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Effluent Decontamination systems

Design, operation and safety

Biosafety and Biotechnology Unit

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Introduction

In the frame of biosafety regulation[1], aiming at protecting human health and the environment from biohazards, all bio-hazardous waste generated in biocontainment facilities must be decontaminated prior to discharge. This includes all microbiologically contaminated solid and liquid waste. In the case of high containment facilities decontamination may also be required (Biosafety Level 4) or optional (Biosafety Level 3) for potentially biologically contaminated effluents depending on the used biological agent (for instance required for notifiable animal pathogens of risk class 4 such as the Foot and Mouth Disease virus[2], and for poliomyelitis vaccine production from wild poliovirus[3]).

Effluent decontamination systems (EDS) are used to treat large amounts of biologically contaminated liquid effluents from biocontainment facilities such as large scale production plants, animal holding facilities and research laboratories. Biologically contaminated effluent originates typically from sinks, showers, autoclave chambers and floor drains.

The used decontamination systems must ensure inactivation of all viable micro-organisms including survival structures (e.g. spores) and in that respect the process must be validated by microbial challenge testing. In this document different process types are discussed as well as biosafety aspects and qualification. Effluent decontamination systems based on UV irradiation for treating drain water of laboratories or greenhouses for work with plant pathogens will not be discussed here.

Effluent decontamination methods

Potentially biologically contaminated effluents can be treated in different ways: by chemical or thermal methods, or a combination of both, and possibly combined with pressure.

For chemical treatment oxidizing agents such as sodium hypochlorite (NaOCl) and peracetic acid (CH_3CO_3H) are generally used as they have a broad-spectrum antimicrobial activity. The chemical is generally mixed at a known concentration directly with the effluent at a determined ratio, held for a specific contact time and heated if required. Although chemical processing can be quite simple in terms of equipment and process requirements, it has several drawbacks: it requires specific construction materials to withstand corrosion (such as high grade stainless steel or corrosion-resistant metal alloys (hastelloy)); it needs adequate mixing and chemicals do not penetrate any solids that enter the system and are prone to clogging; it leaves harmful biocides or reaction products that need to be neutralised before discharge in the sewage system to comply with the local waste water regulations in terms of pH, temperature, chemical/metal content, suspended solids, oil/grease and biochemical oxygen demand; it can also release harmful vapours or chemicals in the work area or environment. (Tremblay et al, 2010, Daugelat et al, 2008).

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[1] European Directive 2009/41/EC on the contained use of genetically modified micro-organisms, in the Belgian legislation enlarged to include contained use of GMO (transgenic animals & plants) and pathogenic organisms.

[2] "Minimum standards for laboratories working with FMD *in vitro* and *in vivo*", established by the European Commission for the control of Foot-and-Mouth Disease of the Food and Agriculture Organisation, 38th session, Rome, April 2009.

[3] WHO Technical Report Series, No. 926, 2004, Annex II "Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses (Addendum, 2003, to the Recommendations for the Production and Quality Control of Poliomyelitis Vaccine (Inactivated)) http://www.who.int/biologicals/publications/trs/areas/vaccines/polio/Annex%202%20(65-89)TRS926Polio2003.pdf

For thermal-based liquid waste treatment methods, a combination of heat and pressure is needed to ensure that all potentially dangerous biological agents are destroyed in the effluent. In contrast to chemical-based systems, solids (in the effluent) can be sterilised and are less susceptible to clogging. Usually, effluent decontamination systems operate between 121°C and 134°C or higher, depending on the chosen system and characteristics of the biological agent to be inactivated. In some cases, for example for a laboratory where only virus work is done, a lower temperature of 93°C could be used as most viruses are destroyed at this temperature (Stahl, 2008). In comparison to chemical treatment, one must consider additional energy consumption for heating - however the system can be designed with heat recovery - and the need for a pressure vessel. The high temperatures will also increase the rate of tank corrosion.

