COMMISSIONING AND VALIDATION OF A UNIQUE THERMO-CHEMICAL EFFLUENT DECONTAMINATION SYSTEM

Rebecca Langer[1], Lana Hudgins[1] and Gilles Tremblay[2]
Baylor College of Medicine[1], Houston TX and Merrick & Company[2], Atlanta GA

BCM
Baylor College of Medicine

Abstract

An effluent decontamination system (EDS) is one of many enhancements that might be required in a high-containment laboratory. However, an EDS is perhaps the only agricultural enhancement that can present great variability in process design and functional operation, in part due to limited industry standards. Pathogen treatment options for an EDS range from heat or chemical only, to a combination of heat/chemical and/or pressure. Non-pressurized thermo-chemical EDS's are being considered by many facilities as an economical way to meet stricter regulations with respect to waste treatment. The caveat is that the facility is responsible for determining the correct temperature and chemical combination for their needs. This is because the heat/chemical combination that kills one agent may not necessarily kill the agent in an adjacent laboratory within the facility. Furthermore, there are few publications available within the scientific literature describing the procedure for validating the inactivation efficacy of an EDS against select organisms. We will present a comprehensive report of the commissioning (verification of heat, pH, valve function, etc) and validation (spore challenge) process for one of the first known thermo-chemical units. The EDS used in our application is a non-pressurized thermo-chemical unit designed to treat laboratory waste water for 1 hour at 200◦F under extreme alkaline conditions (pH 11). Detailed discussion of commissioning process will describe the problems and eventual resolutions of the system's physical and operational parameters (i.e. potential leaks and "cold" spots within the unit). Biological validation results will demonstrate that the choice of model non-pathogenic organisms along with the correct combination of heat/chemical is critical for producing reliable and reproducible inactivation kinetics. In conclusion, unique systems can be applied to high containment laboratory designs as long as the facility personnel develop and implement the appropriate system validation procedures.

Purpose

- Evaluate the installation and operations of all system components associated with the Bio-Response Solutions Thermo-Chemical Effluent Decontamination System (EDS – **Picture 1**) against design specifications.

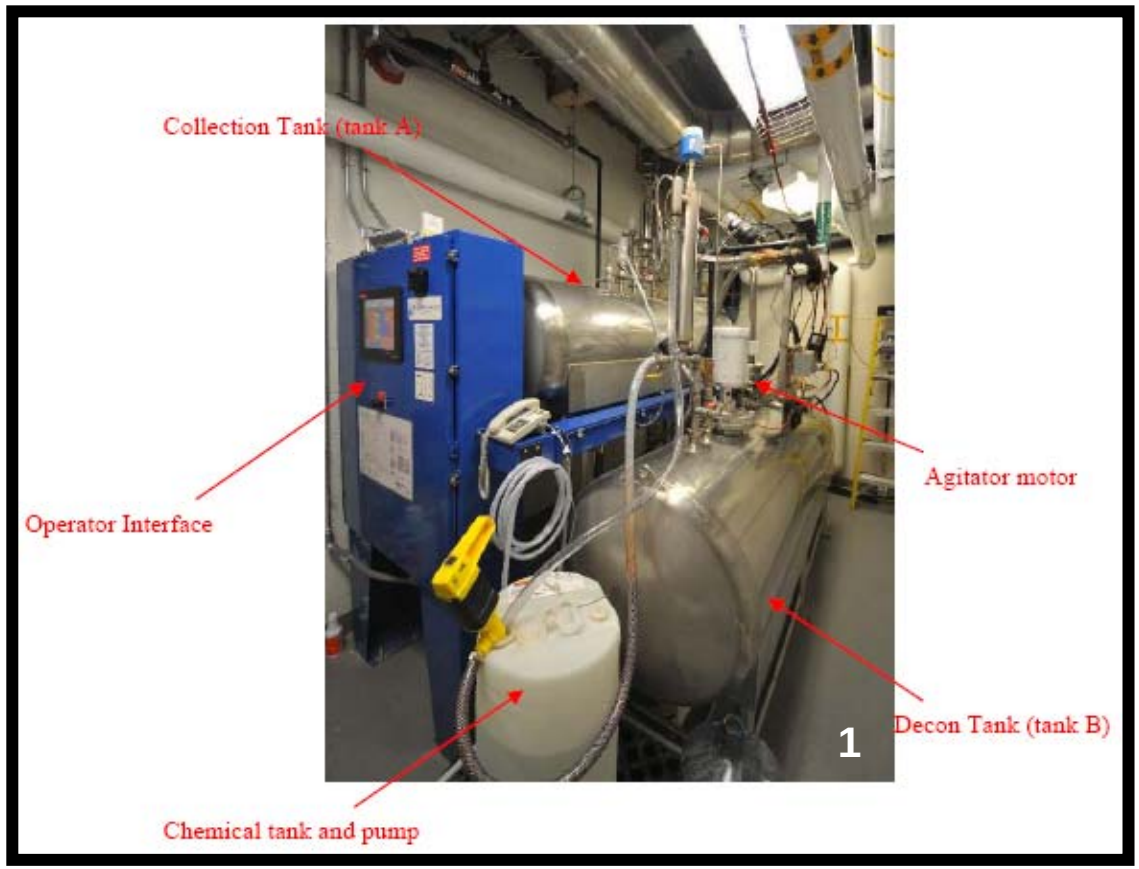
    - Provide measured values of parameters that would allow for adjustments to system components and/or control logic that would meet design intent and owner requirements.

