The Design and Testing of a Continuous Effluent Sterilization System for Liquid Waste

Sabine Daugelat[1], Sabai Phyu[2], Charles Taillens[1], Hooi Leong Wee[3], Juha Mattila[4], Teppo Nurminen[4], and Gerald McDonnell[5]

[1]Novartis Pharma AG, Basel, Switzerland, [2]Novartis Institute for Tropical Diseases, Singapore, [3]Novartis Singapore Pharmaceutical, Manufacturing Pte Ltd., Singapore, [4]STERIS Finn-Aqua, Tuusula, Finland, and [5]STERIS Limited, Hampshire, United Kingdom

Abstract

The disposal of microbiologically contaminated or potentially contaminated liquid waste is an important consideration for research facilities and other installations. Traditional methods include batch steam sterilization and chemical, thermo-chemical, or thermal disinfection kill-tanks. This report describes a new continuous effluent decontamination system that allows for the continuous collection, thermal sterilization, and cool-down of waste water prior to discarding from the facility. The verification of the safety, efficacy, and maintenance of the process required the development of specific microbiological test methods, which are described and discussed. The benefits of and considerations for using the system for liquid waste treatment and disposal—in comparison to traditional kill tanks—are discussed.

Introduction

The safe disposal of microbiologically contaminated liquid waste is a significant challenge to research facilities. Waste water can be biologically inactivated (preferably sterilized) by chemical or thermal means (CDC, 1999; Jennette, 2002; McDonnell, 2007). For the purpose of this discussion, "disinfection" is generally defined as a *reduction* in the microbial load in the waste water, whereas "sterilization" is defined as a validated process to render the waste water free of all viable microorganisms.

For liquid waste treatment, thermal-based methods are generally preferred. A traditional thermal disinfection process consists of a series of phases which can include receiving liquid into a holding vessel, heating up to a specified exposure temperature, holding at that temperature for a predetermined period of time, cooling down, sampling, and discharging to drain when verified safe. Under these conditions, the requirements of disinfection or sterilization can be achieved depending on the temperature and time of exposure to the process. Performance tests for kill tanks (e.g., as described by Schultz, 2002) and other batch processes are normally performed by taking samples of the decontaminated batch prior to discharge or by using biological indicators within the waste.

Chemical disinfection is an alternative method that requires minimal or no heat control for antimicrobial efficacy and can be somewhat simpler in terms of equipment and process requirements. However, liquid chemical treatment systems often require specific construction materials. In addition, some chemical processes can be flammable, can require adequate mixing for the required contact time, and can introduce harmful vapors/chemicals (including biocide residuals or reaction products) into the work area or the environment, depending on the biocidal processes used. They are generally recommended for smaller-scale applications (PHAC, 2004). Chemical biocides used include oxidizing agents such as sodium hypochlorite and peracetic acid, due to their broad-spectrum antimicrobial activity. The chemical (added directly or as part of a formulation) is generally mixed at a known concentration directly into the effluent batch at a sufficient ratio; it can be heated (if required) and held for a specific retention (contact or dwell) time. A specific example includes the use of 1N NaOH to inactivate prions at ambient or high temperature conditions (WHO, 1999). Similar to thermal systems, efficacy can be verified by direct sampling and microbiological analysis.

The conventional methods of liquid waste disinfection are batch-based processes, such as kill tank systems and various sterilizer designs. In these processes the effluent is collected, treated, and discarded as one batch at a time. Due to the waste control demands of research facilities, especially at Biosafety Level 3 and 4 laboratories, continuous waste reprocessing systems are becoming more desirable and the demonstration of their safety and effectiveness is an important consideration. These have included the use of some smaller flow-through heat-based systems leading to fast and convenient decontamination for small waste loads, but these also can be high cost when more than one system is needed for higher volume applications. In this report we discuss the design and installation of a high-capacity, continuous thermal sterilization processing system, its operation, and subsequent testing as a sterilization process.