Thermo-chemical treatment has the advantage that no pressure vessel is needed, and no such high temperatures are needed as systems based on heat alone, which also reduces tank corrosion (Stahl, 2008). Also the system can switch between either chemical or thermal only treatment: in case of steam utility failure a chemical only cycle can be run, or alternatively a thermal only cycle can be chosen with a longer exposure time to ensure complete inactivation. However, the adequate temperature and chemical combination need to be determined for inactivating the agents used in the facility. Also, the use of chemical disinfectant may still require adjusting the physical and chemical parameters of the effluent to comply with waste water regulations before release in the sewage system.

Effluent Decontamination systems (EDS)

In order to choose an effluent decontamination system that offers the best solution for the facility in which it will operate, it is important, among other factors, to first determine a load profile. In determining this, not the flow from the facility during peak periods, but rather the flow throughout the entire day is taken into account: for most of the facilities, peak periods will occur at the end of the afternoon when laboratory personnel is showering, autoclaves are run and animal facilities are cleaned. Also the characteristics of the flow such as load composition including presence of solids or chemicals must also be taken into account. Besides, not only common sources of load but also uncommon sources such as fire protection system load (e.g. sprinklers) or system failure (leaks, human error) should be considered (Tremblay et al, 2012).

There are two main types of design for liquid waste decontamination: a batch operated process or a continuous process. In a batch operated process the effluent is collected, treated and discharged as one batch at the time. Decontamination can be done either by chemical or thermal treatment. A continuous process is a heat-based flow-through system consisting of a series of heating and cooling exchangers and a dedicated pipe section for sterilization under defined pressure, temperature and time. Due to a uniform heat distribution in a compact and restricted area, ensuring improved effectiveness and reliability, continuous waste processing systems are particularly suited for biosafety containment 3 and 4 levels (Daugelat et al, 2008).

A description of both methods is given below.

