

WALK-THROUGH SURVEY REPORT
CONTROL TECHNOLOGY FOR FERMENTATION PROCESSES

AT

Novo Biochemical Industries, Inc.
Franklinton, North Carolina

REPORT WRITTEN BY:
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NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Physical Sciences and Engineering
Engineering Control Technology Branch
4676 Columbia Parkway
Cincinnati, Ohio 45226

PLANT SURVEYED

Novo Biochemical Industries, Inc.
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P.O. Box 567
Franklinton, North Carolina 27525

SIC CODE:

2869

SURVEY DATE:

June 8, 1983

SURVEY CONDUCTED BY:

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No Employee Representatives

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 Novo Biochemical Industries, Inc. (NBI), 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:

Novo Biochemical Industries, Inc. (NBI) is located in Franklinton, North Carolina, and has produced enzymes for its parent company, Novo Laboratories, Inc., Wilton, Connecticut, since March, 1979. Novo Laboratories, Inc. is the U.S. branch of Novo Industri A/S, an international manufacturer and supplier of industrial and health care products headquartered in Bagsvaerd, Denmark. Novo Industri A/S is the world's largest producer of enzymes for industrial applications. Enzymes manufactured at NBI are distributed in the U.S. and Canada.

NBI's employee population is separated into 6 departments; administration, production, maintenance, quality control, finance, and personnel. Approximately 50% of the manufacturing workforce (a segment of the production department) is composed of women. Enzyme production is maintained 7 days per week, 24 hours per day in 12 hour work shifts. An individual manufacturing employee will work 36 hours one week and 48 hours on the next consecutive work week. This helps to reduce the problems of shift changes. Manufacturing employees are not permitted to work more than 12 hours in any 24 hour period.

Process Description:

The processes surveyed at NBI involve the production of two industrial enzymes, α -amylase and amylglucosidase with microbial strains of *Bacillus licheniformis* and *Aspergillus niger*, respectively. Both strains of microorganisms are non-pathogens. The manufacture of the industrial enzymes is accomplished in five basic process steps: selection of a microorganism; maintenance of the selected culture; fermentation; concentration and purification of the enzyme product; and standardization of the activity of the enzyme. Neither the selection or the maintenance of the microorganisms is conducted at NBI.

The selection or screening process for microorganisms determines each culture to be used for a specific enzyme production operation based on their tested ability to produce a commercial quantity of the desired enzyme. Selected cultures must be identified and tested for pathogenicity and their desired inability to co-produce harmful products or toxins, such as mycotoxins or enterotoxins.

The next process step, maintenance of the selected culture, must ensure that the isolated culture supplied to NBI for large-scale manufacture is a pure, uncontaminated culture medium. This requires that the culture be regrown at intervals. Single colonies are selected for regrowth usually on the basis of culture morphology. The selected culture is grown, harvested, sub-divided, and stored at the appropriate conditions to maintain its viability and purity. Before the culture is used for large-scale fermentation, it is tested to determine whether any desirable characteristics have been lost or undesirable characteristics have appeared. All operations through the first two process steps are conducted in the laboratory using sterile equipment with aseptic transfer.

NBI 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 through a pipe network 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 a pre-sterilized nutrient medium, is inoculated with the biomass broth from the seed fermentor. This new broth mixture is aerated, mechanically agitated, and allowed to ferment for continued 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.

The raw materials used to prepare the fermentation nutrient medium are either food grade materials or are tightly controlled to prevent contaminants that would inhibit organism growth or enzyme production -- the raw materials should not contain toxic or harmful compounds that could be carried through the process into the final product. Each raw material is contained in a separate tank before being combined in a batching tank to make up the nutrient medium. There is no employee contact with the raw materials after they have been deposited into their individual tanks. Sterilization of the nutrient medium is accomplished with steam in the seed fermenter or large fermenter tank, depending on where the nutrient is to be used.

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 that can be monitored are the %CO₂ and O₂ 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 all the fermenter tanks for analysis in the laboratory for microbial morphology, pH, dissolved solids, percent mycellium volume, viscosity, stray organism contamination, etc.

Upon completion of the fermenting cycle, the enzyme slurry is cooled and piped to a refrigerated holding tank, where agitation is maintained, to await the concentration and purification processing step. Filter aids, pH adjusters, preservatives, etc. are subsequently added to the slurry as a pretreatment to the processing operation. The enzyme slurry is pumped to a rotary vacuum drum filter (filter aids are 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 filter aid material. The sludge from the mycellium is steam sterilized and diluted, it is then applied to land surrounding the plant, owned by NBI, as fertilizer for hay crops. Waste water, water used as a liquid wash for process operations, from the plant is perfused through a primary treatment system (activated sludge digestion) to reduce the BOD (Biochemical Oxygen Demand) and this treated water is then used to spray irrigate the hay fields. Further concentration and purification of the enzyme will be accomplished utilizing a vacuum evaporator, ultrafiltrator, and bacterial filter. During this process step, samples are periodically extracted and analyzed for enzyme activity and other properties. Tight controls are necessary to ensure the process is economic and that the final enzyme product will be of food grade quality where applicable.

The final step in the NBI enzyme manufacturing process will be to standardize the activity of the purified enzyme concentrate. This is accomplished by simply blending the enzyme with inert, food grade ingredients. The final product is then packaged in structurally and chemically appropriate containers or drums.

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. As indicated above, the microorganisms utilized in the processes surveyed at NBI for their enzyme operations (*Bacillus licheniformis* and *Aspergillus niger*) are non-pathogens.

Filter aids, which may be diatomaceous earth (amorphous silica), are 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/m³/SiO₂ averaged over an eight hour work shift. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a maximum exposure of 1.5 mg/m³ of respirable dust 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 be primary irritants of 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 of or skin irritation due to the contact to enzymatic 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 NBI is discussed below.

