Battling Biohazardous Liquid Laboratory Waste

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"So, what happens with your Biosafety Level 3 lab waste?" I asked the laboratory manager. "Our safety assessment says to just mix it with sterilant wash it down the drain - there is worse stuff down there already." "How about genetically modified material?" "Yeah, that goes the same way - It'll die in the drains".

Treating biologically hazardous waste in such a way is not uncommon in the UK - the combination of chemical sterilants and a well-developed system of sewerage and treatment plants can handle a wide range of biologically active substances, but it is not a failsafe system.

The 2007 foot-and-mouth disease outbreak within the county of Surrey, UK, is believed to be the result of a damaged drainage pipe leading from a Containment Level 4 laboratory [1].

The facility had been permitted to dispose of liquid waste containing small quantities of live foot-and-mouth virus. After chemical sterilisation in an Effluent Decontamination System (EDS), liquid waste from the laboratory's experiments was dispatched to the drain. The laboratory decontamination showers – deemed less likely to contain the Category 4 virus - emptied directly into the drain [2].

The unsterilised wastewater from the decontamination showers may have been the point of egress for the virus. By following the UK's Health and Safety Executive (HSE) guidelines, the laboratory would have taken a different approach: collecting all wastewater from sinks and showers and deactivating the biologically hazardous material contained within it before discharging to the sewer [3]. This process, recommended for Containment Level (CL) 4 facilities, is also in most instances a requirement, if not strongly recommended¬ for CL3 environments across varied settings from animal research [4] and genetically modified organism [5] labs, to large-scale biotechnology plants [6]. Wastewater from the decontamination showers could have been sterilised by handling it in the same way the laboratory's liquid waste was; through sending it to the EDS.

An EDS is a single-purpose device that sterilises wastewater and effluent. While designs vary, these systems usually fall into one of two categories: Thermal EDS and Chemical EDS. The Thermal EDS uses heat to sterilise waste, while a Chemical EDS - as used by the laboratory at the centre of the 2007 foot and mouth outbreak - employs chemical sterilants. The following article will review both methods with the aim of better understanding which method is more suited to the modern laboratory.

The Chemical Effluent Decontamination System

The EDS used by the laboratory employed chemical sterilisation to destroy hazard group 4 pathogens. Typically, such forms of Chemical EDS collect effluent either in a sterilisation tank (also known as a kill tank), or in a storage tank. Once an adequate volume of liquid waste has been amassed, it is mixed with a chemical sterilant in the kill tank and held until sterile [7] (see *Figure 1*).

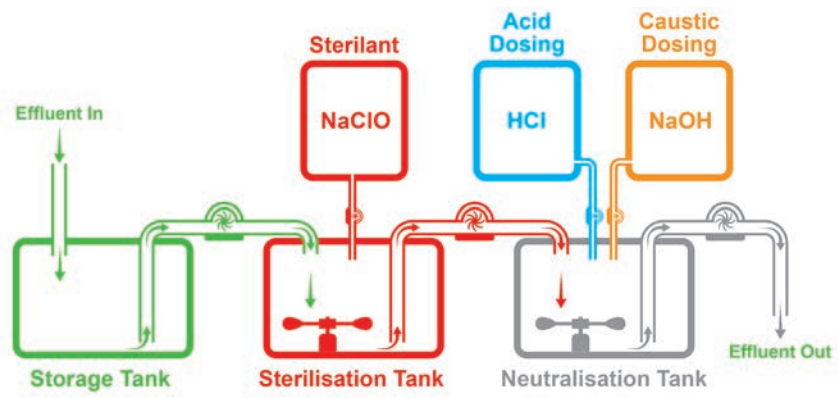

Figure 1. Schematic Diagram of a Chemical Effluent Decontamination System, (Adapted from 'Figure 8. Schematic drawing of the effluent decontamination system'. - Vijayan, V., & Ng, B. (2016). Validating waste management equipment in an animal biosafety level 3 facility. Applied Biosafety, 21(4), 185-192.)

In a Chemical EDS, composition of the effluent forms a key factor determining sterilisation time. Organic and particulate matter in the mixture can encapsulate biologically hazardous agents shielding them from chemical sterilants. Consequently, the larger the amalgamation of material, the lower the efficacy of the sterilisation. Mechanical maceration and blending of the effluent may reduce the shielding effect, but to be effective, the sterilant must come in contact with the pathogen [8].