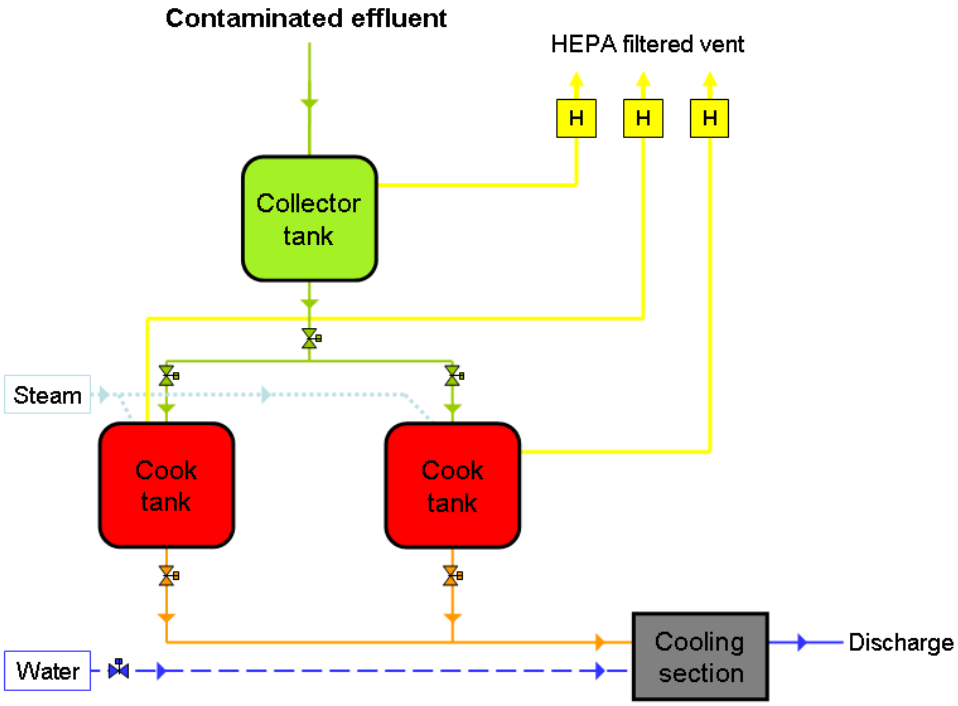

Figure 1: Schematic of a thermal batch operated effluent decontamination system

Batch operated decontamination system

Batch-based EDS systems (see fig. 1) vary in design and operation: usually they consist of a collecting or storage tank and a treatment tank, but systems with one storage tank and two treatment tanks are also a possibility. Effluent waste can even be collected directly in a series of treatment vessels (e.g. one running, one filling and one in standby) (Gordon et al, 2009). Tank size needs to be estimated to match the maximum daily peak volume of potentially contaminated effluent from the installation (Daugelat et al, 2008, Mattila, 2011). It may vary from 1200L (most common) to 3000L. It is important to note that batch operated treatment of large daily effluent load requires a lot of technical space for the high tech decontamination equipment with voluminous storage and decontamination tanks including emergency containment basins in case of accidental leaks. This makes batch processing more suitable for small facilities with low effluent generation (< 400L per day). On the other hand, one advantage of batch processing is that it allows for solids in the effluent waste, whereas a continuous system would rather not as the small diameter tubing specific of the piping-based system can trap solids and plug it (Andersen et al 2011, Tremblay et al 2010).

Depending on the chosen treatment (chemical or thermal), a batch operated process involves the following steps:

1. **Filling step**: effluent is either pumped or gravity drained to a collecting vessel via inlet waste lines. When the effluent waste reaches a high level sensor the inlet will close diverting any further waste effluent to one of the remaining tanks and decontamination or sterilization will start (sterilisation start in one tank will automatically set the next tank in receiving mode).

2. In case of chemical treatment a chemical is either manually or automatically added at a known concentration into the effluent at a known ratio. In case of thermal treatment the collecting tank is heated up either by indirect (heating coils or jacket heating) or direct steam injection. In jacket heating, steam is injected in a steam jacket located between the inner shell and the outer jacket of the vessel, whereby the entire wall is acting as a heating surface (Mattila, 2011, Gordon et al, 2009). Direct steam injection into the liquid load has the advantage of accelerating the heating process as well as agitating the liquid (Palani, 2006). Agitation of the effluent is essential to avoid 'cold spots' (heat treatment) or incomplete mixing (chemical treatment) in the effluent load. For this purpose, a recirculation pump ensures a uniform distribution of temperature or chemical across the system and prevents solids to build up on the bottom of the tank which could affect heat transfer from the heating jacket (Biosafe Life sciences, Tremblay et al, 2010).

3. **Decontamination/sterilization step**: the effluent batch is held for a specific retention time (chemical treatment) or at the specified exposure temperature for a predetermined period of time (thermal treatment) while the recirculation pump continues to function for uniform mixing. The batch process temperatures are standard steam sterilizer cycle temperatures (121 to 134°C) and the process is operated at steam supply pressures of 3 to 4 bar. Exposure time ranges from 15 to 60 minutes (Mattila, 2011). For heat-based systems, sterilisation above 121°C for 30minutes is an accepted practice (Tremblay et al, 2010).

4. In case of heat treatment**, a cooling step** is required**:** after the sterilization phase is completed, the tank is cooled down and depressurized by jacket cooling or cooling coils.

5. **Holding and Release step**: After decontamination is completed (chemical treatment) or the effluent is cooled down at the desired temperature it may require sampling and analysis prior to release in the sewage system. As the analysis can take 3 to 5 days, the batch system may need additional tank volume for batch holding while the results are confirmed free of viable organisms allowing effluent to be released (Mattila, 2011). Validation of the decontamination/sterilization process can be achieved using biological indicators within the effluent waste (chemical treatment) or in a dry well for spore testing (thermal treatment) (Tremblay et al, 2010, Daugelat et al, 2008). Alternatively, a biological agent representative of the biological load can be used instead (Gordon et al, 2009). When the tank is empty it will automatically shut down and go in standby mode until the other processing vessels of the system have completed a cycle. At this point the valves will open again and the tank will proceed to the receiving mode and start a new cycle.