- Assure that biological waste leaving the laboratories is sterilized and safe for disposal, and to further test for potential or abnormal conditions to assure they fail to a known condition or result.

    - Demonstrate that 1 X 10^6 spores/ml (conventional application) is not necessary when working with large systems where this concentration of spores would be cost prohibitive.

Bio-Response Solutions Thermo-Chemical Effluent Decontamination System

Indirect Method



Direct Method

Biological Validation

Spores

- *Bacillus subtilis* 5230 - for monitoring low temperature (115° C/239° F) steam sterilization (SGM Biotech, Inc)
- *Geobacillus stearothermophilus* – for monitoring high temperature (121 C/ 250 F) steam sterilization (SGM Biotech Inc)
- *Bacillus atrophaeus* – for monitoring low temperature steam sterilization (SGM Biotech, Ind)

Indirect Method:

- Spores (1×10^6) were placed collected.
- Spore suspension was plated on Tryptic Soy in small vials (glass capsules, glass vials or plastic boil proof tubes)
- Vials contained media or water, that was supplemented with chemical if applicable
- Vials suspended in mesh tea balls attached to a wire that was secured in place by hooking to the heating coil (**Picture 2 & 3**)
- EDS ran according to protocol (1 hr at 200° F), at completion of run vials were Agar plates, incubated for 7 days at the appropriate temperature and observed for growth (**Picture 4**)

Direct Method:

- Spores (5.2×10^9 = *B. atrophaeus*; 1.25×10^9 = *B. subtilis*) were inoculated into the holding tank just prior to the water level reaching the 150 gallon liquiphant, which automatically triggers a treatment cycle. (**Picture 5**)
- EDS ran according to protocol (1 hr at 200° F with desired chemical) and 500 ml samples collected from sample port. (**Picture 6**)
- Samples were collected immediately after the decon tank filled, when the tank reached 100° F, 150° F, 200° F and every 15 minutes thereafter until the cycle was complete.
- Samples (118 ml = 1×10^6 *B. atrophaeus* spores or 454 ml = *B. subtilis* spores) were concentrated using Nalgene 145 analytical test filter funnels (**Picture 7**). Filters were placed directly onto Tryptic Soy Agar plates, incubated for 7 days at the appropriate temperature and observed for growth (**Picture 8**).

Biological Validation Results

***B. atrophaeus* spores are inactivated by low temperature (200° F) only, while *B. subtilis* spores are not.**

The addition of KOH to a low temperature treatment cycle provides a protective effect for *B. atrophaeus* spores, resulting in no inactivation.

