

IN-DEPTH SURVEY REPORT:  
CONTROL TECHNOLOGY ASSESSMENT OF ENZYME FERMENTATION PROCESSES

AT

Gist-Brocades USA, Inc.  
Kingstree, South Carolina

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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; abrasive blasting,<sup>1</sup> the plastics and resins industry,<sup>2</sup> foundry operations,<sup>3</sup> spray painting and coating,<sup>4</sup> and coke oven emissions.<sup>5</sup> 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.

### BACKGROUND FOR THIS STUDY

NIOSH's research responsibility extends to both existing and emerging technologies which may affect worker health and safety. The attempt to examine new technologies for potential occupational hazards specifically focuses on those technologies which have high growth potentials or for which exposures to particular agents have not been fully characterized. In past research activities, NIOSH has been instrumental in the development of recommendations for safeguarding the workers health from exposure to occupational hazards. Implementation of safeguards and protective engineering controls early in the growth of an industry will minimize occupational health problems and avoid expensive retrofitting of production systems.

NIOSH is currently interested in evaluating the potential hazards and their control for applications of biotechnology and recombinant DNA (rDNA). ECTB's involvement in this NIOSH evaluation is to assess the control technology being

employed to minimize the potential for occupational health hazards in the enzyme fermentation industry. The results of this control technology assessment will be used to develop an informational database that could be extrapolated to other fermentation product technologies. Previous NIOSH research into biotechnology includes a study of six companies employing rDNA techniques in their research activities or their process operations. This earlier study, conducted by the Division of Surveillance, Hazard Evaluation, and Field Studies, was published in a NIOSH report and a journal article.<sup>6,7</sup>

The ECTB study focused on conventional enzyme fermentation process operations. Several factors contributed to the final decision to focus this research project. First, the products manufactured in the overall fermentation industry, although dissimilar entities, are produced with a somewhat standardized process technology. Product recovery operations may vary with the product properties, source microorganisms, and base solvents used, but the basic fermentation technology remains essentially the same. Second, the diversity of the fermentation industry would require different environmental air sampling and analytical methodologies for each product and source microorganism studied. Narrowing the field of investigation satisfied the need to limit the "products" studied in order to minimize sampling and analytical methods development requirements. Third, there was a good probability of finding well controlled processes in the enzyme industry. Last, there existed limited resources (including manpower and finances) with which to conduct this study and time constraints on its completion. Initial studies of various enzyme production plants identified several well controlled processes. Additional studies may evaluate other areas of the fermentation industry including antibiotic, hormone, and steroid production.

This control technology assessment of enzyme fermentation processes attempted to identify effective controls applicable to processes involving microorganisms, processing chemicals, and biologically active products or intermediates. The documentation of effective controls and recommendations to minimize exposure in the enzyme fermentation industry are among the primary objectives of this assessment. Recognizing that the enzyme industry only represents a small segment of the biotechnology industry, the collected data and subsequent evaluation will help to establish a baseline of information on the equipment (and related safety and health programs and practices) currently used in enzyme fermentation operations. This baseline of information will be available for transfer to other fermentation technologies, either those involved with rDNA technology or those utilizing conventional technology.

#### BACKGROUND FOR THIS SURVEY

Selection of plants for inclusion in this study of enzyme fermentation processes as in-depth surveys was based on a number of criteria. First, the plant (or parent company) should be a major manufacturer of industrial enzymes or have extensive experience related to fermentation technology. Second, the process operations should be technically current to insure the transferability of the survey results to other fermentation industries -- including those recombinant DNA companies scaling up operations to commercial production capacity. Third, the plants should exhibit an expressed concern for the safety and health of the workers. This would involve adherence to any or all

of the aspects of control technology to protect the worker including engineering controls, personal protective equipment, work practices, and industrial hygiene monitoring.

Gist-Brocades USA, Inc. (GB) met all three of the in-depth survey selection criteria requirements. GB's parent corporation, Gist-Brocades, nv, based in the Netherlands, is a major manufacturer of enzymes, yeast, and antibiotic products. As such, GB has available a broad base of experience, from the parent company, related to fermentation technology concerning enzymes, in addition to yeast and antibiotic production. Although the fermentation equipment has remained relatively unchanged since its construction in 1957, a new semi-automatic recovery operation was constructed in 1983. GB is currently in the process of developing a Health Assurance Committee to oversee all health related issues that will establish programs for occupational health hazards in the plant.

The in-depth survey of GB was conducted on June 24 - July 3, 1985 to evaluate the controls and containment capabilities of their proteolytic enzyme manufacturing process. This report documents the information pertinent to that evaluation.

## II. PLANT AND PROCESS DESCRIPTION

### Plant Description:

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 eight 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 -- Baxter-Travenol continues to operate a large manufacturing plant adjacent to the GB complex. GB is headquartered in Charlotte, North Carolina.

GB employs a total of approximately 160 workers at the Kingstree plant facility. The plant is separated into six Departments; Personnel, Engineering, Maintenance, Quality Assurance, 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 process surveyed at GB involves the production of the industrial enzyme protease using a microbial strain of *Bacillus subtilis*. This *Bacillus* strain is a non-pathogen and believed to be weakened or debilitated. The manufacture of the industrial enzyme is accomplished in three process steps; laboratory, fermentation, and recovery. The laboratory is located in a separate building (with offices) away from the building 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 pure and uncontaminated 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 (air is sterilized with a 0.22  $\mu$ m pore size filter). 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. Prior to inoculation, the fermentor tank is cleaned with a caustic solution and then sterilized empty with steam for 45 minutes. The fermentor tank, containing a pre-sterilized nutrient medium, is then 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 or enzyme production, and produce a finished product which, in the case of a food grade enzyme product, meets the Food and Drug Administration's 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 fermentor tank for analysis in the laboratory.

In the recovery process step, the enzyme slurry, from the fermentor tank, is purified, concentrated, and stabilized. Acetone, filter aids, pH adjusters,

stabilizers, etc. are added to the slurry as a pretreatment to processing in the mash treatment tank. The enzyme slurry is pumped to a plate and frame filter press where a major portion of the suspended solids (mycellium and other solids) are separated from the enzyme liquid. An operator "knocks" the cake off of the filter cloth when the press is open. The filter cake is discharged to a dumpster via a belt conveyor where it will be subsequently picked up and later transported to a landfill. The enzyme liquid is then pumped to a vacuum evaporator for removal of the acetone. The acetone is transferred to a recycling process. Further concentration and purification of the enzyme will be accomplished utilizing a candle filter, ultrafiltrator, and bacterial filter (plate and frame press). Liquid wastes from these processes are treated in the plant sewage treatment system.

The final process is to standardize the activity of the purified protease enzyme concentrate with propylene glycol. The finished product is pumped into shipment tanks and sold as bulk liquid.

#### Potential Hazards:

The potential for exposure to hazards in the occupational environment within the fermentation industry in general 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 microorganism utilized in the process surveyed at GB (*Bacillus subtilis*) is a non-pathogen. However, increasing attention is being focused upon the potential for immunologic response, after repeated inhalation, to a variety of organic materials including microorganisms. There are currently no reports of these effects in the enzyme industry. Cases of hypersensitivity pneumonitis have been documented in individuals exposed, in the occupational environment, to fungi, thermophilic actinomycetes, as-well-as animal proteins.

Filter aids, such as diatomaceous earth (amorphous silica), are used in the concentration and purification processing step. 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 the quotient of  $30 \text{ mg/m}^3$  divided by the percent of silica present. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a maximum exposure of  $1.5 \text{ mg/m}^3$  of respirable amorphous silica over an eight hour work shift.

Acids and bases are used to adjust pH levels of biomass broth mixtures or concentrated enzyme liquids throughout the enzyme production process; both

will cause burns. Depending on 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.

Acetone is added to the enzyme rich broth prior to the recovery process. Repeated contact exposure (percutaneous absorption) to acetone may produce dry, scaly, and fissured dermatitis. Inhalation of high concentrations of acetone vapors may irritate the conjunctiva and mucous membranes of the nose and throat. Systemic reactions to high concentrations include headaches, nausea, light headedness, vomiting, dizziness, incoordination, and unconsciousness. The current OSHA standard for acetone is 1000 ppm averaged over an eight hour workshift.

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. ACGIH recommends a maximum exposure of 0.8 Delft Units (DU) per m<sup>3</sup> of air of subtilisin proteolytic enzyme over an eight hour work shift. A DU is calculated from the amount of proteolytic enzyme that is required to act on a protein substrate in a specified amount of time.

### III. METHODOLOGY

To effectively evaluate the controls and equipment in place at GB, environmental air samples were taken at strategic locations believed to duplicate workplace exposures and indicate emission sources. The major pieces of equipment used in this evaluation are listed in Table I. of the Appendix.

#### MEASUREMENT OF CONTROL PARAMETERS

##### Viable Sampling

To determine concentrations of airborne microorganisms around unit processes, the Andersen 2-stage viable sampler was used at a flow rate of 1 cubic foot per minute (CFM). Locations for viable samples include the laboratory, inoculum tank, fermentor tank, filter press, and in areas outdoors and in the office building -- the latter two samples sites were selected to give approximations of normal background levels. Some area samples were taken as side-by-side (two Andersens) samples to monitor variability of the microbial air samplers. The samples were collected over a five day period to detect day-to-day variability, if any. Sample times varied from 20 minutes down to



2-1/2 minutes depending on the sample location. For example, a sampling time of 20 minutes was used in areas where microbial concentrations (in the laboratory) were expected to be low and a 2-1/2 minute sampling time was used in areas of high microbial concentration (around filtering operations). Standard Methods Agar was used as the sampling media in each stage of the viable sampler. The 50% effective cutoff diameter for the top stage of the Andersen viable sampler is 8.0 um -- larger, non-respirable particles are collected on the top stage, smaller, respirable particles are collected on the bottom stage.

Analysis of the viable samples was conducted on-site by a Center for Disease Control (CDC) microbiologist and a NIOSH biologist. The primary goal of the microbiological analysis was to determine the numbers of the production microorganism in the air at different locations in the plant. All air sampling plates were counted at 24 hours using standard colony counters. Colonial morphology was compared with that of the production strain of the same age and on the same medium. Where possible, colonies resembling the production strain were included as a separate count. A percentage of these typical colonies were streaked to Standard Methods Agar (with Manganese) for isolation and identification. Colonies were identified by gram stain and/or the Rapid CH kit manufactured by API System, S.A. This identification scheme consists of 49 biochemical tests read at 24 and 48 hours.<sup>8</sup> Results were compared to the Rapid CH profile of the index strain. Each step of the microbial identification process by itself is not an absolute indicator of the production strain. However, combining the results of these identification tests will give the microbiologist the ability to produce reliable conclusions concerning the production strain.

Sample results are in terms of Colony-Forming Units per cubic meter of air (CFU/m<sup>3</sup>) with percentages of the production strain, where available. Sample concentrations around process operations are compared to control samples to help ascertain the degree of microorganism release from manufacturing processes.