Materials and Methods

Test System

The waste treatment system was a STERIS FINN-AQUA CED™ Continuous Effluent Decontamination

system (model number 300-CED-1000, serial number COA42179A; STERIS Finn-Aqua, STERIS FINN-AQUA, Finland) (Figures 1 and 2). The system is designed for biohazardous waste applications from non-hazardous up to category 4-classified microbiological facilities (BS EN 1620, 1997). These systems are available in a number of capacity sizes and are constructed of stainless steel. The CED system consists of a tank module (including an effluent buffer tank with associated equipment and piping) and a decontamination module (including a control system, effluent pumps, heat exchangers, as well as piping, valves, sensors, and other instrumentation; Figure 1). The tank module collects and stores liquid effluent, which is monitored by level sensors to control the operation of the unit. It operates at atmospheric pressure and includes a vent HEPA filter (which is constantly heated to +80°C for safety reasons). The tank module also includes an effluent prefilter (strainer) for screening solids larger than 1.2 mm in diameter. The full tank module (including filter, tank, and pre-filtering system) are automatically steam-sterilizable in place. The decontamination module consists of a pump assembly (two centrifugal pumps in series) and heat exchanger systems designed for heating the effluent to greater than 150°C (for sterilization under defined pressure, temperature, and time conditions) and cooling down to a safe level for discharge according to local guidelines (for example, in Singapore at 45°C). Decontamination is carried out at a constant flow rate in a dedicated length of pipe with a temperature sensor at the beginning and at the end of the pipe. Since the mass flow rate, pipe diameter, minimum holding temperature, and pipe length are known; it is possible to determine the minimum microbial lethality. Because the process pressure within the decontamination section is kept above the saturation pressure curve, water is not evaporated but superheated. Sterilization efficacy can therefore be defined and confirmed at an overkill F_0 value with sufficient margin (Joslyn, 2001; McDonnell, 2007). For example, a holding time of 1.16 seconds at 150°C equals 15 minutes at +121.1°C (therefore an $F_0 = 15$); this is discussed further below.

Overall, the system has five modes of operation: a processing effluent mode, self-test mode (used for start-up), steam sterilization mode, sectional sterilization mode, and service mode. During all modes, the unit continuously monitors the critical parameters (time, temperature, and pressure) during automated operation and self-diagnostics to ensure safe operation and records each mode and batch record electronically.

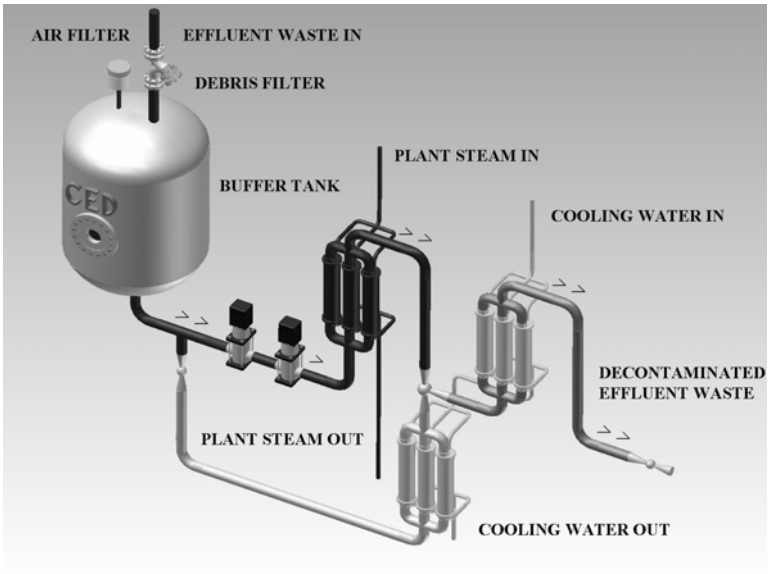
Installation and Commissioning

Waste liquid at the facility is treated in two ways. High-risk liquid microbial wastes (such as cultures), water from cleaning floors (which may contain an excess of various insoluble materials), and waste animal drinking water are chemically treated and autoclaved in batches. The larger-volume general waste water (including water from showers, hand-washing, etc.) is collected and treated through the CED system.