Heat Only Treatment (200° F)

Spores	Cook Time	10^6 reduction
Bacillus atrophaeus	1 hr	Yes
Bacillus subtilis	1 hr	No
Bacillus subtilis	10 hr	No
Geobacillus stearothermophilus	1 hr	No

Thermo-chemical Treatment of *Bacillus atrophaeus* (Direct Method)

Conditions:	**Results:**
Heat • 200° F for 1 hour Chemical • KOH to pH 11	(+) = growth • failure to achieve 10^6 reduction (-) = no growth • successful 10^6 reduction

Samples	*B. atrophaeus*: Heat Only	*B. atrophaeus*: Heat + KOH
Plate only	-	-
Plate and Filter Only	-	-
H_2O - holding tank; no spores	-	-
Spores Only	+	+
Holding Tank inoculated with 5.2 X 10^9 spores		
H_2O - holding tank	+	+
Chemical injected into Decon tank; Holding tank discharges to the Decon tank		
H_2O – decon tank at 100° F	+	+
H_2O – decon tank at 150° F	+	+
H_2O – decon tank at 200° F	-	+
H_2O – decon tank at 200° F for 15 min	-	+
H_2O – decon tank at 200° F for 30 min	-	+/-
H_2O – decon tank at 200° F for 45 min	-	+/-

B. atrophaeus spores are inactivated by low temperature in the presence of acidified bleach, H2O2 or iodine.

Test for Alternate Chemical (Indirect Method)

Conditions:	**Results:**
Heat • 200° F for 1 hour Spores • *Bacillus atrophaeus*	(+) = growth • failure to achieve 10^6 reduction (-) = no growth • successful 10^6 reduction

Pilot Test

Chemical	Growth
Acidified Bleach (1% v/v household bleach and vinegar)	-
Hydrogen peroxide (1.5% v/v)	-
Iodine (1% v/v)	-
Water Only (control)	+

H2O2 combined with low temperature (200° F) can inactivate even the hardiest _Bacillus_ spores when used at a concentration of 0.2% vol/vol.

H_2O_2 Titration (Indirect Method)

Conditions:	**Results:**
Heat • 200° F for 1 hour	(+) = growth • failure to achieve 10^6 reduction (-) = no growth • successful 10^6 reduction

Conditions	*G. stearothermophilus*	*B. subtilis*	*B. atrophaeus*
Water only	+	+	+
Water only + 200° F	+	+	-
H_2O_2 (1%) + 200° F	-	-	-
H_2O_2 (0.5%) + 200° F	-	-	-
H_2O_2 (0.375%) + 200° F	-	-	-
H_2O_2 (0.3%) + 200° F	-	-	-
H_2O_2 (0.25%) + 200° F	-	-	-
H_2O_2 (0.2%) + 200° F	-	-	-
H_2O_2 (0.15%) + 200° F	+	-	-
H_2O_2 (0.1%) + 200° F	+	+/-	-
H_2O_2 (0.05%) + 200° F	+	+/-	-

Component Verification

An eight point thermocouple string (**picture 9**) was installed for monitoring purposes with a data logger recording readings every 15 seconds. The thermocouples were installed at these points:

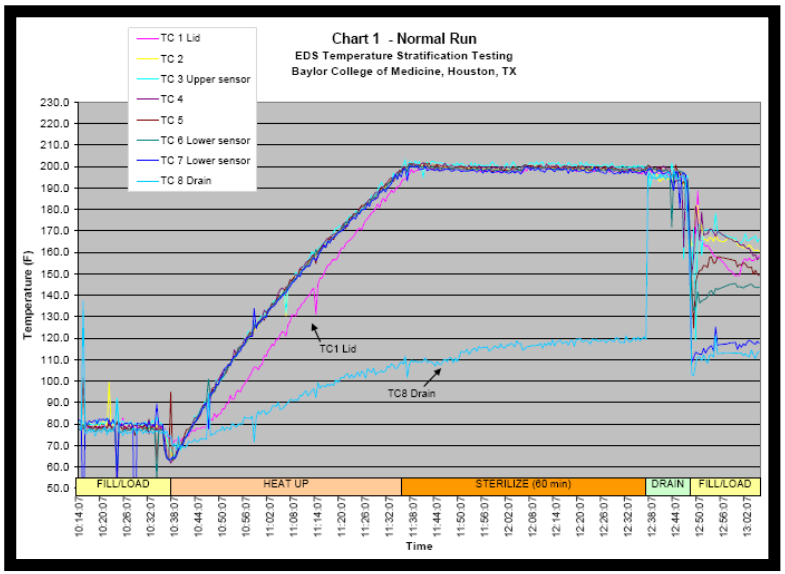
- below access hatch (air gap)
- above water surface - below access hatch
- liquid – attached to EDS upper sensor
- liquid – at 1' intervals between EDS upper and lower sensors
- liquid – attached to EDS lower sensor
- liquid – inserted 6" into drain pipe