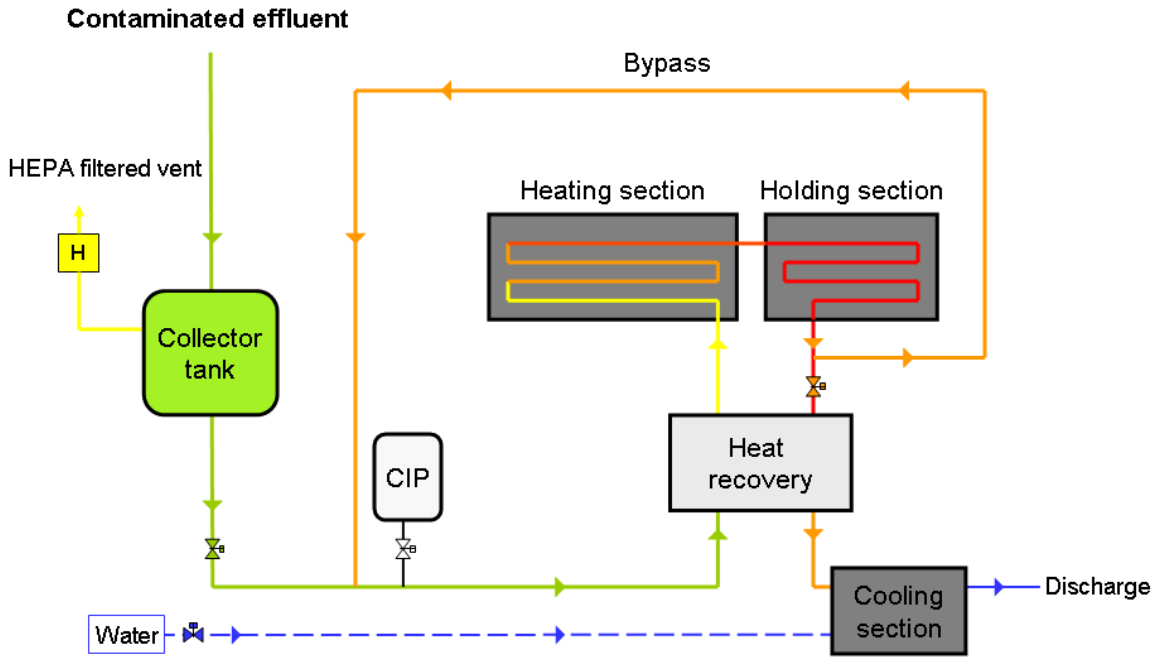

Figure 2: Schematic of a heat-based continuous effluent decontamination system

Continuous decontamination system

In a heat-based continuous decontamination system (see fig. 2), the effluent waste is also collected and stored in a collecting tank similar to a batch operated system. The tank can be automatically steam sterilized in place if needed and is equipped with a filtered vent. The collecting tank is also monitored with a level sensor. If present, solid particles can be removed from the effluent stream, for example by means of a separator vessel with strainer which is sterilized by heat injection. After sterilization and cooling, the solids are drained to a common drainage system (Steris Life Sciences).
The decontamination starts and stops automatically, according to user-defined start and stop levels of the tank and measured by level transmitter or level switches. Effluent from the collecting tank is pressurized and pumped as a continuous flow through a dedicated pipe section after heat exchangers raising the temperature at the desired set point and exposure time. The exposure time is dependent on the unit size (constant liquid flow rate), exposure section length and diameter. The decontamination exposure section of the system is a dedicated length of pipe between two temperature probes. After the decontamination pipe section, the effluent is driven trough a cooling pipe section with cooling heat exchangers and cooled down (generally to 60°C) prior to discharge.

Before the actual effluent processing starts, the system is tuned up by means of heating and cooling in a closed loop. Once the heating up period is achieved, the process will proceed to continuous processing mode, taking effluent from the collecting vessel for heating and cooling in a one way process until the stop level of the collecting vessel is reached. Temperature sensors at the beginning and at the end of the pipe section control exposure temperature and time. Typical temperatures vary between 130°C and 165°C depending on the required inactivation. Due to high temperature continuous systems require higher steam pressure than batch operated systems (5 to 7 bar) but much shorter exposure times (between 3 and 10 seconds to maximum a few minutes). Indeed, for

sterilization in general, increasing temperature decreases exposure time in an exponential way[4]. For instance 1.16 seconds at 150°C equals 15 minutes at 121.1°C) (Mattila, 2011).
Decontamination process and system status are continuously monitored and recorded by the self diagnostic automatic control system.
Unit capacities range from 2-300L/hour (small facility) to over 10.000L/hour (large scale facility) (Mattila, 2011, Daugelat et al, 2008). For the treatment of large effluent volumes, continuous systems are a better option than a batch operated system, since capacity can be expanded simply by increasing heat exchange surfaces, flow rates and instrumentation sizes.
As the system is based on a continuous flow, validation can only be performed by spiking the system and taking samples from the discharge. Spore trips cannot be introduced in the system (Tremblay et al, 2010).

