

WALK-THROUGH SURVEY REPORT:
CONTROL TECHNOLOGY FOR FERMENTATION PROCESSES

AT

Gist-Brocades USA, Inc.
Kingstree, South Carolina

REPORT WRITTEN BY:
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REPORT DATE:
August 1984

REPORT NO.
116-19a

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Physical Sciences and Engineering
Engineering Control Technology Branch
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PLANT SURVEYED: Gist-Brocades USA, Inc.
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SIC CODE: 2869

SURVEY DATE: February 14, 1984

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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.

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. 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 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 that would be 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 Gist-Brocades USA, Inc. (GB), 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:

Gist-Brocades USA, Inc. (GB), formerly known as GB Fermentation Industries, Inc., is located in Kingstree, South Carolina, and has produced industrial grade enzymes at this plant for the last seven years. The Kingstree fermentation plant was originally constructed in 1957 by the Wallerstein Company, a wholly-owned subsidiary of Baxter-Travenol, Inc. The plant was purchased by Gist-Brocades, nv in 1977 -- Wallerstein continues to operate a large manufacturing plant adjacent to the GB complex. Gist-Brocades, nv, based in the Netherlands, is a major manufacturer of enzymes, yeast, and antibiotic products. GB is headquartered in Charlotte, North Carolina.

GB employs a total population of approximately 160 workers at the Kingstree plant facility. This population is separated into six Departments; Personnel, Engineering, Maintenance, Quality Control, Laboratory, and Fermentation and Recovery. The plant operates continuously using three (3) workshifts weekdays and two (2) workshifts on weekends (12 hours each), 24 hours per day, seven (7) days per week. Enzyme production is maintained with five work crews -- two crews are off duty on any given weekday, three on weekends.

Process Description:

The processes surveyed at GB involve the production of three industrial enzymes; α -amylase, amyloglucosidase, and protease using microbial strains of *Bacillus subtilis*, *Bacillus licheniformis*, and *Aspergillus niger*. The amyloglucosidase is produced from a pure culture fermentation of *Aspergillus niger*. Both strains of microorganisms are non-pathogens and are generally weakened or debilitated. The manufacture of the industrial enzymes is accomplished in three process steps; laboratory, fermentation, and recovery. The fermentation and recovery process steps are conducted in two buildings each employing similar process equipment -- although, the equipment in the "west" building is chronologically younger and technically more advanced than that in the "east" building. The laboratory is located in a separate building (with offices) away from the east and west buildings housing the fermentation and recovery equipment.

The laboratory process step includes the selection and maintenance of microorganism cultures. 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. Maintenance of the selected culture must ensure that this isolated microbial culture is a pure, uncontaminated culture medium before being inoculated into the inoculum tanks. 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 (from stock cultures and propagated in shaker flasks), harvested, sub-divided, and stored at the appropriate conditions to maintain its viability and purity. Microbial cultures are transferred manually and aseptically inoculated, maintaining pure cultures, into the inoculum tank for the first segment of the fermentation process.

The fermentation process step is segmented into two parts. In the first part, 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). In the second part, where "fermentation" essentially occurs and the product of interest is biologically synthesized, a submerged, batch fermentation process is employed using a 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. Some of the reactor vessels are cooled with a jacket located on the outside. 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 nutrient medium used in the inoculum and fermentor tanks is prepared in mix mash tanks using raw materials which are of a suitable purity, free of harmful substances. The ingredients used are tightly controlled to prevent contaminants that would inhibit microorganism growth, enzyme production, and produce a finished product which, in the case of a food grade enzyme product, meets the specification for enzymes contained in the Food Chemicals Codex (FCC III).

Measurements are performed continuously during the fermentation process step to check specific parameters of the biomass broth. These measurements include control of process parameters such as temperature, pH, and dissolved oxygen. Manual samples are also extracted periodically from a sampling port on the fermenter tank for analysis in the laboratory.

The recovery process step is where the enzyme slurry, from the fermentor tank, is purified, concentrated, stabilized, and spray-dried (where applicable). Filter aids, pH adjusters, stabilizers, etc. are added to the slurry as a pretreatment to processing. 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 solid wastes from the vacuum filter are discharged to dumpsters and transported to landfills. Further concentration and purification of the enzyme will be accomplished utilizing a vacuum evaporator, ultrafiltrator, and bacterial filter. The enzyme source microorganism determines which concentration process will be used; ultrafiltration, vacuum evaporator, or a combination of both. Liquid wastes from these processes are treated in the plant sewage treatment system. The small percentage of enzymes that are to be sold as solids are atomized in an enclosed spray-drying system.