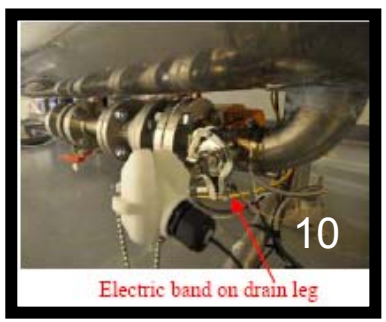
Four sinks in the BSL-3E space were used to fill the holding tank (200 gal), and operating parameters (200 F, pH 11) were tested. System was tested under normal operating conditions and failure conditions.

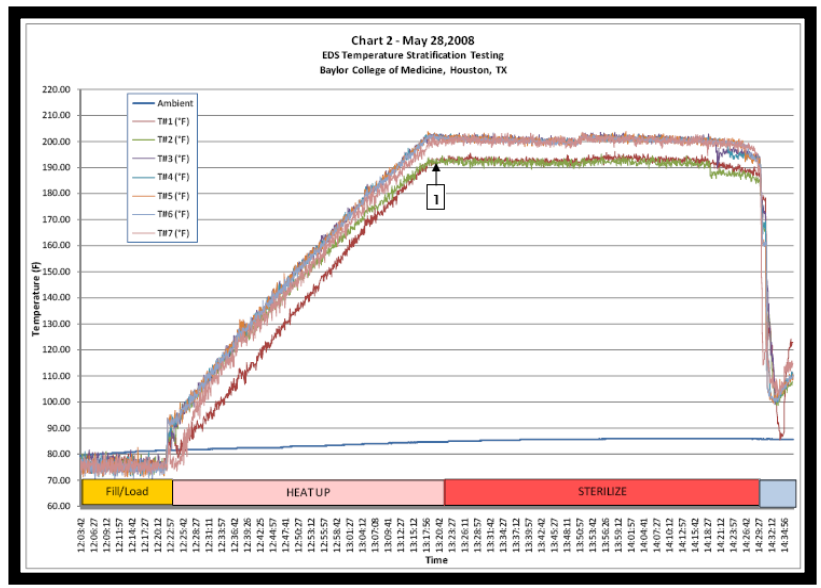
- Typical heat up time: 50-60 minutes
- Sterilize time: 60 minutes
- Typical drain time: 15 minutes

Component Verification Results

A “cold” spot identified in the drain pipe was corrected through the addition of an electric heater band (picture 10)

