# WALK-THROUGH SURVEY REPORT:

# CONTROL TECHNOLOGY FOR FERMENTATION PROCESSES

AT

Miles Laboratories, Inc.
Elkhart, Indiana

REPORT WRITTEN BY:
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NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Physical Sciences and Engineering
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| PLANT SURVEYED: | Miles Laboratories, Inc.<br>1127 Myrtle<br>Elkhart, Indiana 46515 |
| SIC CODE: | 2869 |
| SURVEY DATE: | July 12, 1983 |
| SURVEY CONDUCTED BY: | Kenneth F. Martinez<br>James H. Jones |
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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 particular 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 Miles Laboratories, 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 Miles enzyme operation is contained in the larger Miles Laboratories, Inc. plant complex in Elkhart, Indiana. Miles has been producing carbohydrase enzymes since March 1982. The parent company, based out of Germany, is Bayer A/G.

Enzyme production is a 4 shift operation maintained 7 days per week, 24 hours per day in 12 hour workshifts. The workers are represented by the United Steelworkers of America, Local 12273.

Process Description:
Of the processes observed at Miles, the production of the industrial enzymes α-amylase and glucoamylase were selected for inclusion in the preliminary survey. Aspergillus niger, a eucaryotic fungus, is used for the production of glucoamylase, and Bacillus licheniformis, a procaryotic bacterium, is used for the prodcution of α-amylase. Both strains of microorganisms are non-pathogens.

The manufacture of the industrial enzymes is accomplished using a six step process flow: raw materials - medium preparation; laboratory - microbial preparation; inoculation - microbial growth; fermentation - product biosynthesis; process recovery - product extraction; and final product packaging. All process steps of the enzyme operation are executed in the same plant building. The process flow follows a "horseshoe" pattern through the building — raw materials entering on one side of the building and the final, packaged product exiting on the same side, adjacent to the raw materials.

The raw material specifications used in the nutrient preparation process step are tightly controlled to prevent contaminants that would inhibit organism growth or enzyme production -- the raw materials should not be carried through the process into the final product. Requirements for the nutrient medium include: water; carbon from carbohydrate sources; nitrogen from proteins and amino acids; minerals; and a buffer system. The raw materials are deposited into individual hoppers to be subsequently mixed with the remaining required nutrients in a batching tank. This mixture is sterilized and added to the deep-tank reactor vessels, the seed and fermentor tanks, during the fermentation process step.

The laboratory and inoculation process steps are where initial development, preparation, growth, and maintenance of the selected microorganism cultures are accomplished before being used for large-scale fermentation. All pertinent microbiological operations within the laboratory are conducted using sterile equipment with aseptic transfer to ensure pure, uncontaminated culture mediums. The selected culture is grown (from stock cultures and propagated in shaker flasks), harvested, subdivided, and then stored at the appropriate conditions to maintain its viability and purity. Microbial cultures are transferred manually and aeseptically inoculated, maintaining pure cultures, into the seed tank for the first segment of the fermentation process step. The laboratory is not only used for seed preparation but also in-house quality control work.

Miles utilizes a two-phase operation in their large-scale fermentation process step — this minimizes the possibility of contaminating large quantities of culture media and optimizes the use of expensive equipment. In the first phase, the seed fermentor containing a sterile nutrient medium is inoculated with the selected microbial culture prepared in the laboratory. The seed fermentor is designed to promote the growth of the microbial population to the level necessary for proper fermentation in the deep-tank reactor vessel. The batch mixture is aerated and mechanically agitated until the optimum level of biomass is achieved. The final contents of the seed fermentor is aseptically transferred to the large fermentor (deep-tank reactor vessel). The second phase of the fermentation process step is where "fermentation" essentially occurs and the product of interest is biologically synthesized. A submerged, batch fermentation process is employed using a standard deep-tank reactor vessel with a top-mounted mechanical agitator and a bottom air sparger. Proper temperature conditions are maintained with cooling coils inside the reactor vessel. The fermentor tank, containing the pre-sterilized nutrient medium from the batching tank, is inoculated with the biomass broth from the seed fermentor. This new broth mixture is aerated, mechanically agitated, and allowed to ferment for biomass growth and final production of the desired enzyme. The composition of the medium used in each phase is carefully controlled to promote maximum growth of the organism and/or enzyme production.

Measurements are performed continuously during the fermentation process step to check specific parameters of the biomass broth. These measurements are predominantly computer controlled or monitored and include process parameters such as temperature, pH, nutrient addition, anti-foaming agent addition, air flow rate, back pressure in the vessel, etc. Other typical measurements monitored are the $\%CO_2$ and $O_2$ in the exhaust gas, the power consumption of the agitator motor and the RPM's of the agitator. Manual samples are also extracted periodically from a port valve on the large fermentor tank for analysis in the laboratory.