By contrasting the results of two studies, it is possible to see the effect of effluent composition upon sterilisation time in the Chemical EDS. Using sodium hypochlorite (bleach) as a sterilant at a concentration of 700 parts per million (ppm), it was possible to sterilise a mixture of Bacillus subtilis and water in 30 minutes [9]. However a two-hour sterilisation time was required when using a concentration of 5700ppm of bleach to deactivate bacillus spores in a mixture of animal effluent, humic acid, and fetal bovine serum [10]. While a more viscous and solid-rich effluent does provide a greater challenge for chemical sterilants, it is the organic material's reaction with the sterilant that explains the requirement for a stronger concentration of sodium hypochlorite in the latter example. Chlorine from the bleach reacts with protein, forming iV-chloro compounds and reducing the amount of active sterilant available to deactivate microorganisms and viruses [11, 12]. To counter this, increased quantities of sodium hypochlorite are required.

Concentration of sterilant and processing time is also dictated by the type of biologically hazardous agents in the wastewater. Higher resistance to chlorine-based disinfection is shown within some genera of bacteria, (including Mycobacterium, Bacillus, Legionella, Pseudomonas and Sphingomonas [13]) fungi (such as Aspergillus [14]) and viruses. In the later instance, adaption to tolerate warmer waters been shown to result in a greater tolerance to chlorine-based disinfectants [15].

Once the effluent is disinfected, the mixture requires pH neutralisation before it can be released into the sewer. This process sees acids and alkalis - for example hydrochloric acid and sodium hydroxide - added incrementally to the effluent until a pH range acceptable for the specifications of the local water board is achieved [9]. Only then can the effluent be dispatched to the sewer system.

Once it has left the EDS, the effluent may contain residual chlorine which can be carried into the sewer system. In 2019 alone, UK water companies overflowed untreated sewage into rivers and streams in over 200,000 instances for a combined 1.5million hours [16]. With as little as 100-300 µgL of residual chlorine [17, 18] shown to be toxic to aquatic life [18, 19], wastewater that has been chemically disinfected may create environmental damage even when free of pathogens.

While sodium hypochlorite is not the only chemical sterilant available, it is the most cost effective – an important consideration for laboratories outputting large volumes of biohazardous liquids. Yet all chemical sterilants come with drawbacks. By their nature they are toxic and reactive substances which require specialist and dedicated storage, especially when used on the scale of an EDS. They are also less effective against solid-rich effluent, have varying levels of efficacy on different pathogens, and can be difficult to validate [7].

The shortcomings of utilising Chemical EDS were highlighted in the HSE's Final Report after the 2007 foot and mouth outbreak, which recommended a review of the process, stating "It is our experience that chemical treatments, while reducing the amount of pathogen in the liquid, may not render the liquid completely pathogen-free" [2].

The Thermal Effluent Decontamination System

Chemical sterilisation of biohazardous material is a process commonly used by laboratories, in scales ranging from a vial of liquid waste to a whole facilities effluent. However, analysing the Chemical EDS and how its use contributed to the 2007 foot and mouth outbreak highlights the problems inherent with the sterilisation method. This is not the only method of liquid waste sterilisation available to laboratories. A simpler, more comprehensive method that does not rely on a hazardous consumables is available: heat sterilisation.

Heat sterilisation of biologically hazardous material is a commonplace process, with autoclaves becoming ubiquitous in laboratories since their invention in 1879 [20]. By heating material to between 121°C and 134°C in a pressurised environment for between three and fifteen minutes, autoclaves can destroy all biologically active material [21]. Adapting the autoclave process to the singular task of wastewater treatment has resulted in the Thermal EDS, a device for sterilising effluent via heat.

The structure of a thermal EDS can be varied according to need. Facilities ceaselessly expelling wastewater free of solid material can accommodate a Continuous Flow EDS [7] - a length of heated pipe hot enough for the effluent to maintain sterilisation temperature, and long enough so that the flowing liquid has sufficient time to sterilise. For laboratories with a more varied and variable output, a Thermal Batch EDS is more applicable.

All types of Thermal Batch EDS collect a specified quantity of effluent, then heat it to a sterilisation temperature for long enough to destroy any biologically hazardous materials. Differentiation in their construction allows these units to handle increasingly complex effluent types. For instance, biohazardous liquids that are little more than water containing pathogens require the simplest of Thermal Batch EDS. Sterilisation of such effluent can be achieved with a sealable pressure vessel containing an internal heating element, which can heat the effluent to the correct temperature for long enough to ensure sterility.