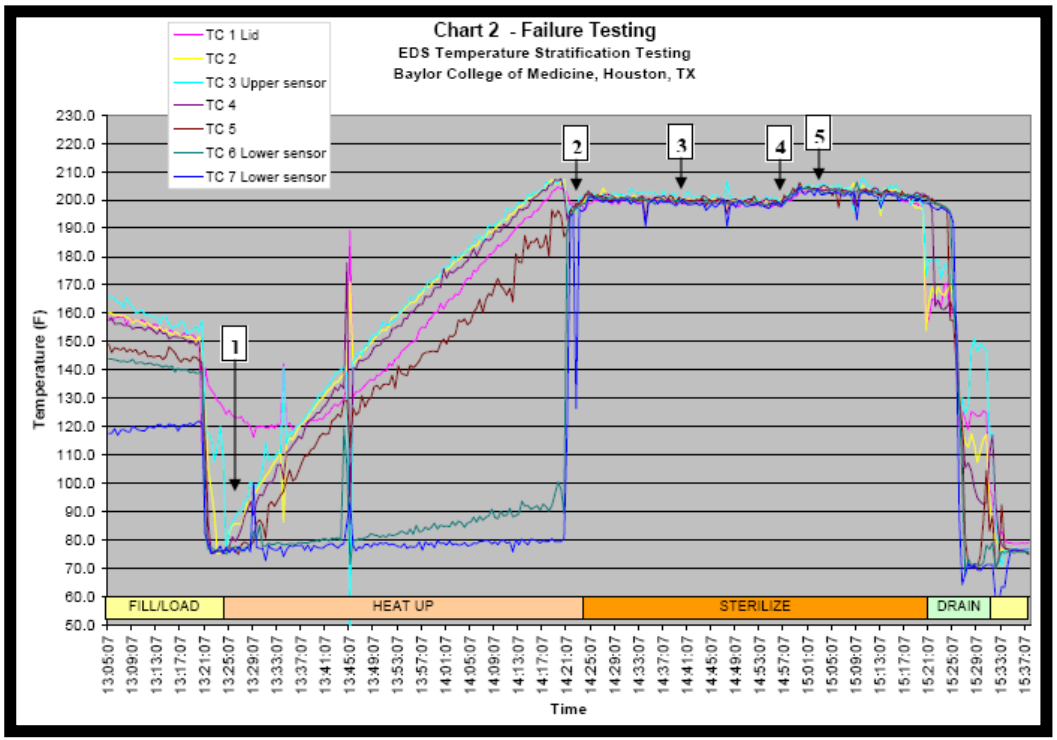






Temperature stratifications created by agitator motor failure, were quickly eliminated when the agitator motor was re-enabled.

Failed alarm notifications for various sensors were corrected by altering the computer program.



Temperature Stratification Testing		
ID	**Event**	**Observation**
1	Agitator Motor Failure	Temperature stratification occurs.
2	Agitator motor re-enabled	Temperature stratification eliminated.
3	Failed upper temperature sensor	No alarm generated. Steam valve remained off. Sterilizer counter continued to count down and did not reset on a temperature failure.
4	Failed lower temperature sensor	Thermocouple alarm generated. Sterilizer counter reset back to 60 min. Upper temperature levels climbed in excess of 207° F and would have exceeded boiling point if left on it's own.
5	Failed holding tank level sensors for full and overfill	No alarm generated for full level sensor failure. Alarm generated on an overfill level sensor.

Conclusions

Don't be deterred from developing your own validation system if conventionally accepted techniques do not meet the needs of your facility or equipment.

- Conventional application indicated that 1 X 10^6 spores/ml were needed, so that 1 ml could be tested directly. This would have been cost prohibitive as the total number of spores needed would have been 5.68 X 10^{11} for a 150 gallon tank.
- A method was devised to use fewer spores and still be able to assess for the reduction of 1 X 10^6 spores.

***B. atrophaeus* spores alone are not an appropriate control for the described EDS.**
KOH cannot be used in combination with low temperature to successfully inactivate *Bacillus* spores.

- Additional *Bacillus* species, not killed by heat only, were needed to confirm the desired killing through a mechanism of thermo-chemical treatment.
- Previous scientific publications [Setlow, J Appl Micro 101 (2006) 514-525] indicated the protective effect we observed with KOH was not unexpected.
    - Resistance to heat when suspended in an aqueous environment is a signature property of bacterial spores.
    - Mineralization of the spore core, with Ca, Mg, Mn, K, or Na is generally associated with higher spore wet heat resistance.
    - Strong alkali treatment thought to cause spore inactivation, may not sufficiently kill spores as they can be revived.

H_2O_2 at a concentration of at least 0.2% vol/vol is the appropriate chemical for the described EDS.

- Various chemicals had been anecdotally described to successfully kill anthrax spores through surface disinfection. However, we needed a chemical that would be compatible with stainless steel and would work successfully in combination with heat during a liquid decontamination.

- We chose to try acidified bleach, Iodine and H_2O_2 at concentrations greater than 1% vol/vol. All three chemicals worked and we narrowed out choice to H_2O_2, believing it would work best with our intended applications.

Problems identified during component verification were easily corrected by working directly with the manufacturer.

Unique systems can be applied to high containment laboratory designs as long as the facility personnel develop and implement validation procedures.

- Do not accept manufacturer's recommendations as the law, perform onsite validation to assure the equipment meets the needs of your facility.

- Have an active working relationship with the manufacturer to correct any identified problems.

Acknowledgements:

We would like to thank Bio-Response Solutions and TEQS for their continued support in optimizing the operation of this unit.