#### Enzyme Sampling

Environmental monitoring of the airborne enzyme concentrations were conducted using General Metalworks high-volume samplers and high efficiency (pre-weighed) 8" by 10" glass fiber filters at a flow rate of approximately 40 CFM. The samplers were strategically positioned at fixed locations in the plant best suited to estimate exposure conditions and isolate points of enzyme aerosol release. Locations for the high-volume samplers include the fermentor, ultrafilter, candle filter, blending tank, and one outdoor background location. Samples were collected for eight hour workshifts over a four day period. Analysis of the enzyme samples was conducted on-site by a NIOSH chemist.

The 8" x 10" glass fiber filters were weighed before sampling on a Mettler AE 163 balance. The instrumental precision for one sitting is 0.01 mg. After sampling, the filters were equilibrated in the laboratory environment (cooled and dehumidified) and reweighed on the same balance. The difference in filter weights were recorded as total weight per filter. After gravimetric analysis,

each filter was agitated with a sonic bath in 100 ml of sodium tripolyphosphate/Brig 35 solution to elute the proteolytic enzyme from the filter. The remaining liquid was passed through a 0.45 um PTFE filter.

Samples and standards in duplicate were reacted with the substrate and incubated under stringent temperature, pH, and time controls as described in the GB protease enzyme activity method.<sup>9</sup> Standards were prepared from a GB manufactured protease of a known Delft Unit (DU) per gram. After an incubation period, the reaction was stopped, excess protein precipitated, and the absorbance of the supernatant measured on a spectrophotometer at a wave length of 275 nm. A calibration curve was prepared daily for each set of samples using a polynomial regression program on all of the calculations. The lower limit of detection and the lower limit of quantitation were determined from plots of the media blanks and the three lowest standards on one curve. The lower limit of detection is defined as the amount of material that can be distinguished from the blanks -- determined to be 50 DU per filter. The lower limit of quantitation is defined as the concentration that has a precision greater than 10% and is routinely three times the limit of detection or 150 DU per filter.

#### Total Dust Sampling

Total dust samples were collected on 37 mm, 5 um pore size PVC filters at an approximate flow rate 2.5 liters per minute (lpm) with Dupont 2500 pumps according to the NIOSH method No. 0500.<sup>10</sup> Samples were collected for eight hour workshifts over a four day period. The pumps were calibrated prior to the field survey. Sample locations included material dump stations, most areas where viable and/or enzyme samples were located, and those areas believed to approximate background dust levels for the plant.

The PVC filters were pre-weighed in the GB laboratory (on a Mettler AE 163 balance) and re-weighed under the same conditions after sampling. The difference between the initial weight and the weight after sampling is given as total weight per filter.

#### Acetone Sampling

Acetone samples were collected according to NIOSH method 1300.<sup>10</sup> Samples were collected with Dupont P-200 pumps at a flow rate of 50 milliliters per minute (ml/m) through standard 150 milligram charcoal tubes. The pumps were calibrated prior to the field survey. After sampling, the charcoal tubes were desorbed for 30 minutes in 1.0 milliliter of carbon disulfide containing 1 microliter/milliliter of hexane as an internal standard. A Hewlett-Packard gas chromatograph (model 5711A) equipped with a flame ionization detector was used for sample analysis. The Column was a 12' x 1/8" stainless steel, 10% TCEP on 80/100 Chromosorb P (AW). Oven conditions were set at 80°C, isothermal. Sample locations were focused around the filter press, for one hour intervals during times when the filter press was in operation and airborne levels would be expected to be at their highest. Samples were also collected at other locations within the recovery area (eg. acetone recovery).

#### IV. RESULTS

The results of the viable air sampling analysis is reported in the appendix in Table II and summarized in Table III. Background samples located outside were grouped into a single classification. This classification of outside background level was based on the assumption that uncontrollable environmental factors (eg. climatic conditions, surrounding traffic, etc.) had the only significant effect upon sample location variability. Effects on outside background samples due to plant unit processes, if any, were assumed to be uniform from sample location to sample location. The results were also assumed to be log normally distributed. The geometric mean of the outside background samples was 123.2 CFU/m<sup>3</sup> with a standard deviation of 7.7. Viable samples collected around selected unit processes ranged from geometric averages of 4.4 CFU/m<sup>3</sup> (clean room) to 10599.4 CFU/m<sup>3</sup> (open filter press). The highest microbial levels occurred around the filter press, dumpster, sample port (in use), and the agitator shafts. These levels were all significantly different from the outside background concentration. Viable samples were not collected around certain areas of the recovery process (mash treatment tank) due to the explosive nature of the acetone and the non-intrinsically safe label on the sampling pumps. All samples were blank corrected.

Results of the samples collected with the high-volume air sampler are reported in Table IV. Average enzyme levels ranged from 0.317 DU/m<sup>3</sup> at the candle filter to 0.45 DU/m<sup>3</sup> at the ultrafilter and the blending tank. There appears to be no statistically significant differences between sampling locations. All samples were blank corrected.

Acetone sample results (from the filter press and the mash treatment tank) are reported in Table V. The average concentration of all samples was less than 3.6 mg/m<sup>3</sup>. All samples are blank corrected.

Total dust sample results (from samples collected with the 37 um pore size PVC filters) are reported in Table VI. Average concentrations ranged from 0.02 mg/m<sup>3</sup> in the incubation room to 1.21 mg/m<sup>3</sup> at the dump station in the recovery area. Concentrations were comparable with the total dust results from the high-volume samplers. All samples are blank corrected.

#### V. CONTROL EVALUATION

##### 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 the fermentation industry, a debilitated production strain can also be an effective means of reducing the microbial level around unit process operations.

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 insure 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.

#### ENGINEERING CONTROLS

GB's enzyme production process is a predominately closed system (with few exceptions) once the process has graduated from the laboratory to the fermentation and recovery process steps. The process equipment is designed to keep microbial contaminants in the ambient environment from getting into the production culture. All growth and holding tanks are closed during process operations. The culture broth is transferred between separate unit operations in the fermentation process step by a steam sterilizable 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.

#### Laboratory Process Step:

There are emission sources of the production microorganism, *Bacillus subtilis* (BS), during the laboratory process step but these sources are at very low levels due to the small quantity of the microorganism being used. Emissions in the main laboratory room were only possible during biochemical analysis of broth samples from the seed and fermentor tanks. General work practices of the lab workers constituted the greatest determinant of exposure to viable emissions. For example, mechanical devices were used for pipetting wet solutions and microbial cultures but oral pipetting was also observed during the microbial transfer process in the clean room. The laboratory air quality was controlled with the building ventilation (heating and cooling) system. Two fume hoods were accessible, and adjacent to one another, in the wet chemistry area of the main laboratory for chemistry work. Viable samples in the main laboratory room indicated a microbial level of 147.3 CFU/m<sup>3</sup> with a standard deviation of 1.1. The percentage of counted colonies identified as the production strain was an average of 34.

Quality control analysis was conducted in a separate laboratory (MLT laboratory) and building (east building) from the main laboratory. The

geometric average of the microbial level in the MLT laboratory was 159.1 CFU/m<sup>3</sup> with a geometric standard deviation of 1.6 (an average of 35% of the counted colonies were determined to be the production strain). The survey microbiologic analytical team conducted their microbial analysis in this room and may have affected levels.

Possible emissions sites were also observed in the clean room -- during transfer of the BS cultures from vial to test tube, test tube to flask, and flask to inoculating devices. The clean room contains a horizontal laminar flow hood which purifies recirculated air with a High Efficiency Particulate Air (HEPA) filter. The hood is designed to pass purified air over the work zone, towards the lab technician, to protect the microbial cultures. As a consequence of the airflow directed away from the hood, possible microbial emissions are introduced into the technicians breathing zone. However, the large volume of air recirculated by the hood effectively reduces the concentration of any microbial emissions by diluting the air. The geometric average of the microbial level in the clean room was 4.4 CFU/m<sup>3</sup> with a geometric standard deviation of 5.1. The percentage of counted colonies identified as the production strain was an average of 76.

Flasks inoculated with the BS culture are transferred to an incubation room adjacent to the clean room. The incubation room is kept at a constant temperature and humidity for proper propagation of the microbial culture. The flasks (sealed with a cotton gauze stopper) are agitated on a shaker assembly for a required amount of time. The geometric average of the microbial level in the incubation room was 346.4 CFU/m<sup>3</sup> with a geometric standard deviation of 29.2. The percentage of counted colonies identified as the production strain was an average of 30.

The microbial culture is manually moved from the laboratory to the seed tank in a sterile, stainless steel inoculating device which serves as containment device during the transfer. The inoculating device is then connected to a steam sealed line on the seed tank and the microbial culture is released into the seed tank. The inoculating device is returned to the laboratory and autoclaved.

#### Fermentation Process Step:

Minor potential for release of aerosolized viables and/or enzymes exists at certain sites around the seed and fermentor tanks. These sites include the broth sampling ports, agitator shafts, and scrubber for the tank exhaust gases. Broth sampling at the seed and fermentor tanks was an intermittent operation. The sample port valve is closed and continuously steam sealed when not in use to prevent contamination of the culture broth. The steam seal also appeared to be effective in preventing the escape of viables from the sample port. During sampling, the steam seal is turned off and a shake flask and/or beaker is filled with broth. After sampling the valve is shut off, the steam is increased to clear the valve of remaining contaminants. The steam release observed was a completely opened valve which aerosolized any microorganisms remaining in the valve. This resulted in a visible brown haze released from the sample port. Proper company procedure is to open the steam valve only enough to gently wash any remaining microorganisms into a catch basin. No engineering controls or protective equipment was used during sampling; a

concrete curb surrounds the area below the fermentor tank to help contain spills. The sampling procedure was the same for the seed tank and the fermentor tank. The geometric average of the microbial level around the sampling port during manual broth sampling was 1668.3 CFU/m<sup>3</sup> with a geometric standard deviation of 1.6 and the percentage of the colonies identified as the production strain was an average of 77. The geometric average of the microbial level around the sampling port when there was no external activities was 195.5 CFU/m<sup>3</sup> with a geometric standard deviation of 2.3 and the percentage of the colonies identified as the production strain an average of 19. The former was statistically different from the average outside background levels for the sampling week whereas the latter microbial level was not indicating the release and viability of production strain BS during the sampling procedure.

The agitator shaft of the seed and fermentor tank is steam sealed with an additional bearing at the bottom of the tank to steady the shaft. Sampling results around the fermentor tank agitator shaft indicated a geometric average microbial level of 339 CFU/m<sup>3</sup> with a geometric standard deviation of 3.1. The percentage of counted colonies identified as the production strain was an average of 48. Sampling results around the seed tank agitator shaft indicated a geometric average microbial level of 1633.9 CFU/m<sup>3</sup> with a geometric standard deviation of 1.5. The percentage of counted colonies identified as the production strain was an average of 23. Both locations had levels that were statistically different from the average outside background level indicating the possibility of minor leaks around the agitator shafts.