The CED system was installed within the containment zone of a Biosafety Level-3 (BSL-3) facility at the Novartis Institute for Tropical Diseases (NITD) in Singapore (Figure 2; Behrmann et al., 2007). This model has a liquid waste tank capacity of 1000L. The tank size and decontamination unit were optimized to match the required application (estimated to meet the maximum peak volume of potentially contaminated effluent from the facility) and planned daily effluent schedule, allowing for some capacity upgrade potential. The system was mounted with a Siemens S7-300 Programmable Logic

Figure 1

Representation of a STERIS FINN-AQUA CED™-continuous effluent decontamination system.



Controller (PLC) with a Simatic Operator Interface Panel (OIP), which was installed outside of the containment area for ease of operation and maintenance. The control system monitored and automatically controlled all process operations and functions. All pre-qualification and qualification tests were performed by Bovis Lend Lease (Bovis Lend Lease Pharmaceutical Pte Ltd., Singapore). All process qualification tests were carried out after installation and site acceptance tests had been successfully completed. These tests included:

- Verification of the CED flow rate and kill zone temperature profile (temperature mapping) under normal operation
- Challenge of the CED under normal decontamination operation with a biological challenge (described below)
- Challenge of the effluent tank module decontamination during a maintenance (sectional) cycle with thermal mapping and biological indicators (see below)
- Challenge of the CED decontamination module for maintenance (sectional) cycle with thermal mapping and biological indicators (see below)

Verification of Flow Rate and Temperature Profiles

All equipment and test instrumentation were calibrated before use. Thermal mapping and temperature profiling were conducted using a Kaye Validator 2000 System and HTR-400 temperature reference unit (GE Sensing, Billerica, Massachusetts). For thermal mapping within the decontamination zone, three thermocouples were placed in the vicinity of each of the controlling temperature sensors in the zone. Thermocouple temperatures were recorded at intervals of two seconds for 140 minutes during normal effluent decontamination mode and sterilization test cycles. The flow rate of the pump was also verified during this mode when the effluent tank was at maximum setting (750L), half full (350L), and low (100L). For each sample, discharge water was collected over a period of two minutes and the volume of water collected was recorded, in triplicate.

Microbiological Supplies and Analysis

Geobacillus stearothermophilus endospores were used for verifying the capacity of the decontamination processes of the CED system. Biological indicator discs were obtained from Raven Biological Laboratory (product #3-6100ST, Omaha, Nebraska) at a spore population of ~1.0 x 10^6 CFU/indicator. Following exposures, discs were inoculated into 8mL of Raven Tryptic Soy Broth (TSB) and incubated at 60°C for 24 hours to detect the presence/absence of spore growth. Spore suspensions included Sportrol (NAMSA SUS-08, 1.01 x 10^8 CFU/mL; Biomedical Research and Support Services, Pte Ltd., Singapore) and Spordex™ (5.3 x 10^8 CFU/mL and $D_{121.1°C}$ = 2.1; STERIS Corporation, Mentor, Ohio). Liquid spore samples were quantified by serial dilution and/or direct filtration through 0.45µ filter membranes and inoculated into Soybean Casein Digest (SCD) agar (Oxoid, Bloxwich Pte Ltd., Singapore). Plates were incubated at 55°-60°C for seven days to detect the presence/absence of growth. The population of each batch of biological indicators and spore suspensions was used as positive controls.

Biological Challenge-Flow-Through Tests

To verify the efficacy of the sterilization process during normal waste processing, biological challenge testing procedures were carried out within the entire process using a flow-through test system. Tests were designed under normal operating procedures using a liquid bacterial spore suspension introduced into the system and sufficiently sampled at the discharge outlet port of the decon-

Figure 2

The 300-CED-1000 liquid waste decontamination system installed. The steam generator is shown on the right, the collection tank module is shown at the middle and the decontamination module (enclosed) on the left.

tamination module. Prior to each test cycle, the effluent tank was emptied and the related parts of the process where sterilized through the Tank and Piping Sterilization Modes. After successful completion of these cycles, the tank was filled with water to the required start level. The incoming lines, which provided effluent to the unit, were equipped with a bypass line (for the purpose of testing) consisting of a 20L atmospheric container and a three-way valve for selection between tank and test liquid container (Figure 3). Samples could then be taken from the effluent outlet port of the unit.