Safety issues

The effluent decontamination process equipment is of primary importance for maintaining environmental safety but it is also an occupational safety device with respect to maintenance staff. Therefore system designers and equipment builders must anticipate problems in case of failure of the system. Conventional engineering and plumbing principles are not appropriate for this highly specialized matter. The system may need to be constructed to withstand corrosive disinfectants or heat that may shorten the life of the stainless steel vessel and create potentially dangerous situations (Stahl, 2008). Hence protective clothing should always be worn for servicing.

Potential risks associated with EDS might be:

- Overflow
- Leaks of pipes, of fittings, of sensors,…
- Back-flow into the laboratory due to failed valves or undersized systems
- Bypassing the heat treatment system leading to exposure to the environment
- Insufficient decontamination (problems inherent on chemical/heat treatment, see below)
- Areas in a heat treatment system that are not exposed to heat, such as a dead leg on a drain outlet before a drain valve could impair adequate sterilisation of the effluent
- Aerosolisation due to the use of pneumatic pressure for transfer of effluent from collection tank to treatment tank
- Poor maintenance resulting in exposure of maintenance staff (faulty pumps, valves, fittings, filters, corroded tank or pipes,…)
- Severe burns from accidental release during a heating cycle
- Exposure to chemicals used within the EDS system

(Tremblay et al, 2010, Stahl, 2008)

As already mentioned above, chemical treatment systems can be seriously impaired by insufficient mixing, or by the presence of contaminating soils (either organic as inorganic) since they can react with the disinfectant and neutralize its biocidal activity. Potential toxic or corrosive by-products generated during the process may cause a concern for the environment as well as for workers, should they be accidentally released in the working area. Heat-based processes too can be compromised by

[4] The relation between exposure temperature and treatment time is defined in the following formula :
Fo = t x 10exp (T-121,1/10) where F0 = number of equivalent minutes of steam sterilization at temperature of 121,1°C at minimum, to lethality required , t = treatment time in minutes, T= treatment temperature in °C. Based on waterlike solutions and reference organism *Geobacillus stearothermophilus.*

inadequate heat distribution within the load due to insufficient agitation or insufficient heat transfer from heat transfer surfaces.
For this reason, solid particles in the waste effluent (i.e. animal cage residues, faeces, hair, …) should ideally be removed from the effluent stream and sterilized separately, even particles smaller than 10mm (Mattila, 2011). Direct steam injection in a relatively small volume can break down the solid mass and penetrates through the material, resulting in a reliable sterilization. Also chemical pre-treatment of solids by dissolution of proteins with alkaline is effective to prevent fouling, for example in the case of egg proteins used in vaccine production (Mattila, 2011).

Risk management - Safety measures

EDS have to be validated, controlled and monitored to ensure their effectiveness and safety.
With respect to these aspects, a number of important design and engineering considerations have been highlighted in guidelines, norms and by experts in the field, as described below.

In its guidance on the use of infectious waste treatment and radiation facilities outside registered space, the CDC (CDC - Select Agent Registry[5]) recommends the EDS of entities working with select agents[6] should comply with the following:

- *The pipe leading from the containment area to the EDS is sealed and checked at least annually for leaks. A double walled pipe is preferred[7].*
- *There are procedures and structures in place to contain more liquid than the EDS system would process should there be a spill of the liquid from the EDS.*
- *Procedures are in place for the cleanup of a spill from the EDS.*
- *There is documentation that the decontamination cycle is adequate for the material to be processed.*
- *There are records of routine maintenance of the EDS.*
- *In cases where there are high levels of contamination or high matrix content such as in infected animal holding areas there should be procedures for waste treatment prior to leaving containment.*
- *There are records that EDS operation staff have been trained on how to respond to leaks and spills.*