The water scrubber (located on a platform 30 feet from fermentor tank agitator shaft) theoretically cleans the exhaust gases from the seed and fermentor tanks. Worker activity around the water scrubber was minimal other than for maintenance. Samples collected next to the scrubber showed a geometric average microbial level of 345.1 CFU/m<sup>3</sup> with a geometric standard deviation of 4.2. The percentage of counted colonies identified as the production strain was an average of 38. This level of microbials was statistically different from the outside background concentration indicating the ability of the water scrubber to only minimize the release of entrained production microorganisms. In situations requiring more stringent controls this may not be adequate.

A high-volume air sampler was placed in the vicinity (within 15 feet) of the fermentor tank agitator shaft and the water scrubber. Enzyme levels at this location were 0.325 DU/m<sup>3</sup> with a standard deviation of 0.248.

Broth samples from the mash treatment tank are manually collected from a port in the top of the tank. The tank is opened by the worker and the sample is taken with a dipper cup. Emission sources of the BS culture, proteolytic enzyme, and acetone could occur during this procedure. The total exposure time of the worker is small. Viable and enzyme air samples could not be taken at this location due to the explosive nature of the acetone and the sampling pumps were not intrinsically safe. Acetone samples were less than 0.01 mg/m<sup>3</sup>.

#### Recovery Process Step:

After acetone addition in the treatment tank, the microbial/enzyme broth is transferred through pipe to the automated filter press for an approximate two hour cycle. This cycle will occur many times during one enzyme fermentation batch. Automation of the filter press does not preclude worker interaction with the process; the worker must intervene at the end of a cycle to manually remove (with a wood oar) the filter cake. Levels during removal of the filter cake ranged as high as 28990 CFU/m<sup>3</sup> on a single sample. Geometric average microbial levels when the filter press was closed were 3906.1 CFU/m<sup>3</sup> with a standard deviation of 2.5. Geometric average microbial levels when the filter press was open were 10599.4 CFU/m<sup>3</sup> with a standard deviation of 1.8. Average levels would decrease by an approximate factor of 2 when the filter press was closed. Geometric average microbial levels when the filter press was closing after the filter cake had been removed were 8757.5 CFU/m<sup>3</sup> with a standard deviation of 1.4. Counts of the production strain on these samples were not made due to time constraints and other analytical factors but the production strain was noted as being the predominant strain at this location by the microbiologist. Geometric average microbial levels at the dumpster were 2400 CFU/m<sup>3</sup> with a standard deviation of 1.5 -- like the filter press, counts of the production strain were not available but were noted as being the predominant organism. Acetone samples collected around the filter press showed one sample at 3.64 mg/m<sup>3</sup> and two other samples less than 0.01 mg/m<sup>3</sup>. Acetone samples collected around the conveyor belt and at the mash treatment tank were all less than 0.01 mg/m<sup>3</sup>. Enzyme air samples could not be taken at this location due to the explosive nature of the acetone and the sampling pumps were not intrinsically safe. Only general dilution ventilation was observed including: two ceiling fans, two louvered windows, and one air supply duct on the top floor; and three wall fans, three louvered windows, and one air supply duct on the ground floor.

A local exhaust ventilation hood (with a pulse jet dust collector) was in operation at a dump station in the recovery area for various material additions to the enzyme liquid (diatomaceous earth, calcium carbonate, carbon black, etc.). The hood has a lid with a cylindrical opening into which specially designed bags fit when the lid is down -- effectively enclosing the dumping operation. Unfortunately, the hood was not used by the operator as designed. The operator, as observed, would normally leave the lid open and pour the raw material directly into the hopper negating the purpose of the "enclosed" system design. The total dust level at this location was 1.21 mg/m<sup>3</sup>. The total dust level in the recovery room at a location away from the dump station and filter press was 0.09 mg/m<sup>3</sup>.

During cleaning and polishing, the acetone-free enzyme liquid is processed through a candle filter. Immediately prior to this operation, filter aids (diatomaceous earth) are manually added to the liquid in a collection tank through a hatch at the top of the tank. Some of the dust generated from this dumping action was observed passing through the workers breathing zone on its way to a ceiling fan. Worker interaction after this point is limited to equipment maintenance and enzyme sample extraction. General area levels of enzyme were 0.317 DU/m<sup>3</sup> with a standard deviation of 0.126 around the candle filter and 0.45 DU/m<sup>3</sup> with a standard deviation of 0.232 around the blender tank. High-volume samples were also extracted around the ultrafilters with

levels of enzyme observed at  $0.45 \text{ DU/m}^3$  with a standard deviation of 0.228. Only dilution ventilation was observed in this area including four ceiling fans and two small louvered windows. One high-volume sample was taken outside between the laboratory and the recovery area with a level of  $0.269 \text{ DU/m}^3$ .

#### WORK PRACTICES

GB maintains an adequate housekeeping program around unit processes -- generally, to reduce the possibility of contaminating an enzyme broth that is in production. This housekeeping program also helps to minimize 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 two safety committees for a number of years. 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.



## VI. CONCLUSION

Viable sample concentrations around selected unit processes were compared (using the t-statistic for comparing two means) to background concentrations to ascertain the degree of containment of those processes.<sup>11</sup> The results indicate that emissions of the viables are most likely to occur at high-energy operations (where there is probable aerosolization) such as the agitator shafts, belt conveyor, filter press, and sampling ports. Another source of emission are the exhaust gases from the fermentor and seed tanks (345.1 CFU/m<sup>3</sup> with 38% production strain) indicating that the water scrubber does not completely control the release of entrained production microorganisms. In situations requiring more stringent controls, a water scrubber may not be adequate.

Work practices of the operators or technicians can be a determining factor in the degree of exposure at the filter press, at the sampling ports, and in the laboratory. Microbial levels at the filter press during removal of the filter cake (the operator knocks the cake off with a wood oar) ranged as high as 28990 CFU/m<sup>3</sup> on a single sample with an geometric average concentration of 10599.4 CFU/m<sup>3</sup> (the predominant colony was the production strain). Average levels would decrease by an approximate factor of 2 when the filter press was closed (3906.1 CFU/m<sup>3</sup>) and minimal surrounding activity was observed. Technicians at the sampling ports could possibly lower emissions by exercising proper company procedure by opening the steam valve gently. In the laboratory, pipetting of any solution by mouth is contrary to the safety procedures of any laboratory.

Enzyme levels at all sampling locations were below the ACGIH recommended Threshold Limit Value of 0.8 DU/m<sup>3</sup>. There appears to be no statistically significant differences between sampling locations.

Acetone levels in the enzyme recovery area were less than 0.01 mg/m<sup>3</sup> at all sampling locations with the exception of one.

## VII. REFERENCES

1. Enviro-Management & Research, Inc., "Abrasive Blasting Operations (Engineering Control and Work Practices Manual)," March 1976, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 76-179.
2. Enviro Control, Inc., "Engineering Control Technology Assessment for the Plastics and Resins Industry," March 1978, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 78-159.
3. Envirex, "An Evaluation of Occupational Health Hazard Control Technology for the Foundry Industry," October 1978, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 79-114.
4. O'Brien, D., and Hurley D., "An Evaluation of Engineering Control Technology for Spray Painting," June 1981, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 81-121.
5. Sheehy, J., "Control Technology for Worker Exposure to Coke Oven Emissions," March 1980, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 80-114.
6. Elliott, L., Carson, G., Pauker, S., West, D., Wallingford, K., Griefe, A., "Industrial Hygiene Characterization of Commercial Applications of Genetic Engineering and Biotechnology," September 1983, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health.
7. Landrigan, P., Cohen, M., Dowdle, W., Elliott, L., Halperin, W., "Medical Surveillance of Biotechnology Workers: Report of The CDC/NIOSH Ad Hoc Working Group on Medical Surveillance for Industrial Applications of Biotechnology," Recombinant DNA Technical Bulletin, 5(3): 133-138, September 1982.
8. The Identification of Bacilli Using the dms Rapid CH, dms Laboratories, Inc., Flemington, New Jersey.
9. Determination of the Protease Activity According to the Delft Manual Method, Gist-brocades USA, Inc., Charlotte, North Carolina, November 1982.
10. NIOSH Manual of Analytical Methods, Third Edition, 1985, U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Publication No. 84-100.
11. Miller, I. and Freund, J., Probability and Statistics for Engineers, Second Edition, Prentice-Hall, Edgewood, New Jersey, 1977.

TABLE I. Equipment Used on Field Survey

Item	Model	
Automatic balance	Mettler AE 163	gravimetric analysis
Automatic psychrometer	Vista Scientific Corporation	temperature and humidity measurements
Colony Counter	New Brunswick Scientific	colony counts and identification
High-volume air sampler	General Metal Works	enzyme and total dust sampling
Personal sampling pump	Dupont 2500	total dust sampling
Personal sampling pump	Dupont P-200	acetone sampling
Smoke tubes	Draeger	air flow patterns
Viable cascade impactor	Andersen 2-stage	microbial air sampling

TABLE II. Viable Sampling Results

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED	TOTAL	% RESP	CFU/m <sup>3</sup>	BS
						COUNT	CFU			
Background - RR tracks	6/24	1:30	1000	RESP	10	13	39	33%	137.3	NC
Background - RR tracks	6/24	1:30	1001	NON	10	26				NC
Background - RR tracks	6/24	1:45	1002	RESP	5	5	5	100%	35.2	NC
Background - RR tracks	6/24	1:45	1003	NON	5	0				NC
Background - RR tracks	6/24	2:15	1006	RESP	5	2	3	67%	21.1	NC
Background - RR tracks	6/24	2:15	1007	NON	5	1				NC
Background - RR tracks	6/24	2:25	1008	RESP	2.5	0	0	0%	0.0	NC
Background - RR tracks	6/24	2:25	1009	NON	2.5	0				NC
Background - RR tracks	6/24	2:40	1010	RESP	5	0	5	0%	35.2	NC
Background - RR tracks	6/24	2:40	1011	NON	5	5				NC
Background - RR tracks	6/24	3:18	1012	RESP	10	0	0	0%	0.0	NC
Background - RR tracks	6/24	3:18	1013	NON	10	0				NC
Background - RR tracks	6/24	3:36	1014	RESP	5	0	1	0%	7.0	NC
Background - RR tracks	6/24	3:36	1015	NON	5	1				NC
Background - RR tracks	6/24	3:50	1016	RESP	5	0	0	0%	0.0	NC
Background - RR tracks	6/24	3:50	1017	NON	5	0				NC
Background - RR tracks	6/24	4:08	1018	RESP	2.5	0	0	0%	0.0	NC
Background - RR tracks	6/24	4:08	1019	NON	2.5	0				NC
Background - RR tracks	6/24	4:15	1020	RESP	2.5	2	2	100%	28.2	NC
Background - RR tracks	6/24	4:15	1021	NON	2.5	0				NC
Background - RR tracks	6/24	4:26	1022	RESP	10	1	11	9%	38.7	NC
Background - RR tracks	6/24	4:26	1023	NON	10	10				NC
Background - RR tracks	6/27	9:39	1300	NON	10	38	128	70%	450.7	1
Background - RR tracks	6/27	9:39	1301	RESP	10	90				0
Background - RR tracks	6/27	9:54	1302	NON	15	120	260	54%	610.3	1
Background - RR tracks	6/27	9:54	1303	RESP	15	140				0
Background - RR tracks	6/27	10:14	1304	NON	10	46	91	49%	320.4	0
Background - RR tracks	6/27	10:14	1305	RESP	10	45				0
Background - RR tracks	6/27	10:29	1306	NON	10	49	100	51%	352.1	3
Background - RR tracks	6/27	10:29	1307	RESP	10	51				0
Background - RR tracks	6/27	10:46	1308	NON	10	97	222	56%	781.7	0
Background - RR tracks	6/27	10:46	1309	RESP	10	125				0
Background - RR tracks	6/27	11:01	1310	NON	10	28	91	69%	320.4	1
Background - RR tracks	6/27	11:01	1311	RESP	10	63				0
Background - RR tracks	6/27	11:17	1312	NON	10	10	10	0%	35.2	0
Background - RR tracks	6/27	11:17	1313	RESP	10	0				0
Background - RR tracks	6/27	11:33	1314	NON	10	66	195	66%	686.6	9
Background - RR tracks	6/27	11:33	1315	RESP	10	129				0
Background - RR tracks	6/27	11:47	1316	NON	10	114	243	53%	855.6	5
Background - RR tracks	6/27	11:47	1317	RESP	10	129				0
Background - RR tracks	6/26	11:58	1116	NON	10	13	27	52%	95.1	NC
Background - RR tracks	6/26	11:58	1117	RESP	10	14				NC
Background - RR tracks	6/26	2:05	1118	NON	5	0	10	100%	70.4	0
Background - RR tracks	6/26	2:05	1119	RESP	5	10				0