Each sample volume was one litre and a total of five samples were taken during each test. The effluent decontamination rate of the unit was 600L/hour, which lead to a sampling period of 55 seconds plus the initial 10 seconds, with an initial spore inoculum of 10L (from the testing tank). Each test/sample was timed in a planned, synchronized schedule. Because the samples were collected manually and the test period was short, two testing persons were required to complete the test. All test samples were collected and analyzed for spore survival at an independent microbiological laboratory. The test was carried out using four different exposure temperatures (+150°C, +140°C, +130°C, +120°C). The log reduction observed under each condition was defined as the logarithm of input population divided by output population. Only the sample containing the highest remaining population at specific test temperature was noted. Samples with low spore populations were confirmed by membrane filtration.

Biological Challenge—Additional Tests

Maintenance sterilization cycles are available to separately sterilize the effluent tank or the decontamination module. Both sterilization cycles were validated using biological spore suspensions and biological indicator discs. The biological spore suspension was introduced via the effluent tank. The effluent tank was filled with water to a volume of 300L, inoculated with >1 x 10^8 spores, and mixed. The system was run in effluent process mode to allow contamination of the CED decontamination module and then placed in standby to allow circulation of the biological suspension. The sterilization cycle of the decontamination module was then run in triplicate, collecting four 500mL samples discharged from the module on completion of each cycle.

For effluent tank testing, the tank was contaminated as described above and, in addition, biological indicator discs were also placed within the tank. Five indicators were placed within the tank (above the 300L level of the suspension test volume), with a further four indicators placed within the module strainer and four within the filter vent. The effluent strainer, vent filter, and tank sterilization cycles were then tested (in triplicate). Following cycles, ten 500mL tank discharge samples were collected and all indicators were recovered aseptically for laboratory analysis.

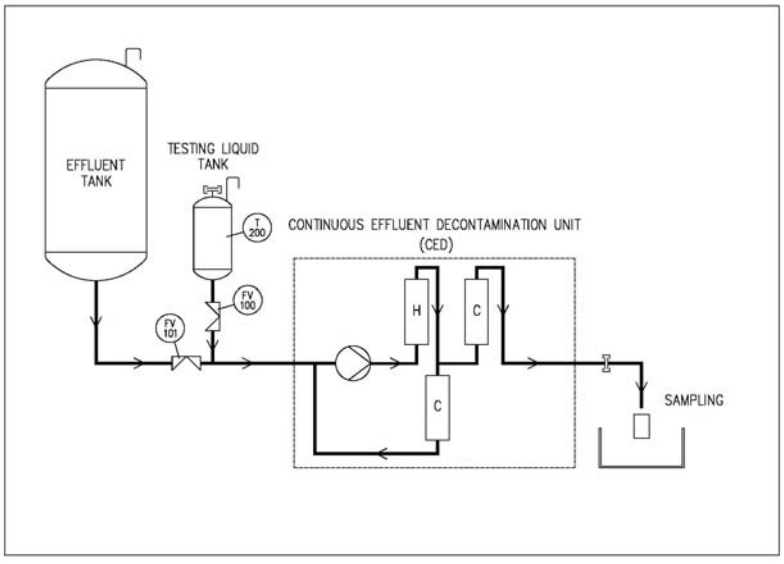
Results

Parametric Tests

The flow rate through the sterilizing zone of the CED system during normal operation has a set point of 300L/hour but an operating range of 300L/hour ± 15L/hour. The flow rate was tested by collecting effluent water from the system in triplicate experiments and at three different levels of effluent tank volume (to simulate