In process recovery, two separate techniques are utilized to extract the product enzyme from the biomass broth mixture; one, glucoamylase is perfused through a belt filter and two, $\alpha$-amylase is passed through a rotary vacuum drum filter system. In the first method involving the belt filter, the filter media is not caulked to the drum but is made to form a continuous loop which is flat against the drum during the form and dewatering portions of the filtering cycle, it is then transported over a series of rollers in the discharge area. After the cake is removed, the belt is washed before returning to the drum. In the second method, the enzyme slurry is pumped to a rotary vacuum drum filter system (diatomaceous earth is used as a precoat) where a major portion of the suspended solids (mycellium and other solids) are separated from the enzyme liquid. A stellite doctor blade shaves off the filter cake and a fraction of the diatomaceous earth precoat. The solid wastes from these operations are discharged to dumpsters and transported to landfills. The enzyme liquid must then be concentrated with an ultrafiltration system, used mainly to assist in keeping the evaporator energy efficient, and then concentrated again using an evaporator. The last step in process recovery, is the final polishing or purification of the concentrated enzyme accomplished with a filter to remove unwanted bacterial contamination.

The final processing step in the Miles enzyme manufacturing process, final product packaging, is to formulate and package the concentrated enzymes. Formulation involves standardizing the activity of the liquid enzyme and adding preservatives in a mechanically agitated mixing tank. The finished enzyme product is then packaged in headpacks, drums, or a bulk tank truck.

Potential Hazards:
The generic potential for exposure to hazards in the occupational environment within the general 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. Miles utilizes strains of Bacillus licheniformis and Aspergillus niger microorganisms, non-pathogens, for the selected enzyme manufacturing operations.

Diatomaceous earth (amorphous silica) is used in the concentration and purification processing step as a precoat on the drum of the rotary drum vacuum filter. 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 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 through-out 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 potential, 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 Miles Laboratories, Inc. is discussed below.

Engineering Controls:
Miles' enzyme production operation is a predominantly closed system once the process has graduated from the laboratory to the large-scale fermentation process steps. There appears to be extremely 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 closed during process operations. Batch broth mixtures or concentrated liquid enzymes are transferred between separate unit operations from the fermentation process step to the enzyme standardization process step by a steam sterilized pipe network. Employee contact with the production process operation, once the raw materials have been deposited into their individual container vessels, is minimal other than for equipment maintenance or manual broth sample extraction. The inoculating procedure not only ensures that a pure culture is delivered to the seed fermentor, but also reduces the possibility of employee contact with the microbial biomass.

There are minor potentials 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. A local

exhaust hood which is attached to the sampling port valve helps to reduce this potential for exposure during the manual broth sampling. All dumping stations are equipped with local exhaust ventilation hoods with bag filters built into each exhaust. The hoppers, into which the raw materials are deposited, are equipped with interlocked doors which turn on the exhaust fans when the doors are opened. The vapor emissions system, which includes emissions from the belt filters, rotary vacuum drum filters, ultrafiltrator, and processing tank, is connected to two water-sprayed rotoclones. All controlled vapors and remaining dust in the plant is exhausted through the rotoclones. There is some recirculation of the exhausted air, but this is dependent upon the outside environmental conditions (climate controlled). The offices, labs, and computer control room are on a separate ventilation system from the production area.

The overall process technology is recent. The majority of the large-scale process operations are capable of being either controlled or monitored by a computer system. This "automation" aids in limiting direct employee involvement, and therefore potential hazard exposure or contact, with the process operations.

Work Practices:
Miles maintains a relatively clean occupational environment -- generally, to reduce the threat of contaminating an enzyme broth. But, this attitude also benefits the workers by helping to prevent the unnecessary exposure to hazardous agents or conditions. If an enzyme spill occurs, it is washed (flushed) down into the plant sewer system. Diatomaceous earth spills are removed with an industrial vacuum cleaner.

Monitoring:
The environmental health program for the Miles enzyme operation is monitored on the corporate level. The responsibilities of the Safety and Health and Medical Departments are for the entire plant complex and its employees. Part of the environmental health program, as it applies to enzyme production, has been the conduct of settling plate samples. These samples indicated strictly enzyme producing or non-producing colonies. Miles is attempting to develop a total (quantitative) colony count sampling methodology. They are also attempting to develop a procedure (activity test) for detecting minute quantities of enzyme in the ambient air -- some bulk samples have been conducted.

Pre-placement medical evaluations are conducted including a complete medical history, pulmonary function test, audiometric test, visual exam, cardiogram, CBC, urine analysis, and a SMA-14. Periodic medical evaluations are selectively performed. If a problem is encountered with an enzyme production employee, the appropriate actions are taken to determine and correct the problem, for example, an allergy battery and interpretation or a radioallergosorbent test (RAST).

Personal Protective Equipment:
Inhalation of diatomaceous earth (amorphous silica) is possible during the dumping of bags of diatomaceous earth. Disposable respirators (3M model 8710) are used when employees are engaged in this operation. Goggles, faceshields,

and gloves are required during the bag dumping and handling of acids and caustics.

Miles employs a confined space procedure when an employee is required to enter a tank for maintenance or other purposes. Emergency escape units are available during tank entry operations.

## 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 include 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 may be useful in detecting sensitization of any exposed workers.

Miles is using a system of control measures which are intended to limit the potential for exposure to the microorganisms, process chemical intermediates, and/or the biological products of the enzyme operation. These measures seem to be based on sound design principles, based on this preliminary survey. Therefore, the Miles Laboratories, Inc. enzyme production plant will probably be recommended for an in-depth survey to evaluate these control measures.