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED	TOTAL	%	CFU/m3	BS
						COUNT	CFU	RESP		
Background - RR tracks	6/26	2:15	1120	NON	16	57	126	55%	277.3	NC
Background - RR tracks	6/26	2:15	1121	RESP	16	69				NC
Background - water tower	6/27	1:41	1318	NON	10	74	151	51%	531.7	5
Background - water tower	6/27	1:41	1319	RESP	10	77				1
Background - water tower	6/27	1:56	1320	NON	10	34	76	55%	267.6	3
Background - water tower	6/27	1:56	1321	RESP	10	42				5
Background - water tower	6/27	2:11	1322	NON	10	16	30	47%	105.6	5
Background - water tower	6/27	2:11	1323	RESP	10	14				1
Background - water tower	6/27	2:24	1324	NON	10	107	169	37%	595.1	71
Background - water tower	6/27	2:24	1325	RESP	10	62				34
Background - water tower	6/27	2:39	1326	NON	10	35	88	60%	309.9	5
Background - water tower	6/27	2:39	1327	RESP	10	53				0
Background - water tower	6/27	3:11	1328	NON	10	54	137	61%	482.4	0
Background - water tower	6/27	3:11	1329	RESP	10	83				5
Background - water tower	6/27	3:25	1330	NON	11	51	192	73%	614.6	6
Background - water tower	6/27	3:25	1331	RESP	11	141				0
Background - water tower	6/27	4:19	1332	NON	10	41	108	62%	380.3	5
Background - water tower	6/27	4:19	1333	RESP	10	67				1
Background - water tower	6/27	4:32	1334	NON	10	17	26	35%	91.5	1
Background - water tower	6/27	4:32	1335	RESP	10	9				0
Background - water tower	6/27	4:45	1336	NON	10	12	15	20%	52.8	0
Background - water tower	6/27	4:45	1337	RESP	10	3				0
Background - water tower	6/27	4:58	1338	NON	10	281	424	34%	1493.0	NC
Background - water tower	6/27	4:58	1339	RESP	10	143				NC
Background - water tower	6/27	5:13	1340	NON	10	216	306	29%	1077.5	NC
Background - water tower	6/27	5:13	1341	RESP	10	90				NC
Background - behind offices	6/25	10:45	1050	NON	10	40	71	44%	250.0	NC
Background - behind offices	6/25	10:45	1051	RESP	10	31				NC
Background - behind offices	6/25	11:03	1052	NON	5	1	1	0%	7.0	NC
Background - behind offices	6/25	11:03	1053	RESP	5	0				NC
Background - behind offices	6/25	11:16	1054	NON	2.5	0	2	100%	28.2	NC
Background - behind offices	6/25	11:16	1055	RESP	2.5	2				NC
Background - behind offices	6/25	12:33	1056	NON	5	0	4	100%	28.2	NC
Background - behind offices	6/25	12:33	1057	RESP	5	4				NC
Background - behind offices	6/25	12:45	1058	NON	5	11	12	8%	84.5	NC
Background - behind offices	6/25	12:45	1059	RESP	5	1				NC
Background - behind offices	6/25	1:09	1062	NON	10	28	47	40%	165.5	NC
Background - behind offices	6/25	1:09	1063	RESP	10	19				NC
Background - behind offices	6/25	1:30	1064	NON	5	0	1	100%	7.0	NC
Background - behind offices	6/25	1:30	1065	RESP	5	1				NC
Background - behind offices	6/25	1:40	1066	NON	5	0	0	0%	0.0	NC
Background - behind offices	6/25	1:40	1067	RESP	5	0				NC
Background - behind offices	6/25	2:10	1068	NON	2.5	14	25	44%	352.1	NC
Background - behind offices	6/25	2:10	1069	RESP	2.5	11				NC

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED COUNT	TOTAL CFU	% RESP	CFU/m <sup>3</sup>	BS
Background - behind offices	6/25	2:20	1070	NON	5	9	14	36%	98.6	NC
Background - behind offices	6/25	2:20	1071	RESP	5	5				NC
Background - behind offices	6/28	10:02	1410	NON	10	50	118	58%	415.5	NC
Background - behind offices	6/28	10:02	1411	RESP	10	68				NC
Background - behind offices	6/28	10:16	1412	NON	11	43	76	43%	243.3	NC
Background - behind offices	6/28	10:16	1413	RESP	11	33				NC
Background - behind offices	6/28	10:31	1414	NON	10	82	202	59%	711.3	NC
Background - behind offices	6/28	10:31	1415	RESP	10	120				NC
Background - behind offices	6/28	10:46	1416	NON	10	47	125	62%	440.1	NC
Background - behind offices	6/28	10:46	1417	RESP	10	78				NC
Background - behind offices	6/28	11:02	1418	NON	10	113	264	57%	929.6	NC
Background - behind offices	6/28	11:02	1419	RESP	10	151				NC
Background - behind offices	6/28	11:17	1420	NON	10	76	201	62%	707.7	NC
Background - behind offices	6/28	11:17	1421	RESP	10	125				NC
Background - behind offices	6/28	11:46	1424	NON	10	114	310	63%	1091.5	NC
Background - behind offices	6/28	11:46	1425	RESP	10	196				NC
Background - field	6/28	8:17	1400	NON	10	22	222	90%	781.7	NC
Background - field	6/28	8:17	1401	RESP	10	200				NC
Background - field	6/28	8:31	1402	NON	11	44	245	82%	784.3	NC
Background - field	6/28	8:31	1403	RESP	11	201				NC
Background - field	6/28	8:46	1404	NON	10	58	194	70%	683.1	NC
Background - field	6/28	8:46	1405	RESP	10	136				NC
Background - field	6/28	9:14	1408	NON	8	97	130	25%	572.2	NC
Background - field	6/28	9:14	1409	RESP	8	33				NC
Background - cafeteria	6/26	8:25	1100	NON	10	27	59	54%	207.7	19
Background - cafeteria	6/26	8:25	1101	RESP	10	32				14
Background - cafeteria	6/26	8:43	1102	NON	5	23	43	47%	302.8	16
Background - cafeteria	6/26	8:43	1103	RESP	5	20				6
Background - cafeteria	6/26	8:56	1104	NON	10	44	89	51%	313.4	10
Background - cafeteria	6/26	8:56	1105	RESP	10	45				6
Background - conference room	6/26	9:27	1106	NON	10	53	171	69%	602.1	35
Background - conference room	6/26	9:27	1107	RESP	10	118				48
Background - conference room	6/26	9:43	1108	NON	5	40	88	55%	619.7	28
Background - conference room	6/26	9:43	1109	RESP	5	48				24
Background - conference room	6/26	9:54	1110	NON	5	59	111	47%	781.7	34
Background - conference room	6/26	9:54	1111	RESP	5	52				26
Background - conference room	6/26	10:05	1112	NON	10	109	218	50%	767.6	30
Background - conference room	6/26	10:05	1113	RESP	10	109				39
Background - conference room	6/26	10:22	1114	NON	5	58	107	46%	753.5	43
Background - conference room	6/26	10:22	1115	RESP	5	49				22
Background - locker room	6/26	2:51	1124	NON	10	0	2	100%	7.0	NC
Background - locker room	6/26	2:51	1125	RESP	10	2				0
Background - locker room	6/26	3:16	1126	NON	5	13	19	32%	133.8	NC
Background - locker room	6/26	3:16	1127	RESP	5	6				NC

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED	TOTAL	%	CFU/m <sup>3</sup>	BS
						COUNT	CFU	RESP		
Background - office	6/26	3:32	1130	NON	20	111	211	47%	371.5	0
Background - office	6/26	3:32	1131	RESP	20	100				NC
Background - office	6/26	4:01	1132	NON	10	115	213	46%	750.0	NC
Background - office	6/26	4:01	1133	RESP	10	98				NC
Filter Press	6/24	1:24	2003	RESP	4	244	959	26%	9439	NC
Filter Press	6/24	1:24	2004	NON	4	715				NC
Filter Press	6/24	1:42	2008	RESP	5	227	1118	21%	8803	NC
Filter Press	6/24	1:42	2007	NON	5	891				NC
Filter Press	6/24	2:57	2011	RESP	4	223	597	38%	5876	0
Filter Press	6/24	2:57	2012	NON	4	374				NC
Filter Press	6/24	3:19	2015	RESP	3	406	2209	19%	28990	NC
Filter Press	6/24	3:19	2016	NON	3	1803				NC
Filter Press	6/24	3:42	2019	RESP	4	170	384	45%	3780	NC
Filter Press	6/24	3:42	2020	NON	4	214				NC
Filter Press	6/24	3:55	2023	RESP	4	87	237	38%	2333	NC
Filter Press	6/24	3:55	2024	NON	4	150				NC
Filter Press	6/24	1:24	2001	RESP	4	276	1871	15%	18343	NC
Filter Press	6/24	1:24	2002	NON	4	1595				0
Filter Press	6/24	1:42	2006	RESP	5	264	1379	19%	10816	NC
Filter Press	6/24	1:42	2005	NON	5	1115				NC
Filter Press	6/24	2:57	2009	RESP	4	231	1394	17%	13667	NC
Filter Press	6/24	2:57	2010	NON	4	1163				NC
Filter Press	6/24	3:42	2017	RESP	4	2221	2406	92%	23588	NC
Filter Press	6/24	3:42	2018	NON	4	185				0
Filter Press	6/24	3:55	2021	RESP	4	64	170	39%	1667	NC
Filter Press	6/24	3:55	2022	NON	4	106				NC
Filter Press	6/25	8:10	2024	NON	4	36	155	80%	1520	10
Filter Press	6/25	8:10	2025	RESP	4	119				10
Filter Press	6/25	8:22	2028	NON	4	36	112	72%	1098	28
Filter Press	6/25	8:22	2029	RESP	4	76				13
Filter Press	6/25	8:53	2032	NON	3	195	343	45%	4484	165
Filter Press	6/25	8:53	2033	RESP	3	148				101
Filter Press	6/25	9:01	2036	NON	3	236	407	43%	5320	197
Filter Press	6/25	9:01	2037	RESP	3	171				NC
Filter Press	6/25	9:18	2040	NON	2.5	253	447	45%	7012	NC
Filter Press	6/25	9:18	2041	RESP	2.5	194				NC
Filter Press	6/25	10:20	2044	NON	2.5	224	597	63%	9365	NC
Filter Press	6/25	10:20	2045	RESP	2.5	373				NC
Filter Press	6/25	11:11	2048	NON	5	39	126	73%	988	NC
Filter Press	6/25	11:11	2049	RESP	5	87				NC
Filter Press	6/25	12:41	2056	NON	4	701	985	29%	9657	NC
Filter Press	6/25	12:41	2053	RESP	4	284				NC
Filter Press	6/25	1:01	2058	NON	4	150	265	45%	2598	NC
Filter Press	6/25	1:01	2059	RESP	4	115				NC