EDS used in low level containment facilities of Biosafety level 1 or 2 (for ex pharmaceutical plants) are generally located in utility areas, providing disinfectant is available in case of a leak situation. On the contrary, EDS for high containment facilities might require a proper biocontainment room meeting the demand of high level containment. Given this technical area is contained it is recommended that in case of emergency situations all required safety measures are carried out outside before entering the equipment room. Remote control and monitoring should be in place to allow the user to evaluate the equipment status (Mattila, 2011)

[5] http://www.selectagents.gov/Guidance_on_the_Use_of_Infectious_Waste_Treatment.html

[6] Biological agents and toxins that could cause a severe threat to public health , animals and plants and are subject to regulation for possession, use and transfer in the frame of bioterrorism preparedness and protection and require registration. The current list is available at the Centers for Disease Control (CDC) and (Animal and Plant Health Inspection Service (APHIS) websites.

[7] Note that a double wall piping – although recommended if situated above public areas – is difficult to inspect, decontaminate and repair and should have a leak detection system, preferentially a pressurized system where the annulus between the carrier pipe and exterior pipe is pressurized and monitored (Tremblay et al, 2010).

EDS systems are preferentially located directly below the containment facility. This allows for a gravity flow into the system, avoiding additional intermediate equipment such as pumps. If possible, effluent load should be reduced as much as possible by avoiding unnecessary sources of liquid effluent or solids, reducing room space, equipment and volume that could leak. A level indicator on the collection vessel will prevent overflow and the system should be equipped with vent filters (HEPA) for treating off-gas. Dead legs on a drain outlet (see above) can be wrapped with a small steam heater or flush mounted valves can be installed (Tremblay et al, 2010).

With respect to construction, containers for liquid waste should be constructed in such a way that they are: 1) impermeable to prevent leakage, 2) of sufficient rigidity and strength to contain the intended load and 3) made of materials which are compatible with the intended treatment method (EN 12740:1999). Performance classes are established for kill tanks on the basis of leaktightness (classes LI-A, LI-B, LI-C), cleanability (CI-A, CI-B, CI-C) and sterilisability (SI-A, SI-B, SI-C) (EN 13311-1:2001). The appropriate class of kill tank is to be chosen on the basis of a risk analysis. When risk group 3 or 4 micro-organisms need to be inactivated in the load, a type II kill tank is recommended. A type II kill tank including auxiliary equipment (e.g. piping and valves) should meet well defined requirements for the above mentioned criteria: with respect to leaktightness escape of target micro-organism should be controlled and quantified according to defined conditions and should be below detection limit (LI-C), cleanability should be tested and quantified or calculated in function of specified criteria (CI-B), and they should be sterilizable (SI-C) (EN 13311-5:2001).

EDS: State of the Art

In a review article presenting design considerations of both batch and continuous thermal decontamination systems, J. Mattila recommends the following (Mattila, 2011):
Stainless steel high grade 316/316/L is an accepted standard as it prevents fouling of process surfaces and corrosion and polytetrafluoroethylene (PTFE) based gasket material should be chosen for its chemical and heat resistance.
Also, the number of connections within the systems should be minimized as they might be subject to leakage, and if technically possible orbital welding should be preferred. Temperature sensors subject to frequent calibration should be in welded pockets that are not in contact with the effluent.
For easy servicing, a reduced number of components and moving or rotating parts are preferable. For instance, agitation of effluent can be done by means of magnetic coupling instead of agitators. However, for safety reasons redundancy is needed for critical process components (dual process pumps, temperature sensors, barrier valves (one-way valves), vent filters and electrical heating for vent filters). Also this can keep the EDS operational in case of failure or maintenance.
Beware: in case of heat recovery systems direct heat transfer between hot decontaminated and cold contaminated effluent can cause cross-contamination. Therefore, heat transfer need to be organised in such a way that effluent streams are separated and pressure controlled. This is achieved by ensuring a higher pressure on the decontaminated effluent side.
The system should be continuously controlled via automated monitoring of different parameters (self-diagnostics) and equipped with alarm systems. A strict control system should be in place, with password protection.
In case of an emergency situation in a high containment area, room decontamination from outside the space using a gas (such as vaporized hydrogen peroxide) fed through specific ports should be made possible. Also liquid disinfectant should be available in case of leaks or spills.