Figure 3

The test system used for verifying the efficacy of the CED Decontamination system. The by-pass tank/line for inoculation is labelled "Testing Liquid Tank" (note the additional valves below the tank and to the left of the junction to the effluent tank to allow selected inoculation of the system during testing) and the sampling port is shown on the right.



maximum volume at 750L, half full at 350L, and low volume at 100mL). The flow rate was found to range from 295.5L/hour to 307.5L/hour, with an average rate of 303.5L/hour, with no differences observed among the various tank volumes tested. In addition, the sterilization temperature has a set point of 155°C but has a minimum operation sterilization temperature set at 150°C. Temperatures were monitored during a series of decontamination modes and each individual maintenance sterilization cycle. During normal decontamination operation cycles, temperatures of the effluents were verified at 155°±4°C.

Theory of Operation and F_0 Calculation

Heat sterilization is a function of probability dependent upon the number of microorganisms, their intrinsic heat resistance, and the amount of heat exposure (Joslyn, 2001; PDA, 2002; Pflug et al., 2001). The CED system was tested at four different exposure temperatures (+150°C, +140°C, +130°C, +120°C). Using multiple temperature points allowed the estimation and comparison of sterilization effectiveness within the system at different temperatures, using the theoretical values as reference. The F_0 value is defined as the number of equivalent minutes of steam sterilization at temperature of T_b = +121.1°C at minimum, to lethality required. The relation between exposure temperature T_0 and F_0 can be determined by the following formula:

$$F_0 = L \cdot \int_{t_0}^{t} dt \qquad (1)$$

where L is the lethal rate specified by the process conditions of the CED unit and T_0 = 0 and t = exposure time of the specific process.

The lethal rate is therefore a constant value and is calculated using the following formula:

$$L = 10^{\left(\frac{T_0 - T_b}{z}\right)} \qquad (2)$$

$$L = 10^{\left(\frac{150-121.1}{10}\right)} = 776.25$$

where, T_0 = process exposure temperature [°C].
For the CED unit under normal operation, the T_0 = ≥150°C
T_b = reference temperature of sterilization; T_b = +121.1°C
z = the number of degrees of temperature change necessary to change the D-value by a factor of 10. In this case the z-value is assumed to be 10.

When the exposure temperature T_0 is higher than the reference value of T_b = +121.1°C, the efficacy of sterilization rises exponentially, resulting in much shorter required exposure times. The STERIS CED decontamination exposure temperature is set to T_0 = ≥150°C. The exposure time is therefore dependent on the unit size (i.e., constant liquid flow rate Q), exposure section length(s), and diameter (d). The decontamination exposure section of CED-unit is a dedicated length of pipe between two temperature probes, and this minimum verified section length was used for calculations. Overall, sterilization efficiency estimations are considered worst case and are expected to be greater than the theoretical calculations given.

With this particular installation, the constant liquid flow rate was Q = 600 L/h = $1.67 \cdot 10^{-4}$ m^3/s. The exposure section length s = 1.5 m and inner diameter d = 22.1 mm = 22.1·10-3 m. Therefore, the calculated exposure time was:

$$t = \frac{\pi \cdot d^2 \cdot s}{4 \cdot Q} = \frac{\pi \cdot (22.1 \cdot 10^{-3})^2}{4 \cdot (1.67 \cdot 10^{-4})} = 3.45\ s = 0.0575\ \text{min} \qquad (3)$$

The exposure time was then converted to minutes to comply with F_0 value calculation:

$$F_0 = L \cdot \int_{t_0}^{t} dt = 776.25 \cdot \int_{0}^{0.0575} dt = 44.63 \quad \text{[according to (1)]}$$

where L is derived from formula (2).

The F_0 value for CED-unit at a minimum 150°C process temperature with Q = 600L/h and exposure time of t = 3.45s was therefore estimated to be 44.63.