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED TOTAL		% RESP	CFU/m <sup>3</sup>	BS
						COUNT	CFU			
Filter Press	6/25	8:10	2026	NON	4	69	183	65%	1801	26
Filter Press	6/25	8:10	2027	RESP	4	114				16
Filter Press	6/25	8:22	2030	NON	4	83	178	56%	1752	60
Filter Press	6/25	8:22	2031	RESP	4	95				19
Filter Press	6/25	8:53	2034	NON	3	369	570	36%	7480	10
Filter Press	6/25	8:53	2035	RESP	3	201				162
Filter Press	6/25	9:01	2038	NON	3	669	884	25%	11601	NC
Filter Press	6/25	9:01	2039	RESP	3	215				NC
Filter Press	6/25	9:18	2042	NON	2.5	273	462	42%	7276	NC
Filter Press	6/25	9:18	2043	RESP	2.5	189				NC
Filter Press	6/25	10:20	2046	NON	2.5	183	339	47%	5339	NC
Filter Press	6/25	10:20	2047	RESP	2.5	156				NC
Filter Press	6/25	11:11	2050	NON	5	1069	1136	6%	8945	NC
Filter Press	6/25	11:11	2051	RESP	5	67				NC
Filter Press	6/25	12:41	2054	NON	4	653	938	31%	9232	NC
Filter Press	6/25	12:41	2055	RESP	4	285				NC
Filter Press	6/25	1:01	2060	NON	4	245	349	31%	3435	NC
Filter Press	6/25	1:01	2061	RESP	4	104				NC
Fermentor Sample Port - open	6/24	2:10	3000	NON	4	100	148	32%	1312.1	89
Fermentor Sample Port - open	6/24	2:10	3001	RESP	4	48				42
Fermentor Sample Port - open	6/24	2:10	3003	NON	4	114	139	18%	1215.0	74
Fermentor Sample Port - open	6/24	2:10	3002	RESP	4	25				18
Fermentor Sample Port - open	6/25	1:45	3034	NON	6	190	444	57%	2902.0	NC
Fermentor Sample Port - closed	6/25	1:45	3035	RESP	6	254				NC
Fermentor Sample Port - closed	6/24	2:27	3004	RESP	4	20	40	50%	354.6	4
Fermentor Sample Port - closed	6/24	2:27	3005	NON	4	20				0
Fermentor Sample Port - closed	6/24	2:27	3006	RESP	4	27	33	82%	288.5	0
Fermentor Sample Port - closed	6/24	2:27	3007	NON	4	6				0
Fermentor Sample Port - closed	6/24	2:48	3008	RESP	4	4	21	19%	186.2	1
Fermentor Sample Port - closed	6/24	2:48	3009	NON	4	17				3
Fermentor Sample Port - closed	6/24	2:48	3010	RESP	4	5	11	45%	96.2	3
Fermentor Sample Port - closed	6/24	2:48	3011	NON	4	6				5
Fermentor Sample Port - closed	6/24	3:05	3012	RESP	4	5	7	71%	62.1	0
Fermentor Sample Port - closed	6/24	3:05	3013	NON	4	2				1
Fermentor Sample Port - closed	6/24	3:05	3014	RESP	4	9	9	100%	78.7	0
Fermentor Sample Port - closed	6/24	3:05	3015	NON	4	0				0
Fermentor Sample Port - closed	6/25	2:01	3036	NON	11	78	124	37%	442.1	NC
Fermentor Sample Port - closed	6/25	2:01	3037	RESP	11	46				NC
Fermentor Sample Port - closed	6/25	2:21	3038	NON	10	50	125	60%	490.2	NC
Fermentor Sample Port - closed	6/25	2:21	3039	RESP	10	75				NC
Incubation Room	6/25	8:47	3016	NON	17	76	160	53%	333.8	NC
Incubation Room	6/25	8:47	3017	RESP	17	84				NC
Incubation Room	6/25	9:08	3020	NON	20	60	168	64%	297.9	NC
Incubation Room	6/25	9:08	3021	RESP	20	108				NC



TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED COUNT	TOTAL CFU	% RESP	CFU/m <sup>3</sup>	BS
Incubation Room	6/25	9:39	3026	NON	22	122	237	49%	382.0	NC
Incubation Room	6/25	9:39	3027	RESP	22	115				NC
Incubation Room	6/25	10:10	3028	NON	20	118	178	34%	315.6	NC
Incubation Room	6/25	10:10	3029	RESP	20	60				NC
Incubation Room	6/26	2:38	3078	NON	20					ND
Incubation Room	6/26	2:38	3079	RESP	20	52				29
Incubation Room	6/26	3:59	3084	NON	20	143				42
Incubation Room	6/26	3:59	3085	RESP	20					31
Incubation Room	6/26	2:11	3076	NON	20	102	160	36%	315.0	20
Incubation Room	6/26	2:11	3077	RESP	20	58				20
Incubation Room	6/26	3:32	3082	NON	20	107	175	39%	344.5	30
Incubation Room	6/26	3:32	3083	RESP	20	68				35
Clean Room	6/25	8:56	3018	NON	20	2	6	67%	10.5	NC
Clean Room	6/25	8:56	3019	RESP	20	4				NC
Clean Room	6/25	9:54	3022	NON	9	0	1	100%	3.9	NC
Clean Room	6/25	9:54	3023	RESP	9	1				NC
Clean Room	6/25	10:15	3030	NON	10	0	0	0%	0.0	NC
Clean Room	6/25	10:15	3031	RESP	10	0				0
Clean Room	6/25	10:30	3032	NON	10	0	4	100%	14.0	0
Clean Room	6/25	10:30	3033	RESP	10	4				NC
Clean Room	6/26	8:19	3052	NON	21	32	39	18%	72.8	27
Clean Room	6/26	8:19	3053	RESP	21	7				9
Clean Room	6/26	8:49	3054	NON	16	0	0	0%	0.0	1
Clean Room	6/26	8:49	3055	RESP	16	0				1
Clean Room	6/26	9:11	3056	NON	20	0	0	0%	0.0	0
Clean Room	6/26	9:11	3057	RESP	20	0				0
Clean Room	6/26	11:05	3062	NON	20	0	0	0%	0.0	1
Clean Room	6/26	11:05	3063	RESP	20	0				1
Clean Room	6/26	1:56	3064	NON	20	0	0	0%	0.0	1
Clean Room	6/26	1:56	3065	RESP	20	0				1
Clean Room	6/26	2:25	3066	NON	20	0	0	0%	0.0	1
Clean Room	6/26	2:25	3067	RESP	20	0				1
Clean Room	6/26	2:53	3068	NON	21	16	19	16%	35.5	2
Clean Room	6/26	2:53	3069	RESP	21	3				1
Clean Room	6/26	3:46	3072	NON	20	0	0	0%	0.0	1
Clean Room	6/26	3:46	3073	RESP	20	0				1
Main Laboratory	6/26	9:53	3058	NON	25	21	98	79%	153.7	12
Main Laboratory	6/26	9:53	3059	RESP	25	77				4
Main Laboratory	6/26	10:32	3060	NON	20	29	70	59%	137.3	15
Main Laboratory	6/26	10:32	3061	RESP	20	41				21
Quality Control Laboratory	6/27	2:48	1	RESP	10	26	70	37%	275.6	10
Quality Control Laboratory	6/27	2:48	2	NON	10	44				9
Quality Control Laboratory	6/27	3:04	3	RESP	15	27	69	39%	181.1	6
Quality Control Laboratory	6/27	3:04	4	NON	15	42				10
Quality Control Laboratory	6/27	3:23	5	RESP	10	15	34	44%	133.9	0