Qualification

Equipment and process qualification methods and procedures should be put in place to ensure the reliability and safety of the EDS, and are mandatory for effluent processing systems within biosafety level 3 and 4 facilities.
For that purpose, four types of tests need to be carried out: a Factory Acceptance Test (FAT), a Site Acceptance Test (SAT) or start-up test, a commissioning test under normal and abnormal (failure) operation scenario's and finally biological validation tests (Tremblay et al, 2010):
The Factory Acceptance Test is conducted to ensure all specified components and sequences of operation are covered and is run in normal as well as in simulated (human or electronic) failure situations. The operational test at the factory can be run "dry", that means without liquid or heat, simply by testing the required operational steps (e.g. opening and closing of valves as specified). The supplier should deliver the documentation proving the tests were successful.
The Site Acceptance Test is conducted to validate start-up capability and performance under the exact conditions of use of the system (no simulation). Again, the supplier should deliver the documentation proving the tests were successful.
Commissioning requires a quality assurance process achieving, verifying and documenting the performance of the system to ensure that it does comply with the operation needs within the capabilities of the documented design and the capacities of the equipment. This will include component verification (to meet design intent, technical requirements and installation criteria), tests to ensure functionality under all operational parameters and tests to ensure functionality of interdependent systems.

In addition to the tests mentioned above, the decontamination process must be controlled to be sure all biologically contaminated effluents are decontaminated and are safe for disposal. Process and equipment are qualified by sampling and laboratory analysis of the decontaminated batch prior to discharge to the sewage system and specialized sample ports are required allowing for safe and simple collection of treatment effluent. Additionally, validation is performed by biologically challenge testing of the entire process. Validation must be carried out onsite under strict biological protocols and under the exact conditions the system will be used. This validation is not usually done by the vendor or installation contractor. It should be done by a safety professional with experience in validating such systems. Unfortunately, there are only a few publications within the scientific literature on procedures for validation of the inactivation efficacy of effluent decontamination systems by means of biological challenge testing.
The sampling procedures (including sampling devices) and the test methods used for the detection of viable organisms should be performed in accordance with the European standards. They may be carried out continuously, at regular or irregular intervals, for instance at random control checks. In case of suspected malfunction or operating at or very near to capacity testing should be done more frequently (EN 12740:1999).
Validation is carried out using a model test organism that matches best with its anticipated every day load, or an organism that is more resistant. In case of a facility working exclusively with the same type of organism (e.g. vegetative bacteria or viruses), it may be possible to validate the system with the organism of concern (Tremblay et al, 2010). For facilities with many different micro-organisms in use validating with the most resistant organism, such as Bacillus spores will be required. In cases where the biological indicator is less resistant than the most resistant organism used in the facility, the last one should be used as test model (EN 12740:1999).
As different Bacillus species are available with different resistant properties to treatment methods, it is best to choose a Bacillus that is killed at the used treatment method. In other words, it's not a good idea to use *Geobacillus stearothermophilus* spores in a heat-based decontamination system with

temperatures reaching only 93°C, as at least 121°C is required to kill the spores. In this case *B. atrophaeus* would be a better choice (Tremblay et al, 2010). *Geobacillus stearothermophilus* spores are resistant to wet heat or chemical disinfection (e.g. peracetic acid). As it is generally accepted that the sterility assurance level (SAL) is less than 1 in 10^6 of the starting population, validation will be focused on the reduction of 1x 10^6 spores, as done in three consecutive test runs.
Two types of validation are possible: indirect or direct validation. With indirect validation, self-contained vials containing 1x 10^6 spores are suspended in the tank or placed in a sample port (dry well). At the end of the decontamination process, the vials are collected, incubated and checked for colour change as specified by the manufacturer. This is a simple and quick method. With direct validation, spores at a specific concentration are added to the tank and decontamination cycle performed. Samples in a volume equivalent to 1x 10^6 spores are collected in sterile containers from the sample port at defined time intervals and concentrated samples are plated on agar plates, incubated and checked for growth (before taking a sample, sample port should be purged to be sure samples are taken directly from the tank, Gordon et al, 2009). A direct method is more labour intensive but is considered the best practice. Eventually labour can be reduced by first verifying test procedures and results on a small scale before testing the system itself (Tremblay et al, 2010). The Centers for Disease Control and Prevention (CDC) recommends the biological validation to be carried out annually (CDC, 2009).