The D-value for the challenge microorganism is defined as the time in minutes required for a one log (90%) reduction of the specific microbial population under lethal conditions. For steam or liquid-heat processes, *G. stearothermophilus* spores are known to be the most resistant organisms (McDonnell, 2007) and a reference D-value (or resistance) is specified at 121.1°C ($D_{121.1°C}$) for each lot of commercial spores produced. Therefore, the expected log reduction of *G. stearothermophilus* spores in the CED system at each test temperature was estimated based on the population and reference D-value of spores used for testing (in this case 5.3 x 10^6 CFU/mL and a $D_{121.1°C}$ = 2.1, respectively). The total output population and actual log reductions were calculated by (Note that the number of colonies per 0.5mL sample in a 10L batch was multiplied by 20,000 to give the total population):

$$\text{Output population} = \textit{number of colonies} \cdot 20000$$

$$\text{Actual log reduction} = \log\left(\frac{\textit{input population}}{\textit{output population}}\right)$$

These were estimated at 150°C = 21.3, 140°C = 2.1, 130°C = 0.2, and 120°C = 0.02 (Table 1).

Biological Tests

A summary of the expected and actual bacterial spore log reductions under the different test temperatures in decontamination mode during verification is given in Table 1. Test results showed that the CED equipment performance exceeded the expected log reductions. This performance level was expected due to the estimations of log reduction being based on the *minimum* length of exposure time and temperature in the decontamination module; for example, additional log reduction may be ex-

pected during the heat up (up to 150°C) and cool down (down to 45°C). Overall, the test method worked well enough to ensure the required log reductions, and safety factors could be achieved during the decontamination process, as expected from the F_0 estimations.

The original test method for the decontamination piping sterilization cycles included large-volume sampling from the CED closed loop circulation. This method became impractical due to the system design. Therefore, piping sterilization cycle testing was conducted with biological indicators with a minimum population of 1.0 x 10^6. Similar practical problems were observed with testing the tank sterilization cycle, where the use of biological indicators proved to be more controllable and repeatable. Therefore, the tank sterilization mode tests were tested with biological indicators, as well as with a 300L batch of *G. stearothermophilus* at 1.53 x 10^8 (verified by serial dilutions and plate-counting), which exceeded the minimum requirement (10^6) by a factor of 100. The sterilization conditions in each case were at a minimum 134°C for 15 minutes. Each of the total eight tests performed passed, with no growth observed in any of the biological indicators or suspension tests completed.

Discussion

Biosafety in research facilities is a coordinated approach dependent not only on various methods and practices to contain hazardous microorganisms, including airlocks, air-handling systems, ventilation filtration, biosafety cabinets, personal protective equipment, etc. (CDC, 2007; Fleming & Hunt, 2007; PHAC, 2004), but also on the types of pathogens intended to be handled within these facilities (Behrmann et al., 2007). An important consideration in the design and use of these facilities, in particular for Biosafety Level 3 and 4 facilities, is the treatment of waste water, which is integrated into global security and safety concepts. The waste water itself can range from used tap water (e.g., "gray water" from showers or for hand-washing) to grossly contaminated liquid animal wastes. Therefore, the design of water-treatment systems requires facility-specific risk assessment and design requirements (CDC, 2007; Jennette, 2002; PHAC, 2004). The efficiency of installed treatment methods has to be controlled and monitored to ensure their security, safety, and effectiveness. For example, chemical-based treatments can be dramatically affected by the presence of contaminating soils (both organic and inorganic) that can react with and neutralize biocidal activity. From an environmental perspective, chemical-based processes also need to consider any potential toxic or corrosive by-products that can be generated during the disinfection process and may cause a concern for subsequent release into a sanitary sewer system. Similarly, heat-based processes can be compromised by insufficient heat distribution and scaling on heat transfer surfaces to prevent sufficient heat transfer to and within the load. A further consideration is the volume of waste water that the facility may produce and the efficiency of the treatment system to be able to meet this need.