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED	TOTAL	%	CFU/m <sup>3</sup>	BS
						COUNT	CFU	RESP		
Quality Control Laboratory	6/27	3:23	6	NON	10	19				9
Quality Control Laboratory	6/27	3:39	7	RESP	10	15	75	20%	295.3	9
Quality Control Laboratory	6/27	3:39	8	NON	10	60				19
Quality Control Laboratory	6/27	4:15	9	RESP	10	11	25	44%	98.4	5
Quality Control Laboratory	6/27	4:15	10	NON	10	14				8
Quality Control Laboratory	6/27	4:31	11	RESP	10	10	23	43%	90.6	4
Quality Control Laboratory	6/27	4:31	12	NON	10	13				6
Quality Control Laboratory	6/27	4:44	13	RESP	15	14	35	40%	91.9	2
Quality Control Laboratory	6/27	4:44	14	NON	15	46				NC
Quality Control Laboratory	6/27	5:02	15	RESP	10	5	59	8%	232.3	3
Quality Control Laboratory	6/27	5:02	16	NON	10	20				NC
Dumpster	6/28	9:05	3612	NON	6	281	434	35%	2565.0	NC
Dumpster	6/28	9:05	3613	RESP	6	153				NC
Dumpster	6/28	9:05	3614	NON	6	339	501	32%	2919.6	NC
Dumpster	6/28	9:05	3615	RESP	6	162				NC
Dumpster	6/28	9:05	3616	NON	6	111	177	37%	1161.4	NC
Dumpster	6/28	9:05	3617	RESP	6	66				NC
Dumpster	6/28	10:16	3618	NON	5	234	352	34%	2496.5	NC
Dumpster	6/28	10:16	3619	RESP	5	118				NC
Dumpster	6/28	10:16	3620	NON	5	309	508	39%	3552.4	NC
Dumpster	6/28	10:16	3621	RESP	5	199				NC
Dumpster	6/28	10:16	3622	NON	5	163	262	38%	2063.0	NC
Dumpster	6/28	10:16	3623	RESP	5	99				NC
Dumpster	6/28	12:07	3630	NON	5	243	364	33%	2581.6	NC
Dumpster	6/28	12:07	3631	RESP	5	121				NC
Dumpster	6/28	12:07	3632	NON	5	469	666	30%	4657.3	NC
Dumpster	6/28	12:07	3633	RESP	5	197				NC
Dumpster	6/28	12:07	3634	NON	5	106	174	39%	1370.1	NC
Dumpster	6/28	12:07	3635	RESP	5	68				NC
Fermentor Tank - agitator shaft	6/25	1:56	3040	NON	2.5	19	95	80%	1328.7	NC
Fermentor Tank - agitator shaft	6/25	1:56	3041	RESP	2.5	76				NC
Fermentor Tank - agitator shaft	6/25	1:56	3042	NON	2.5	22	64	66%	907.8	NC
Fermentor Tank - agitator shaft	6/25	1:56	3043	RESP	2.5	42				45
Fermentor Tank - agitator shaft	6/25	2:08	3044	NON	2.5	5	11	55%	153.8	9
Fermentor Tank - agitator shaft	6/25	2:08	3045	RESP	2.5	6				1
Fermentor Tank - agitator shaft	6/25	2:08	3046	NON	2.5	0	14	100%	198.6	13
Fermentor Tank - agitator shaft	6/25	2:08	3047	RESP	2.5	14				4
Fermentor Tank - agitator shaft	6/25	2:24	3048	NON	2.5	3	82	96%	1146.9	82
Fermentor Tank - agitator shaft	6/25	2:24	3049	RESP	2.5	79				4
Fermentor Tank - agitator shaft	6/25	2:24	3050	NON	2.5	4	162	98%	2297.9	161
Fermentor Tank - agitator shaft	6/25	2:24	3051	RESP	2.5	158				0
Fermentor Tank - agitator shaft	6/26	11:25	3129	RESP	5	5	12	42%	83.9	0
Fermentor Tank - agitator shaft	6/26	11:25	3130	NON	5	7				0
Fermentor Tank - agitator shaft	6/26	11:25	3131	RESP	5	0	13	0%	92.2	1

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED TOTAL		% RESP	CFU/m <sup>3</sup>	BS
						COUNT	CFU			
Seed Tank - agitator shaft	6/27	10:45	3422	NON	5	73				5
Seed Tank - agitator shaft	6/27	10:53	3423	RESP	5	194	242	80%	1716.3	3
Seed Tank - agitator shaft	6/27	10:53	3424	NON	5	48				5
Seed Tank - agitator shaft	6/27	10:53	3425	RESP	5	119	190	63%	1328.7	4
Seed Tank - agitator shaft	6/27	10:53	3426	NON	5	71				5
Seed Tank - agitator shaft	6/27	11:11	3427	RESP	5	78	149	52%	1056.7	10
Seed Tank - agitator shaft	6/27	11:11	3428	NON	5	71				25
Seed Tank - agitator shaft	6/27	11:11	3429	RESP	5	105	155	68%	1083.9	13
Seed Tank - agitator shaft	6/27	11:11	3430	NON	5	50				19
Seed Tank - agitator shaft	6/27	11:25	3435	RESP	10	126	467	27%	1656.0	85
Seed Tank - agitator shaft	6/27	11:25	3436	NON	10	341				128
Seed Tank - agitator shaft	6/27	11:25	3437	RESP	10	102	331	31%	1157.3	66
Seed Tank - agitator shaft	6/27	11:25	3438	NON	10	229				114
Seed Tank - agitator shaft	6/27	11:42	3439	RESP	5	102	179	57%	1269.5	18
Seed Tank - agitator shaft	6/27	11:42	3440	NON	5	77				34
Seed Tank - agitator shaft	6/27	11:42	3441	RESP	5	182	264	69%	1846.2	9
Seed Tank - agitator shaft	6/27	11:42	3442	NON	5	82				20
Seed Tank - agitator shaft	6/27	11:52	3443	RESP	5	115	270	43%	1914.9	9
Seed Tank - agitator shaft	6/27	11:52	3444	NON	5	155				55
Seed Tank - agitator shaft	6/27	11:52	3445	RESP	5	133	252	53%	1762.2	22
Seed Tank - agitator shaft	6/27	11:52	3446	NON	5	119				37
Scrubber exhaust	6/26	8:32	3101	RESP	5	43	51	84%	356.6	35
Scrubber exhaust	6/26	8:32	3102	NON	5	8				0
Scrubber exhaust	6/26	8:32	3103	RESP	5	72	77	94%	546.1	45
Scrubber exhaust	6/26	8:32	3104	NON	5	5				1
Scrubber exhaust	6/26	8:50	3105	RESP	5	24	27	89%	188.8	15
Scrubber exhaust	6/26	8:50	3106	NON	5	3				3
Scrubber exhaust	6/26	8:50	3107	NON	5	8	54	15%	383.0	5
Scrubber exhaust	6/26	8:50	3108	RESP	5	46				31
Scrubber exhaust	6/26	9:06	3109	RESP	5	60	63	95%	446.8	46
Scrubber exhaust	6/26	9:06	3110	NON	5	3				0
Scrubber exhaust	6/26	9:06	3111	RESP	5	43	45	96%	314.7	34
Scrubber exhaust	6/26	9:06	3112	NON	5	2				2
Scrubber exhaust	6/26	9:20	3113	RESP	2.5	12	12	100%	170.2	2
Scrubber exhaust	6/26	9:20	3114	NON	2.5	0				0
Scrubber exhaust	6/26	9:20	3115	RESP	2.5	23	23	100%	321.7	22
Scrubber exhaust	6/26	9:20	3116	NON	2.5	0				0
Scrubber exhaust	6/26	9:44	3117	RESP	2.5	103	120	86%	1702.1	61
Scrubber exhaust	6/26	9:44	3118	NON	2.5	17				5
Scrubber exhaust	6/26	9:44	3119	RESP	2.5	75	91	82%	1272.7	29
Scrubber exhaust	6/26	9:44	3120	NON	2.5	16				1
Scrubber exhaust	6/26	9:55	3121	RESP	5	37	70	53%	496.5	NC
Scrubber exhaust	6/26	9:55	3122	NON	5	33				1
Scrubber exhaust	6/26	9:55	3123	RESP	5	31	47	66%	328.7	NC

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED TOTAL		%		CFU/m3	BS
						COUNT	CFI	RESP			
Fermentor Tank - agitator shaft	6/26	11:25	3132	NON	5	13					4
Fermentor Tank - agitator shaft	6/26	11:37	3133	RESP	5	2	17	12%	118.9		0
Fermentor Tank - agitator shaft	6/26	11:37	3134	NON	5	15					7
Fermentor Tank - agitator shaft	6/26	11:37	3135	RESP	5	17	42	40%	297.9		2
Fermentor Tank - agitator shaft	6/26	11:37	3136	NON	5	25					9
Fermentor Tank - agitator shaft	6/26	11:48	3137	RESP	2.5	0	6	0%	83.9		0
Fermentor Tank - agitator shaft	6/26	11:48	3138	NON	2.5	6					2
Fermentor Tank - agitator shaft	6/26	11:48	3139	RESP	2.5	0	8	0%	113.5		0
Fermentor Tank - agitator shaft	6/26	11:48	3140	NON	2.5	8					4
Fermentor Tank - agitator shaft	6/26	11:57	3141	RESP	2.5	26	70	37%	979.0		7
Fermentor Tank - agitator shaft	6/26	11:57	3142	NON	2.5	44					22
Fermentor Tank - agitator shaft	6/26	11:57	3143	RESP	2.5	19	55	35%	780.1		22
Fermentor Tank - agitator shaft	6/26	11:57	3144	NON	2.5	36					19
Fermentor Tank - agitator shaft	6/27	2:03	3500	NON	5	26	35	26%	248.2		1
Fermentor Tank - agitator shaft	6/27	2:03	3501	RESP	5	9					0
Fermentor Tank - agitator shaft	6/27	2:03	3502	NON	5	23	43	47%	300.7		5
Fermentor Tank - agitator shaft	6/27	2:03	3503	RESP	5	20					0
Fermentor Tank - agitator shaft	6/27	2:20	3504	NON	5	8	22	64%	156.0		0
Fermentor Tank - agitator shaft	6/27	2:20	3505	RESP	5	14					0
Fermentor Tank - agitator shaft	6/27	2:20	3506	NON	5	11	15	27%	104.9		2
Fermentor Tank - agitator shaft	6/27	2:20	3507	RESP	5	4					0
Fermentor Tank - agitator shaft	6/27	2:53	3508	NON	5	99	222	55%	1574.5		38
Fermentor Tank - agitator shaft	6/27	2:53	3509	RESP	5	123					35
Fermentor Tank - agitator shaft	6/27	2:53	3510	NON	5	41	132	69%	923.1		7
Fermentor Tank - agitator shaft	6/27	2:53	3511	RESP	5	91					24
Seed Tank - agitator shaft	6/27	9:51	3400	NON	3	146	265	45%	3132.4		51
Seed Tank - agitator shaft	6/27	9:51	3401	RESP	3	119					0G
Seed Tank - agitator shaft	6/27	9:51	3402	NON	3	164	363	55%	4230.8		0G
Seed Tank - agitator shaft	6/27	9:51	3403	RESP	3	199					62
Seed Tank - agitator shaft	6/27	10:07	3404	NON	2.5	74	162	54%	2265.7		28
Seed Tank - agitator shaft	6/27	10:07	3405	RESP	2.5	88					19
Seed Tank - agitator shaft	6/27	10:07	3406	NON	2.5	161	231	30%	3276.6		44
Seed Tank - agitator shaft	6/27	10:07	3407	RESP	2.5	70					9
Seed Tank - agitator shaft	6/27	10:21	3408	NON	5	128	219	42%	1553.2		77
Seed Tank - agitator shaft	6/27	10:21	3409	RESP	5	91					0G
Seed Tank - agitator shaft	6/27	10:21	3412	NON	5	139	234	41%	1636.4		NC
Seed Tank - agitator shaft	6/27	10:21	3413	RESP	5	95					65
Seed Tank - agitator shaft	6/27	10:36	3414	NON	6	101	195	48%	1152.5		38
Seed Tank - agitator shaft	6/27	10:36	3415	RESP	6	94					24
Seed Tank - agitator shaft	6/27	10:36	3416	NON	6	131	211	38%	1229.6		59
Seed Tank - agitator shaft	6/27	10:36	3417	RESP	6	80					29
Seed Tank - agitator shaft	6/27	10:45	3419	RESP	5	101	149	68%	1056.7		1
Seed Tank - agitator shaft	6/27	10:45	3420	NON	5	48					6
Seed Tank - agitator shaft	6/27	10:45	3421	RESP	5	100	173	58%	1209.8		2