The final process is to standardize the activity of the purified enzyme concentrate. This is accomplished by simply blending the enzyme with inert,

food grade (where applicable) 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 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 may pose to the exposed worker. As indicated, the microorganisms utilized in the processes surveyed at GB (*Bacillus subtilis*, *Bacillus licheniformis*, and *Aspergillus niger*) are non-pathogens.

Filter aids, such as 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 $80 \text{ mg/m}^3/\% \text{SiO}_2$ 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 of respirable amorphous silica 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.

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 GB is discussed below.

Engineering Controls:

GB'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, once the raw materials have been added to the mix mash tanks, is minimal other than for equipment maintenance or manual broth sample extraction.

Minor potential for release of aerosolized viables or enzymes exists during manual sampling procedures and circumjacent to the agitator shafts, but the quantities involved probably pose minimal contact or exposure concerns. To minimize the aerosolization potential around the agitator shafts, GB has encased the shafts (where they enter the inoculum and fermentor tanks) with steam sterilizable seals. Aerosolization of microorganisms and liquid enzymes

can occur from the rotary drum vacuum filter, which is open to the environment. Raw materials dumping stations are equipped with local exhaust ventilation hoods to reduce dust levels. Blending and packaging operations (of enzyme solids) are conducted in separate, enclosed rooms with local exhaust ventilation hoods in place. Exposures to enzyme dusts are limited to the period when full containers are replaced with empty containers to be filled. Negative pressure is maintained in the buildings housing the fermentation and recovery equipment with general ventilation -- air flows in through louvers on the side walls and is forced out through fans in the ceiling.

Work Practices:

GB maintains a relatively clean occupational environment -- generally, to reduce the possibility of contaminating an enzyme broth that is in production. This clean environment also helps to prevent any unnecessary exposures to employees from hazardous agents or conditions.

The employees play a major role in the development of safety and health guidelines in the GB plant. Using the concept of "quality circles", employees select safety related projects that they have collectively researched and present them to management for consideration. The employees initiate the engineering studies needed to evaluate the feasibility of these projects but the studies are actually conducted by the Engineering Department. Employees may also submit project studies that are directly related to process operations. As part of their safety program, GB has had organized, for a number of years, two safety committees. The first committee is composed of randomly selected employee representatives of each department who meet once a month. The second committee is composed of management personnel and meets one week after the employee committee meeting to discuss the relevant topics of that meeting. This two-committee structure offers an "umbrella" view of plant safety and health issues.

Monitoring:

GB does not currently have an environmental health program but is in the process of developing a committee to oversee all health hazard issues (a Health Assurance Committee). Specific health hazard issues of concern include; audiometric studies, pulmonary studies, sensitivity studies (to enzymes), and environmental sampling methods. The committee will be composed of personnel from the corporate level and from the production plant (production workers) in Kingstree. Additionally, an industrial hygienist and an occupational health physician, outside consultants, will sit on this committee.

Pre-employment physical examinations are given to all new employees of GB. Subsequent examinations are administered by a South Carolina mobile health unit on an annual basis and include audiometric tests and pulmonary function tests. The parent company, Gist-Brocades nv, has conducted tests for enzyme sensitivity among its employees overseas (using a radioallergosorbent test -- RAST). A RAST is not used at the Kingstree facility.

Personal Protection:

Employees engaging in operations at dumping stations are required to wear disposable dust respirators. Canister type respirators and self-contained breathing apparatuses (SCBA) are available if needed.

IV. CONCLUSIONS AND RECOMMENDATIONS

GB does not currently have a formal occupational health program at their Kingstree facility. They do, however, have experience (over several years) with safety committees and have begun using the concept of "quality circles" for safety related projects. GB is also in the process of developing a Health Assurance Committee which will oversee all health related issues and will establish programs for occupational health hazards in the plant. These current and proposed programs, in addition, to a seemingly effective system of control measures exhibits a high level of concern for worker safety and health. The control measures in place appear to provide for the limited potential of exposure to the microorganisms, process chemical intermediates, and/or the biological products of the enzyme operation. The Gist-Brocades USA, Inc. enzyme production plant is recommended for an in-depth survey.