The CED systems are examples of continuous effluent-treatment processes. These systems have been designed for facilities that handle any bio-hazardous materials and produce waste liquids that may be microbially contaminated (from Risk Group I to Risk Group IV agents; WHO, 2004). This list of facilities includes large-scale pharmaceutical production facilities, research laboratories, hospitals, agricultural and animal research facilities, food industry plants, and governmental or military facilities. The effluent treatment systems can be installed outside or within a given containment area, with the system described in this report installed within the biosafety area but the system control mounted outside for ease of use, maintenance and operation. These systems can manage liquid waste capacity ranges from 100 to 3000L/hour with the same basic design. The design is particularly applicable for handling liquid waste, but this design can be enhanced for screening out solids from the waste water, if required. One of the benefits of a continuous process is the integrity of the decontamination. Since the ac-

Table 1

The expected (estimated) and actual log reductions observed under various test temperature conditions in the CED decontamination system (testing conducted at STERIS Finn-Aqua).

Temperature [°C]	Log Reduction (Log_{10})	
	Expected	Tested
150	21.3	>6.72*
140	2.1	>6.72*
130	0.2	1.72
120	0.02	0.76

*Under the test conditions, no spores survived giving a minimum log reduction of >6.72 (initial bioburden).

tual decontamination takes place in a very compact and restricted area, and under turbulent flow conditions, the heat distribution in the superheated water is intrinsically uniform. Therefore, the success of the decontamination process is not likely to be compromised by air pockets or cold spots. Turbulent flow in the decontamination tube also enables very fast and accurate temperature monitoring which is essential for secure and reliable operation. During installation and subsequent testing of the system onsite, it was highlighted that close attention should be paid to ensure that the correct utilities are provided and subsequent maintenance schedules are followed to allow continuous operation of the system to meet facility capacity needs.

In general, the testing of liquid waste disposal will depend on routine monitoring of the microbial content of the waste following treatment, which can be difficult and costly to perform. With heat-based systems, these systems generally can be more routinely tested and validated based on parametric monitoring. In addition to parametric testing, periodic biological testing is also conducted to verify the efficacy of these systems.

This approach posed some difficulties with a continuous effluent treatment system. The testing defined in this report was defined to demonstrate the overkill of the thermal sterilization process according to well accepted models of moist heat sterilization, as well as test methods to allow routine testing (in parallel to the parametric testing constantly conducted) for validation or re-validation purposes. Testing included the parametric monitoring of the process in order to predict the minimum, predicted microbial lethality with bacterial endospores which are well established as the most resistant organism to moist heat processes (McDonnell, 2007; Pflug et al., 2001). In this testing, the lethality observed during the process under lower and actual temperature/pressure operating conditions was shown to correlate with the predicted log reductions based on the F_0 and resistance of the spore population used for testing. This method allowed the demonstration of the significant overkill observed with these systems during normal operating conditions, which should be expected for most moist-heat sterilization processes. During verification, a combination of more typical direct microbial (bacterial spore suspension) challenge with biological indicators was sufficient (when used in parallel with parametric testing) to demonstrate the efficacy and safety of the process implemented in a typical facility. Routine parametric monitoring during the continuous use of the system can therefore be conducted and archived, with programmed alarms when the process falls below minimum acceptable criteria for the defined process variables (in this case with a set point of >150°C within the decontamination module for the required exposure time).

It is also possible to collect samples directly from the effluent system (in this case with waste water being discarded at < 45°C) and conduct traditional microbial analysis, if deemed necessary. Further, from a maintenance point of view, the system can be sterilized routinely by traditional and equally controlled steam cycles to allow safe access to the system to ensure its integrity. An example of this control logic is on "start-up of the system." A "Self Test Mode" has been programmed where the system automatically tests all functions required for processing effluent. When the unit is "cold-started," the Self Test must be carried out successfully before "Processing Effluent Mode" can be selected. "Self Test Mode" must also be carried out after a critical alarm situation, any sterilization procedure, or a Clean-In-Place (CIP) procedure. During this process phase, the effluent is pumped by magnetic-coupled booster pump through a set of steam-operated heaters to the decontamination section, returned to the suction side of the pumps through the circulation cooling exchangers, and cooled down close to the tank temperature (normally ambient).