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MLN	CORRECTED TOTAL		% RESP	CFU/m3	BS
						COUNT	CFU			
Seed Tank - agitator shaft	6/27	10:45	3422	NON	5	73				5
Seed Tank - agitator shaft	6/27	10:53	3423	RESP	5	194	242	80%	1716.3	3
Seed Tank - agitator shaft	6/27	10:53	3424	NON	5	48				5
Seed Tank - agitator shaft	6/27	10:53	3425	RESP	5	119	190	63%	1328.7	4
Seed Tank - agitator shaft	6/27	10:53	3426	NON	5	71				5
Seed Tank - agitator shaft	6/27	11:11	3427	RESP	5	78	149	52%	1056.7	10
Seed Tank - agitator shaft	6/27	11:11	3428	NON	5	71				25
Seed Tank - agitator shaft	6/27	11:11	3429	RESP	5	105	155	68%	1083.9	13
Seed Tank - agitator shaft	6/27	11:11	3430	NON	5	50				19
Seed Tank - agitator shaft	6/27	11:25	3435	RESP	10	126	467	27%	1656.0	85
Seed Tank - agitator shaft	6/27	11:25	3436	NON	10	341				128
Seed Tank - agitator shaft	6/27	11:25	3437	RESP	10	102	331	31%	1157.3	66
Seed Tank - agitator shaft	6/27	11:25	3438	NON	10	229				114
Seed Tank - agitator shaft	6/27	11:42	3439	RESP	5	102	179	57%	1269.5	18
Seed Tank - agitator shaft	6/27	11:42	3440	NON	5	77				34
Seed Tank - agitator shaft	6/27	11:42	3441	RESP	5	182	264	69%	1846.2	9
Seed Tank - agitator shaft	6/27	11:42	3442	NON	5	82				20
Seed Tank - agitator shaft	6/27	11:52	3443	RESP	5	115	270	43%	1914.9	9
Seed Tank - agitator shaft	6/27	11:52	3444	NON	5	155				55
Seed Tank - agitator shaft	6/27	11:52	3445	RESP	5	133	252	53%	1762.2	22
Seed Tank - agitator shaft	6/27	11:52	3446	NON	5	119				37
Scrubber exhaust	6/26	8:32	3101	RESP	5	43	51	84%	356.6	35
Scrubber exhaust	6/26	8:32	3102	NON	5	8				0
Scrubber exhaust	6/26	8:32	3103	RESP	5	72	77	94%	546.1	45
Scrubber exhaust	6/26	8:32	3104	NON	5	5				1
Scrubber exhaust	6/26	8:50	3105	RESP	5	24	27	89%	188.8	15
Scrubber exhaust	6/26	8:50	3106	NON	5	3				3
Scrubber exhaust	6/26	8:50	3107	NON	5	8	54	15%	383.0	5
Scrubber exhaust	6/26	8:50	3108	RESP	5	46				31
Scrubber exhaust	6/26	9:06	3109	RESP	5	60	63	95%	446.8	46
Scrubber exhaust	6/26	9:06	3110	NON	5	3				0
Scrubber exhaust	6/26	9:06	3111	RESP	5	43	45	96%	314.7	34
Scrubber exhaust	6/26	9:06	3112	NON	5	2				2
Scrubber exhaust	6/26	9:20	3113	RESP	2.5	12	12	100%	170.2	2
Scrubber exhaust	6/26	9:20	3114	NON	2.5	0				0
Scrubber exhaust	6/26	9:20	3115	RESP	2.5	23	23	100%	321.7	22
Scrubber exhaust	6/26	9:20	3116	NON	2.5	0				0
Scrubber exhaust	6/26	9:44	3117	RESP	2.5	103	120	86%	1702.1	61
Scrubber exhaust	6/26	9:44	3118	NON	2.5	17				5
Scrubber exhaust	6/26	9:44	3119	RESP	2.5	75	91	82%	1272.7	29
Scrubber exhaust	6/26	9:44	3120	NON	2.5	16				1
Scrubber exhaust	6/26	9:55	3121	RESP	5	37	70	53%	496.5	NC
Scrubber exhaust	6/26	9:55	3122	NON	5	33				1
Scrubber exhaust	6/26	9:55	3123	RESP	5	31	47	66%	328.7	NC

TABLE II. Viable Sampling Results (Continued)

SAMPLE LOCATION	DATE	TIME	PLATE	FRAC	MIN	CORRECTED	TOTAL	%	CFU/m <sup>3</sup>	BS
						COUNT	CFU	RESP		
Scrubber exhaust	6/26	9:55	3124	NON	5	16				NC
Scrubber exhaust	6/26	10:08	3125	RESP	5	156	182	86%	1290.8	143
Scrubber exhaust	6/26	10:08	3126	NON	5	26				1
Scrubber exhaust	6/26	10:08	3127	RESP	5	143	168	85%	1174.8	131
Scrubber exhaust	6/26	10:08	3128	NON	5	25				8
Scrubber exhaust	6/27	3:13	3512	NON	5	24	110	78%	780.1	0
Scrubber exhaust	6/27	3:13	3513	RESP	5	86				5
Scrubber exhaust	6/27	3:13	3514	NON	5	25	97	74%	678.3	4
Scrubber exhaust	6/27	3:13	3515	RESP	5	72				4
Scrubber exhaust	6/27	3:26	3516	NON	5	9	22	59%	156.0	2
Scrubber exhaust	6/27	3:26	3517	RESP	5	13				1
Scrubber exhaust	6/27	3:26	3518	NON	5	0	0	0%	0.0	1
Scrubber exhaust	6/27	3:26	3519	RESP	5	0				0
Scrubber exhaust	6/27	4:25	3520	NON	5	4	27	85%	191.5	0
Scrubber exhaust	6/27	4:25	3521	RESP	5	23				0
Scrubber exhaust	6/27	4:25	3522	NON	5	12	32	63%	223.8	0
Scrubber exhaust	6/27	4:25	3523	RESP	5	20				0
Scrubber exhaust	6/27	4:35	3524	NON	5	9	18	50%	127.7	1
Scrubber exhaust	6/27	4:35	3525	RESP	5	9				0
Scrubber exhaust	6/27	4:35	3526	NON	5	8	16	50%	111.9	0
Scrubber exhaust	6/27	4:35	3527	RESP	5	8				0
Scrubber exhaust	6/27	4:46	3528	NON	5	31	50	38%	354.6	4
Scrubber exhaust	6/27	4:46	3529	RESP	5	19				6
Scrubber exhaust	6/27	4:46	3530	NON	5	18	33	45%	230.8	2
Scrubber exhaust	6/27	4:46	3531	RESP	5	15				1
Scrubber exhaust	6/27	5:02	3532	NON	5	7	8	13%	56.7	1
Scrubber exhaust	6/27	5:02	3533	RESP	5	1				1
Scrubber exhaust	6/27	5:02	3534	NON	5	14	25	44%	174.8	4
Scrubber exhaust	6/27	5:02	3535	RESP	5	11				0
Scrubber exhaust	6/28	8:08	3600	NON	5	8	211	96%	1496.5	NC
Scrubber exhaust	6/28	8:08	3601	RESP	5	203				NC
Scrubber exhaust	6/28	8:08	3602	NON	5	8	205	96%	1433.6	NC
Scrubber exhaust	6/28	8:08	3603	RESP	5	197				NC
Scrubber exhaust	6/28	8:22	3608	NON	5	18	215	92%	1524.8	NC
Scrubber exhaust	6/28	8:22	3609	RESP	5	197				NC
Scrubber exhaust	6/28	8:22	3610	NON	5	12	209	94%	1461.5	NC
Scrubber exhaust	6/28	8:22	3611	RESP	5	197				NC

TABLE III. Viable Sampling Summary

Sample Location	Geometric		% B1	Minimum Value	Maximum Value
	Mean	St Dev			
Fermentor Tank - agitator shaft	339.0	3.1	NA	83.9	2297.9
Seed Tank - agitator shaft	1633.9	1.5	23	1056.7	4230.8
Scrubber	345.1	4.2	38	0	1702.1
Sample Port - open	1668.3	1.6	77	1312.1	2902.0
Sample Port - closed	195.5	2.3	19	62.1	490.2
Incubation Room	332.4	1.1	31	297.9	382.0
Clean Room	4.4	5.1	NA	0	72.8
Main Laboratory	147.3	1.1	34	137.3	153.7
Quality Control Laboratory	159.1	1.6	35	90.6	295.3
Dumpster	2400	1.5	NA	1161.4	4657.3
Filter Press - open	10599.4	1.8	NA	4484.0	28990.0
Filter Press - closing	8757.5	1.4	NA	5320.0	11601.0
Filter Press - closed	3906.1	2.5	NA	988.0	23588.0
Background - outside	123.2	7.7	7	0	1493.0
Cafeteria	270.2	1.3	42	207.7	313.4
Conference Room	701.5	1.1	51	602.1	781.7
Locker Room	32.9	7.3	NA	7.0	133.8
Office	528.9	1.6	NA	371.5	750.0

TABLE IV. Enzyme Sampling Results

SAMPLE LOCATION	DATE	FILTER NO.	FLOW RATE (ft <sup>3</sup> /min)	SAMPLE TIME(hr)	DUST (ug)	TOTAL DUST (ug/m <sup>3</sup> )	ENZYME DU/m <sup>3</sup>
Ultrafilter	6/25	25-6	45	5.42	70.68	0.171	0.657
Ultrafilter	6/26	25-3	45	6.79	66.99	0.129	0.276
Ultrafilter	6/27	27-3	45	7.57	53.52	0.093	0.230
Ultrafilter	6/28	28-6	45	8.72	136.67	0.205	0.637
Candle Filter	6/25	25-5	46	5.05	144.44	0.366	0.316
Candle Filter	6/26	26-6	46	6.68	45.82	0.088	0.255
Candle Filter	6/27	27-2	46	7.57	35.09	0.060	0.205
Candle Filter	6/28	28-2	46	8.65	92.11	0.137	0.494
Blender Tank	6/25	25-7	42	5.07	49.57	0.137	0.339
Blender Tank	6/26	26-5	42	6.6	45.71	0.097	0.522
Blender Tank	6/27	26-2	42	7.5	35.21	0.066	0.204
Blender Tank	6/28	28-4	42	8.65	79.82	0.130	0.738
Fermentor	6/26	25-4	42	7.44	33.02	0.062	0.209
Fermentor	6/27	27-4	42	7.81	27.48	0.050	0.156
Fermentor	6/28	27-5	42	8.3	39.58	0.067	0.609
Outside-Background	6/28	28-1	44	7.68	99.12	0.173	0.269
Blank		25-1			-0.34		
Blank		25-2			-0.30		
Blank		25-8			-0.01		
Blank		26-1			0.50		
Blank		26-3			0.06		
Blank		26-4			-1.63		
Blank		26-7			-0.58		
Blank		27-1			-0.42		
Blank		27-6			0.23		
Blank		28-3			0.23		
Blank		28-5			0.95		