In case of batch operated systems, qualification seems simple and straightforward. Samples are taken from the decontamination vessel before discharge of the effluent. However in case of large volumes, defining a valid and representative number of samples and a consistent qualification method for non-homogenous batches (including solids) is quite a challenge. For chemical-based batch systems the system can only be validated by spiking effluents with the test organism (spores) and taking samples from the discharge fluid, as spores need to come in contact with the chemical disinfectant to determine efficacy (Tremblay G. et al, 2010). This is not the case for heat-based batch systems, which can be validated by means of the indirect method mentioned above whereby self-contained vials with spores are put in a dry well sample port, not in contact with the effluent. Coagulation of particles within the vessel would generate uneven chemical or temperature distribution during decontamination that is difficult to detect by sensor monitoring. But since warmer water rises, temperature should be monitored at the bottom of the tank. Nevertheless, in heating tanks without agitation differences in temperature between top and bottom have been found to be less than 9°C (Whittmeier, 2001).
Also challenge biological testing by means of direct inoculation into the collecting vessel requires growth of the test spores to the high concentrations needed for efficacy testing (10^6 spores/ml of effluent). To overcome this problem *Bacillus thuringiensis* spores could be used: they can be obtained as a dry biological pesticide and exhibit similar heat resistance as *Bacillus anthracis* spores, being the organism the most resistant to decontamination of the type of pathogenic organisms generally manipulated in high containment facilities (Gordon et al, 2009).

In case of continuous decontamination systems, sampling is performed directly on the outflow of the effluent. As the load is *continuously* discharged in this system, a properly validated inactivation is of primary importance. Because of the continuous flow, the system can only be validated by spiking the system with the test organism (spores) and taking samples from the discharge. Introducing spore strips into the system is impossible. For extra safety, a buffer discharge tank can eventually be installed.
However, these systems offer significant advantages over batch operated systems: momentary processing volumes are minimal (a very small volume is sterilized at any onetime), homogenous and well controlled in terms of temperature and heat transfer thanks to creation of a turbulent flow. Biological challenge testing conducted by S. Daugelat on a continuous effluent decontamination system at a range of 4 set point temperatures (120, 130, 140, 150°C) showed log reductions that

exceeded the expected log reduction of the initial spore population in the used liquid spore suspension (Daugelat et al, 2008). Considering the process is continuously monitored, effluent release could be possible on constant parametric[8] monitoring alone, provided the daily process parameters match those during qualification of the process This constitutes an advantage over batch systems as these may require sampling of each batch before discharge (Mattila 2011, Daugelat et al, 2008).

Conclusion

Choosing the most appropriate effluent decontamination system adapted to type of effluent and micro-organism is not an easy task.
Apart from complex design and technical requirements to be adapted on the situation on site, qualification of the equipment and process on a regular basis is a key aspect, since inactivation of effluent needs to be guaranteed at all times. Comparing two currently used heat-based methods, it appears that a continuous decontamination system has several advantages over a batch operated system in terms of uniform heat distribution and accurate monitoring suggesting it may be a more reliable and safer system and as such preferred for high containment facilities. Whatever system is used, careful attention should be paid to process qualification by means of biological indicators. However, there are only a few publications available in the scientific literature on validation of the inactivation efficacy of effluent decontamination. Considering all these aspects, specific guidelines on process design and equipment as well as on process qualification would be most welcome.

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[8] A parametric release is based on compliance with the defined critical parameters without having to perform the requirements under sterility tests. In other words, this could eventually replace sample testing or biological challenge tests during routine testing

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