Moist-heat sterilization systems are generally regarded to be the most efficient for inactivation of all microorganisms, including atypical pathogens such as prions. In the case of conventional microorganisms such as bacteria, viruses, and protozoa, their heat resistance has been investigated, with bacterial endospores being the most resistant (McDonnell, 2007); however, in the case of prions, their true heat resistance is unknown and is speculated (Taylor et al., 1998). The optimal treatment of prions has been suggested to be extended steam sterilization cycles and/or immersion in a high concentration of alkali (such as 1N NaOH) for extended periods (CDC, 2007; WHO, 1999). More recent studies have suggested that liquid heat-based processes may indeed be more effective than steam sterilization alone, due to the mechanisms of heating and hydration during the process in comparison to vacuum-based steam processes (Fichet et al., 2004). It may be expected that further studies on the mechanisms of prion inactivation will continue to support this observation.

In conclusion, continuous thermal sterilization for biosafety facilities has been shown to be an adequate, efficient, and effective method for the treatment and handling of liquid waste. Following installation and validation tests, the process has been successfully implemented and used by the facility. The test methods described in this report are recommended for the testing of any continuous systems to ensure their safety and efficacy.

References

Behrmann, T., Brunner, A., Daugelat, S., Perrin, M., Phyu, S., Taillens, C., et al. (2007). Regulatory compliance of a BSL-3 laboratory unit in a drug discovery evironment. *Applied Biosafety: Journal of the American Biological Safety Association, 12*(4), 220-238.

BS EN 1620. (1997). European Standard. Large-scale process and production. Plant building according to the degree of hazard.

Centers for Disease Control and Prevention (CDC). (2007). *Biosafety in microbiological and biomedical laboratories* (5th ed.), HHS Pub. (CDC). Washington, DC: U.S. Government Printing Office.

Fichet, G., Comoy, E., Antloga, K., Duval, C., McDonnell, G., & Deslys, J. P. (2004). Novel methods for disinfection of prion-contaminated medical devices. *Lancet, 364,* 521-526.

Fleming, D. O., & Hunt, D. L. (2007). *Biological safety: Principles and practices* (4th ed.). Washington, DC: ASM Press.

Jennette, J. P. (2002, January). Animal waste treatment: Selecting technologies for the disposal of animal waste and wastewater. Paper presented at the 7th National Symposium on Biosafety, Atlanta, GA.

Joslyn, L. J. (2001). Sterilization by heat. In S. S. Block (Ed.), *Disinfection, sterilization, and preservation* (5th ed.) (pp. 695-728). Philadelphia: Lippincott Williams & Wilkins.

McDonnell, G. (2007). *Antisepsis, disinfection, and sterilization: Types, action, and resistance*. Washington, DC: ASM Press.

PDA Technical Monograph No. 1, (2002 Revision). *Industrial moist heat sterilization,* Volume 57 (2003 Supplement).

Pflug, I. J., Holcomb, R. G., & Gomez, M. M. (2001). Principles of the thermal destruction of microorganisms. In S. S. Block (Ed.), *Disinfection, sterilization, and preservation* (5th ed.) (pp. 79-129). Philadelphia: Lippincott Williams & Wilkins.

Public Health Agency of Canada (PHAC). (2004). *Laboratory biosafety guidelines* (3rd ed.). Ottawa: Health Canada.

Schultz, C. C. (2002). Biosafety wastewater sterilization. *PM Engineer,* Issue 11/02.

Taylor, D. M., Fernie, K., McConnell, I., & Steele, P. J. (1998). Observations on thermostable subpopulations of the unconventional agents that cause transmissible degenerative encephalopathies. *Veterinary Microbiology, 64,* 33-38.

World Health Organization (WHO). (1999). *WHO infection control guidelines for transmissible spongiform encephalopathies*. Geneva: World Health Organization. WHO/CDS/CSR/APH/2000.3 (March).

World Health Organization (WHO). (2004). *Laboratory biosafety manual*. Geneva: World Health Organization.