TABLE V. Acetone Sampling Results

SAMPLE LOCATION	DATE	TUBE NO.	FLOW RATE (cc/min)	SAMPLE TIME(hr)	ACETONE (mg)	ACETONE mg/m <sup>3</sup>
Filter press	6/25	165	49.6	0.94	(0.01)	
Filter Press - rail	6/25	162	51.9	0.95	(0.01)	
Filter Press - rail	6/28	173	49.2	0.93	0.01	3.64
Filter Press - rail	6/28	176	49.2	0.95	(0.01)	
Conveyor	6/25	163	52.2	0.93	(0.01)	
Conveyor	6/28	179	51.2	0.95	(0.01)	
Conveyor	6/28	174	51.2	0.85	(0.01)	
Mash Tank	6/28	181	51.9	0.95	(0.01)	
Mash Tank	6/28	177	51.9	0.82	(0.01)	
Mash Tank	6/28	184	51.9	0.99	(0.01)	
Blank	6/28	164			(0.01)	
Blank	6/28	172			(0.01)	
Blank	6/28	178			(0.01)	
Blank	6/28	180			(0.01)	
Blank	6/28	167			(0.01)	
Blank	6/25	171			(0.01)	
Blank	6/25	166			(0.01)	
Blank	6/27	172			(0.01)	

( ) = less than

TABLE VI. Total Dust Sampling Results

SAMPLE LOCATION	DATE	FILTER NO.	FLOW RATE (l/min)	SAMPLE TIME(HR)	TOTAL DUST (ug)	TOTAL DUST mg/m <sup>3</sup>
Blending Tank 1	6/25	102	2.21	6.36	-0.03	0.02
Blending Tank 1	6/26	112	2.20			
Blending Tank 1	6/27	178	2.21	8.27	0.02	0.02
Blending Tank 2	6/25	145	2.20	6.35	0.04	0.05
Blending Tank 2	6/26	109	2.20	8.30	0.02	0.02
Blending Tank 2	6/27	184	2.22	8.22	0.10	0.09
Blending Tank 2	6/28	189	2.21	8.80	0.14	0.12
Candlefilter	6/25	111	2.21	6.38	-0.01	0.02
Candlefilter	6/26	101	2.20	8.28	0.05	0.05
Candlefilter	6/27	154	2.21	8.26	0.05	0.05
Candlefilter	6/28	160	2.20	8.80	0.19	0.17
Dumpster	6/25	123	2.21	6.54	0.05	0.06
Dumpster	6/26	116	2.20	8.43	0.03	0.02
Dumpster	6/27	192	2.20	8.37	0.21	0.19
Dumpster	6/28	157	2.20	9.00	0.06	0.05
Dumpster	6/25	122	2.21	6.50	-0.01	0.02
Dumpster	6/26	114	2.20	8.39	-0.01	0.02
Dumpster	6/27	162	2.20	8.35	0.24	0.22
Dumpster	6/28	153	2.22	9.00	0.31	0.26
Sampling Port	6/25	135	2.21	6.50	0.06	0.07
Sampling Port	6/26	104	2.20	8.44	-0.07	0.02
Sampling Port	6/27	174	2.21	8.35	0.05	0.05
Fermentor Tank	6/25	103	2.20	6.43	-0.05	0.02
Fermentor Tank	6/26	146	2.22			
Fermentor Tank	6/27	148	2.20	8.35	0.07	0.07
Fermentor Tank	6/25	139	2.20	6.42	0.00	0.02
Fermentor Tank	6/26	115	2.20			
Fermentor Tank	6/27	191	2.20	8.37	0.05	0.05
Filter Press - belt conveyor	6/25	128	2.21	6.80	0.07	0.08
Filter Press - belt conveyor	6/26	126	2.20	8.39	0.00	0.02
Filter Press - belt conveyor	6/27	156	2.21	8.29	0.30	0.28
Filter Press - belt conveyor	6/28	176	2.20	8.96	0.11	0.10
Filter Press - left	6/25	131	2.20	6.88	-0.01	0.02
Filter Press - left	6/26	106	2.21	8.38	-0.03	0.02
Filter Press - left	6/27	179	2.20	8.30	0.21	0.20
Filter Press - left	6/28	170	2.21	8.99	0.04	0.04
Filter Press - right	6/25	138	2.20	6.89	0.01	0.02
Filter Press - right	6/26	127	2.22	8.38	0.01	0.02
Filter Press - right	6/27	193	2.21	8.28	0.43	0.40
Filter Press - right	6/28	168	2.21	8.93	0.14	0.12
Incubation Room	6/25	130	2.21	7.03	0.01	0.02
Incubation Room	6/26	117	2.21	8.38	-0.02	0.02
Mash Treatment Tank	6/26	105	2.21	8.16	-0.02	0.02
Mash Treatment Tank	6/27	173	2.20	8.37	0.05	0.05

TABLE VI. Total Dust Sampling Results (continued)

SAMPLE LOCATION	DATE	FILTER NO.	FLOW RATE (l/min)	SAMPLE TIME(HR)	TOTAL DUST (ug)	TOTAL DUST mg/m <sup>3</sup>
QC Laboratory	6/26	110	2.21	8.31	0.06	0.06
QC Laboratory	6/27	180	2.20			
Recovery - dump station	6/25	142	2.20	6.74	0.00	0.02
Recovery - dump station	6/26	107	2.20	8.40	-0.02	0.02
Recovery - dump station	6/27	161	2.21	9.02	4.29	3.59
Recovery - work table	6/25	129	2.20	6.76	0.05	0.06
Recovery - work table	6/26	125	2.21	8.40	0.06	0.06
Recovery - work table	6/27	151	2.21	8.32	0.15	0.14
Scrubber	6/25	121	2.20	6.45	0.01	0.02
Scrubber	6/26	144	2.21			
Scrubber	6/27	175	2.20	8.29	0.04	0.04
Seed Tank	6/26	136	2.20	8.26	0.09	0.09
Seed Tank	6/27	183	2.21	8.18	0.06	0.06
Ultrafilter	6/25	141	2.22	6.30	0.10	0.12
Ultrafilter	6/26	140	2.21	8.27	-0.01	0.02
Ultrafilter	6/27	147	2.20	8.28	0.05	0.05
Blank	6/28	150			0.01	
Blank	6/28	166			-0.02	
Blank	6/28	164			0.02	
Blank	6/26	119			-0.10	
Blank	6/26	120			-0.03	
Blank	6/26	132			-0.06	
Blank	6/26	124			-0.08	
Blank	6/26	108			-0.04	
Blank	6/25	143			-0.32	
Blank	6/25	134			-0.05	
Blank	6/25	137			-0.06	
Blank	6/25	113			-0.06	
Blank	6/25	118			-0.07	
Blank	6/25	133			-0.07	
Blank	6/27	152			-0.04	
Blank	6/27	181			0.02	
Blank	6/27	177			0.02	
Blank	6/27	182			0.09	
Blank	6/27	172			0.01	

TABLE VII. Exploratory Viable Sampling Results

SAMPLE LOCATION	PLATE	MIN	COLONY COUNT	TOTAL CFU	CFU per m <sup>3</sup>
Main Laboratory	1	20	91	151	266.8
Main Laboratory	2	20	60		
Main Laboratory	3	10	37	65	229.6
Main Laboratory	4	10	28		
Main Laboratory	5	2.5	----	----	----
Main Laboratory	6	2.5	9		
Main Laboratory	5	20	40	141.3	
Main Laboratory	8	5	20		
Clean Room	175	2.5	3	4	56.5
Clean Room	176	2.5	1		
Clean Room	177	13	26	88	239.2
Clean Room	178	13	62		
Clean Room	179	20	50	85	150.2
Clean Room	180	20	35		
Fermentor - agitator shaft	81	15	1316	1672	3938.8
Fermentor - agitator shaft	82	15	356		
Fermentor - agitator shaft	83	15	1208	1531	3606.6
Fermentor - agitator shaft	84	15	323		
Fermentor - agitator shaft	86	2.5	22	103	1455.8
Fermentor - agitator shaft	87	2.5	81		
Fermentor - agitator shaft	88	2.5	279	636	8989.4
Fermentor - agitator shaft	89	2.5	357		
Scrubber	91	15	302	1326	3123.7
Scrubber	92	15	1024		
Scrubber	93	2.5	58	110	1554.8
Scrubber	94	2.5	52		
Scrubber	95	5	187	494	3491.2
Scrubber	96	5	307		
Scrubber	97	5	270	592	4183.7
Scrubber	98	5	322		
Filter Press	9	10	244	400	1413.4
Filter Press	10	10	156		
Filter Press	11	20	142	319	563.6
Filter Press	12	20	177		
Filter Press	13	2.5	120	205	2897.5
Filter Press	14	2.5	85		
Filter Press	15	10	834	1060	3745.6
Filter Press	16	10	226		
Filter Press	17	2.5	41	89	1258.0
Filter Press	18	2.5	48		
Filter Press	19	20	2804	3276	5788.0
Filter Press	20	20	472		
Conference Room	30	20	331	618	1091.9
Conference Room	31	20	287		
Conference Room	32	20	361	688	1215.5

TABLE VII. Exploratory Viable Sampling Results (Continued)

SAMPLE LOCATION	PLATE	MIN	COLONY COUNT	TOTAL CFU	CFU per m3
Conference Room	33	20	327		
Conference Room	34	10	197	373	1318.0
Conference Room	35	10	176		
Conference Room	36	10	176	369	1303.9
Conference Room	37	10	193		
Conference Room	38	6	600	711	4187.3
Conference Room	39	6	111		
Conference Room	40	6	191	326	1919.9
Conference Room	41	6	135		
Background - outside	60	20	197	410	367.5
Background - outside	61	20	213		
Background - outside	62	20	241	429	494.7
Background - outside	63	20	188		
Background - outside	66	20	225	406	353.4
Background - outside	67	20	181		
Background - outside	68	2.5	65	228	3222.6
Background - outside	69	2.5	163		
Background - outside	70	2.5	91	239	3378.1
Background - outside	71	2.5	148		
Background - outside	72	2.5	51	126	1780.9
Background - outside	73	2.5	75		
Background - outside	74	5	65	-----	-----
Background - outside	75	5	-----		
Background - outside	76	5	23	111	784.5
Background - outside	77	5	88		
Background - outside	78	5	73	121	855.1
Background - outside	79	5	48		
Background - outside	42	2.5	18	26	367.5
Background - outside	43	2.5	8		
Background - outside	44	2.5	18	35	494.7
Background - outside	45	2.5	17		
Background - outside	46	2.5	14	25	353.4
Background - outside	47	2.5	11		
Background - outside	48	20	251	505	892.2
Background - outside	49	20	254		
Background - outside	50	20	232	393	694.3
Background - outside	51	20	161		
Background - outside	52	20	263	569	1005.3
Background - outside	53	20	306		
Background - outside	54	5	16	34	240.3
Background - outside	55	5	18		
Background - outside	56	5	20	48	339.2
Background - outside	57	5	28		
Background - outside	58	5	8	23	162.5
Background - outside	59	5	15		