Challenged with sterilising effluent containing more varied constituents including solids, a Thermal Batch EDS with a jacketed sterilisation tank provides an effective solution [7] (See *Figure 2*).

The jacketed sterilisation tank is a vessel with hollow walls. Effluent is either pumped into the tank, or flows into it from a source above using gravity. Once the tank is sufficiently full, valves isolate it, and high-temperature pressurised steam is passed through the cavity in the walls of the jacketed vessel. This raises the temperature of the effluence to sterilisation temperature and pressure for long enough to destroy all pathogens within the sterilisation tank [7]. Once sterilisation is completed, the tank is emptied through displacement with high-temperature pressurised steam.

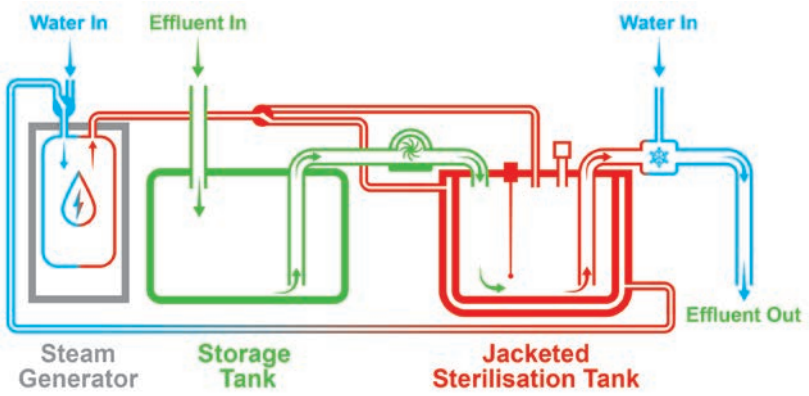

Figure 2: Schematic Diagram of a Thermal Batch Effluent Decontamination System.

A Thermal Batch EDS with a jacket vessel offers certain advantages over other types of EDS. It is not hampered by effluent containing organic material, does not add additional contaminants to the wastewater, and doesn't require any chemicals. It shares these attributes with all forms of Thermal EDS.

However, unlike Continuous Flow EDS and Chemical EDS, the Thermal Batch EDS can sterilise solid material in effluent, is not prone to clogging, and can be easily validated during operational [7]. With capacity to adjust sterilisation times and temperatures, treatment parameters can be varied. While variation in the number and type of tanks used can create systems capable of efficiently handling any effluent flow rate.

Would use of such a jacketed-vessel Thermal Batch EDS mitigated the foot and mouth outbreak in 2007? Maybe so. The penetrative and highly effective capabilities of heat sterilisation would have greatly reduced the possibility of materials leaving the EDS unsterilised. With low running costs, running all liquid effluent from the lab – including the showers – ¬ to the Thermal Batch EDS would be a simple and cost-effective solution during the initial site build.

Furthermore, it's worth noting that this approach would have provided a more environmentally-friendly solution for the laboratory that was also safer for staff. As mentioned, chemical sterilants and neutralisers are hazardous substances that require specialist handling and storage. Not only does this increase site safety protocols to mitigate the potentially harmful effects of the sterilants on staff and the environment, but it also necessitates more infrastructure. Chemicals require frequent shipping to site by road or rail, alongside buildings in which to store them until required; both factors that give the Chemical EDS a large carbon footprint.

In contrast, even the most advanced Thermal EDS can receive all the resources it requires from national energy and water supply networks. With electricity flowing from the grid into the device, and water for steam generation plumbed in, no manual loading is required. Even rare examples of Thermal EDS that use natural gas as an energy source can be supplied through pipelines. This reduction in manual interaction improves staff safety - a factor further enhanced as the only potentially harmful by-product of the Thermal EDS is heat.

While not all laboratories may be working with CL4 pathogen, facilities handling biologically hazardous material of any grade can benefit from a Thermal EDS. AstellBio, sister company to the autoclave manufacturer Astell Scientific, produce Thermal EDS solutions for a variety of uses. From small scale sink units able to provide mobile and packaged EDS functionality, to larger EDS units capable of handling a whole building's effluent. By installing an AstellBio Thermal EDS, you can be assured that all effluence leaving your facility is sterile – without the need for chemical sterilants or disinfectants.

Figure 3: A selection of Thermal Batch Effluent Decontamination Systems.

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