, all enzyme operations were conducted in the new building. The liquid enzyme, concentrated in the ultrafiltration system, is drummed and

transported to the original building. The enzymes that are to be of food grade quality are fed into a refrigerated holding tank and continuously agitated. A precoat is added to the liquid enzyme which is then pumped to a filter press to remove remaining residues and bacteria. The residue is discarded. This final concentrate is placed into another refrigerated holding tank with continuous agitation. The enzyme products that are to be sold as liquids are preserved, stabilized, and packaged; those enzymes that are to be sold as solids are spray-dryed by a contractor.

Broth mixtures or concentrated liquid enzymes are transferred between separate unit operations from the plant inoculum phase to the processing phase by pipe, excluding the transport of drummed enzyme concentrates between buildings.

Potential Hazards:
The potential for exposure to hazards in the occupational environment within the fermentation industry is a three-fold problem. Exposure may involve potentially hazardous microorganisms (innate as well as genetically modified) toxic processing chemicals, and biologically active products or intermediates.

Presently, the microorganisms used by the enzyme industry, inclusive of the overall fermentation industry, for fermentation operations are non-pathogenic in nature. But future involvement with rDNA technology may produce microorganisms in need of more stringent containment requirements and equally stringent programs in occupational safety and health due to the increased health risks that they pose to the exposed worker. Genencor, Inc. utilizes strains of Bacillus subtilis, Aspergillus niger, and Aspergillus oryzae microorganisms, non-pathogens, for their enzyme manufacturing operations.

Diatomaceous earth (amorphous silica) is used in two unit operations of the processing phase; as a precoat on the drum of the rotary drum vacuum filter and as a precoating mixture added to the concentrated enzyme liquid to be perfused through the filter press. Amorphous silica can affect the body if it is inhaled or if it comes in contact with the eyes. Prolonged inhalation of amorphous silica including uncalcined diatomaceous earth may produce x-ray changes in the lungs without disability. Prolonged inhalation of calcined diatomaceous earth may cause silicosis with scarring of the lungs, cough, and shortness of breath. The current OSHA standard for amorphous silica is 80 $mg/m^3/\%SiO_2$ averaged over an eight hour work shift. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a maximum exposure for diatomaceous earth of 1.5 mg/m^3 over an eight hour work shift.

Acid and base compounds are used to adjust pH levels of biomass broth mixtures or concentrated enzyme liquids throughout the enzyme production process; acids are corrosive and irritating, whereas, base compounds are caustic and will cause burns. Dependent upon the compound being used and its degree of hazard potentiality, protective clothing should be worn and the appropriate control techniques implemented to prevent potential contact or exposure to these agents.

The enzyme molecule consists of a chain of amino acids arranged in a specific geometric configuration. This protein structure, as is with the case of many proteinaceous materials, will cause immunologic responses in susceptible

persons due to the inhalation of these antigens. Repeated inhalation of enzyme dust may provoke respiratory allergies (hay fever, asthma) or illnesses (rhinitis) in individuals who have become sensitized to a specific enzyme-protein structure. Sensitization reactions may vary from mild to severe dependent upon the particular individual exposed. Some enzymes, proteolytic enzymes as an example, have been shown to cause contact dermatitis to exposed areas of moist skin, eyes, and mucous membranes. The majority of documented case studies of persons exposed to enzymes has focused upon the immunologic responses due to the inhalation or contact to dusts. There appears to be limited available literature pertaining to individuals exposed to aerosolized liquid enzymes.

III. CONTROLS

PRINCIPLES OF CONTROL

Occupational exposures can be controlled by the application of a number of well-known principles, including engineering measures, work practices, personal protection, and monitoring. These principles may be applied at or near the hazard source, to the general workplace environment, or at the point of occupational exposure to individuals. Controls applied at the source of the hazard, including engineering measures (material substitution, process/equipment modification, isolation or automation, local ventilation) and work practices, are generally the preferred and most effective means of control both in terms of occupational and environmental concerns. Controls which may be applied to hazards that have escaped into the workplace environment include dilution ventilation, dust suppression, and housekeeping. Control measures may also be applied near individual workers, including the use of remote control rooms, isolation booths, supplied-air cabs, work practices, and personal protective equipment.