Engineering Controls:

NBI's 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 microorganisms. 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. All bag dumping stations, which includes the dumping of raw materials, acids, and bases into their separate container vessels and diatomaceous earth for the rotary vacuum drum filter, are equipped with a local exhaust ventilation outlet. There is minor potential for release of aerosols containing viable microorganisms and/or enzymes during manual sampling procedures and circumjacent to the agitator shafts, but the quantities involved pose minimal contact or exposure concerns. Potential

aerosolization of liquid enzyme concentrates or microorganisms into the occupational environment at the rotary vacuum drum filter is limited by a local exhaust ventilation hood.

The overall process technology is recent, within the last three years, and therefore relatively advanced. The majority of the large-scale process operations are either controlled or monitored by a computer system which is centrally located within the production building. This "automation" aids in limiting direct employee involvement, and therefore potential hazard exposure or contact, with the process operations.

Work Practices:

NBI requires that their employees maintain a clean occupational environment; not only to ensure that the final product remains free of contaminants, but also to prevent the workers from being unnecessarily exposed to hazardous agents or conditions. Good housekeeping is promoted as part of this "clean" attitude in the safety procedures. In addition, a spill control procedure has been outlined and implemented within the Manufacturing Area. The procedures attempt to address and resolve two problems; one, control of the spill and clean-up of the spilled material, and two, disposal of the spilled material and its effect on the NBI waste treatment system. The procedures include spills pertaining to food grade ingredients or chemicals, salts, bases, acids, oils and refrigerants, and fuel oils. Employees are expected to include themselves as part of this clean work environment. Clean clothes, provided and cleaned by NBI, are required everyday. Showers are also required at the end of every work day -- lockers are also provided for each employee.

NBI employs a computerized preventative maintenance program as part of their "good" work practice regime. Weekly printouts are provided by the computer detailing the equipment and/or instruments in need of routine maintenance. There is also a monthly, quarterly, or elapsed time, dependent upon the degree of bearing usage, vibration analysis conducted on all bearings.

Monitoring

The environmental health program in effect at the Franklinton plant is monitored by the Quality Control Manager of NBI. Although NBI does not employ a full-time industrial hygienist at the plant, there is a corporate industrial hygienist available on a consulting basis from Novo Laboratories, Inc., Wilton, Connecticut. As part of this program, routine workplace concentration monitoring is conducted for active aerosolized liquid enzymes. Samples are taken at six different monitoring locations utilizing a Galley high-volume sampler. All assays are accomplished in-house at the Franklinton plant laboratory.

NBI implements a relatively complete medical/biological examination and monitoring program. Pre-screening employee physicals are conducted including a complete allergy battery and interpretation. Blood samples are taken annually from all employees for Radioallergosorbent (RAST) Tests to determine whether a response is occurring to specific antigen-producing compounds to which they may be exposed. Exposure records are maintained for each employee. Annual audiometric tests are conducted in order to monitor employees' hearing ability and to note any changes or deterioration that may

occur. Annual physical examinations for employees include urine specimens, pulmonary function, chart eye checks, ear checks for wax accumulation, tetanus toxoid or booster (every 5 years), and a review of employees' previous physical examinations records. A heavy emphasis is placed upon the respiratory evaluation section of the annual physicals. There are no medical practitioners (doctors, nurses, etc.) on call at the plant during normal working hours, however, there are two local physicians used for physicals and medical emergencies. In addition, there is a rescue squad available 3 miles from the plant complex to the west in Franklinton and a hospital located 6 miles to the east in Louisburg.

Personal Protection.

NBI's safety program and operations are guided by a Safety Committee composed of a chairman and two members, one salaried and one hourly, from each of the following Departments, Maintenance, Manufacturing, Farm, and Laboratory. In addition a member of the Personnel Department serves on the Committee. The chairmanship rotates between departments. This committee conducts monthly meetings and makes quarterly safety inspections of all facilities. Quarterly safety lectures for the workers are maintained with additional programs in emergency training and Cardio Pulmonary Resuscitation (CPR). Safety problems are considered a priority. All accidents are documented. NBI claims to have had 3 years with no lost-time accidents.

Personal protection requirements are part of the NBI safety procedures. Safety glasses are required to be worn at all times except when face shields or goggles are required. Safety shoes are required to be worn at all times except for "walk-throughs." Ear protection is required to be worn while working in the evaporator and utility rooms. Disposable dust respirators are required to be worn in all bag emptying processes and areas where enzyme contamination is suspected. Disposable dust respirators are also required when repairing the internal portions of these units where exposure may be expected -- this includes the filter changing operation in the heating and ventilation units. Acid goggles, rubber gloves, and an apron is required to be worn while transporting or handling acids and caustics. A respirator (Willson cannister type - Type H-3), rubber gloves, and a rainsuit are required to be worn whenever a worker is handling formaldehyde.

NBI employs a company procedure for entering a deep-tank reactor vessel. These procedures include a second person as an observer, continuous fresh air replenishment inside the tank during the complete operation, and a safety harness attached to a mechanical lifting device.

IV. CONCLUSIONS AND RECOMMENDATIONS

NBI has provided, for the employees, an occupational environment with an exhibited concern for worker safety and health. An outgrowth of this management attitude, in combination with current state-of-the-art technology, is a seemingly effective system of control measures. These control measures are, in part, responsible for the limited potential for exposure to the microorganisms, process chemical intermediates, and/or the biological products of the enzyme operation. Occupational health and safety conditions appear to be reasonably-well controlled at NBI; therefore, the Novo Biochemical Industries, Inc. enzyme production plant is recommended for an in-depth survey.