In general, a system comprised of the above control measures is required to provide worker protection under normal operating conditions as well as under conditions of process upset, failure, and/or maintenance. Process and workplace monitoring devices, personal exposure monitoring, and medical monitoring are important mechanisms for providing feedback concerning effectiveness of the controls in use. Ongoing monitoring and maintenance of controls to ensure proper use and operating conditions, and the education and commitment of both workers and management to occupational health are also important ingredients of a complete, effective, and durable control system.

These principles of control apply to all situations, but their optimum application varies from case-to-case. The application of these principles at Genencor, Inc. is discussed below.

The enzyme production process is essentially a "closed" system from the plant inoculum phase through the processing phase -- excluding the transport of the drummed liquid enzyme concentrates between buildings. There appears to be limited potential for exposure to the microorganisms involved in the fermentation processes or the enzyme products of these microoganisms. All growth and holding tanks are virtually closed, except when raw materials are periodically added during the fermenting process, and batch cultures are piped between unit operations. There is minor potential for release of aerosolized viables or enzymes during manual sampling procedures and circumjacent to the agitator shafts, but the quantities involved pose minimal contact or exposure concerns. The main form of ventilation present are general or spot ventilation designed for a comfort controlled environment.

Exposure to liquid enzyme solutions is possible during filling and emptying operations or cleaning of enzyme spills. The packaging (including preservation and stabilization) of dry enzymes has the greatest potential for exposure. The packaging operation was contained in a separate room (closed door) to help retard enzymatic dust from being dissipated to other parts of

the building. Local exhaust ventilation is used during the drum filling operation, even-so, dust was present on surfaces in the room.

Potential inhalation of diatomaceous earth (amorphous silica) is evident during the dumping of bags of diatomaceous earth into the rotary vacuum drum filter system. Local exhaust ventilation exists at both dumping operation stations. Additionally, disposable dust masks (3M model 8710) are employed during the dumping of bags of diatomaceous earth.

Potential exposure to acids, used to control pH levels in the enzyme broth, are minimized with personal protective equipment. Safety glasses, rubber boots, face shields, and rubber gloves (PVC or neoprene) are employed when pouring acidic solutions.

Because the operation of the enzyme manufacturing process is conducted by Rohm and Haas employees, all health and safety concerns are coordinated by the Rohm and Haas corporation. As part of the health and safety program, a collaborative corporate-union safety committee is employed. Corporate industrial hygiene audits of the entire plant are conducted every two years -- this includes total dust sampling assays of the mixing and charging (bag dumping) operations.

The occupational medical program is also handled on the Rohm and Haas corporate level. A complete staff of medical practitioners (doctors, nurses, etc.) are on call on the plant premises during normal working hours. Employees are required to take a pre-employment physical including; cardiograms, vision tests, hearing tests, pulmonary function tests, blood chemistry tests, and a review of an individual's medical history. There are also periodic (annual) physical examinations, however, there are no special medical diagnostic tests for workers involved with enzyme production.

IV. CONCLUSIONS AND RECOMMENDATIONS

To help ensure that occupational exposures in the enzyme manufacturing process are being appropriately controlled, some changes in the current medical monitoring program should be made. These changes should include periodic medical examinations which would focus on possible sensitization to the enzymes. This could be accomplished by medical history and physical examinations focusing on dermatitis, rhinitis, and asthmatic symptoms. This should be done approximately every six months with appropriate medical follow-up of any individuals with these symptoms to determine if these symptoms were related to enzyme exposure. Periodic radioallergosorbent tests (RAST) specific for the enzyme being produced would also be useful in detecting sensitization of any exposed workers.

Improved housekeeping in the enzyme warehouse and packaging operation will help in controlling dust levels, as these are the only locations where workers are routinely exposed to enzyme dust particulates.

Considerable information was obtained during the survey pertaining to control technology in fermentation processes, but because of the age of the operation, this plant does not exhibit current state-of-the-art technology. Although occupational health and safety conditions appeared to be reasonably controlled, the Genencor, Inc. enzyme production plant will probably not be recommended for an in-depth study.