Ohio Administrative Code

Rule 3745-570-212 Alternative infectious waste treatment technology efficacy testing.

Effective: April 6, 2025

(A) An applicant for an alternative infectious waste treatment technology approval shall ensure that the efficacy testing protocol is designed to evaluate whether the alternative treatment technology used by the treatment unit is capable of achieving the performance standard for treatment set forth in rule 3745-570-210 of the Administrative Code, complies with the appendix to this rule, and includes the following:

[Comment: It is strongly recommended that the applicant submit the proposed efficacy testing protocol to Ohio EPA prior to testing. Upon request, Ohio EPA will review and provide comments on the protocol.]

(1) Using sound scientific microbial techniques that at a minimum include the following:

(a) Enumeration of all stock suspensions.

(b) Enumeration of a representative sampling of carriers.

(c) Placement of all samples and controls into buffered diluent.

(d) Performance of a minimum of three test runs with a minimum of three samples for each microorganism and control.

(e) Collection of all samples and controls immediately upon completion of the treatment cycle.

(f) Neutralization of the collected samples and applicable controls immediately upon collection from the treatment unit, if the treatment technology utilizes chemical treatment.

(g) Homogenization of each dilution immediately prior to withdrawing an aliquot for plating or continued dilution.

(h) Inoculation of the growth media immediately with the dilutions of processed waste samples and applicable controls, unless immediate inoculation is not possible.

(i) If immediate inoculation is not possible, place the samples in ice for a period of time not to exceed sixty minutes, unless an alternative timeframe for holding the samples has been approved by the director, and then immediately inoculate in accordance with paragraph (A)(1)(h) of this rule.

(j) Plating of dilutions in triplicate.

(k) Utilization of only microbial plates that contain between thirty and three-hundred colonies.

(l) Utilization of only those plate counts that demonstrate a margin of error no greater than five per cent difference between the replicate plates and no greater than a ten per cent difference in individual test runs.

(m) If the difference between the plate counts of the replicate plates in a single run has a quantitative difference of greater than five per cent between them, test another waste load for that particular bacterial spore. If all three of the plate counts have a quantitative difference of greater than five per cent between them, the test run is considered invalid.

(n) If any of the three test runs has a quantitative difference of greater than ten per cent between them, test another waste load for that series. If any one of the three test run plate dilution series has a quantitative difference of greater than ten per cent between them, the test run is considered invalid.

(2) Using a bacterial spore species selected from Table 1 of this rule that is the most resistant to the treatment technology as the challenge microorganism, subject to concurrence by Ohio EPA, using the "D" value as appropriate.

Geobacillus stearothermophilus.
Bacillus subtilis.
Bacillus atrophaeus.

Any other bacterial spore species that is determined to be resistant to all aspects of the treatment technology.

(3) Conducting a recovery test run prior to efficacy testing to determine the amount of bacterial spore loss as a result of the physical aspects of the treatment unit and the ability of the treatment unit to retrieve the bacterial spores from the waste or carrier. The applicant shall use a sufficient number of challenge microorganisms in the recovery test run such that the challenge microorganisms can be retrieved using one of the following methods to quantify the results for each test waste load:

[Comment: The percent number of recoverable microorganisms (%R) calculations are contained in the appendix to this rule.]

(a) Direct inoculation technique. Directly inoculate the infectious waste with enough liquid suspension containing the appropriate bacterial spores to give an adjusted theoretical challenge, calculated in accordance with the appendix to this rule, of at least 1.0×10^7 bacterial spores per gram of waste, or per milliliter of waste if the technology is designed to treat liquid infectious waste.

(b) Carrier system technique. Inoculate the infectious waste with enough recoverable carriers, such as bacterial spore strips, containing the appropriate bacterial spores to give an adjusted theoretical challenge, calculated in accordance with the appendix to this rule, of at least 1.0×10^7 bacterial spores per gram of waste.

(4) Testing only waste loads that satisfy the following:

(a) Are representative of the waste stream that the alternative treatment technology is designed to treat.

(b) Are of sufficient volume to simulate operation of the treatment unit at full capacity.

(c) Are composed such that the test waste load poses the greatest challenge to the alternative treatment technology.

(d) For those alternative treatment technologies that are designed to treat a specific category of infectious waste, are composed entirely of the specific infectious waste category that the alternative

treatment technology is designed to treat.

(e) For those alternative treatment technologies that are designed to treat every category of infectious waste but are sensitive to particular combinations or individual items contained in a waste stream, are composed entirely of the combination or individual item of that specific infectious waste category that poses the greatest challenge to that alternative treatment technology.

[Comment: An example of an alternative treatment technology that would use a test waste load as outlined in this paragraph would be a chemical treatment technology whose active ingredient is a chemical that is "bound" or "consumed" by large quantities of organics that may be present in a waste load. Therefore, the treatment technology would need to use test waste loads composed of one hundred per cent organics. This testing would challenge the treatment technology in a "worst-case" scenario.]

(f) Are comprised entirely of new or unused representative materials or of infectious waste sterilized by autoclaving and cooled to ambient temperature prior to inoculation.

(5) Inoculation procedures to ensure the applicant prepares each test waste load in accordance with paragraph (A)(4) of this rule and the following:

(a) Distributes the inoculum evenly throughout the test waste load such that the ratio of the volume of inoculum to the amount of waste is not less than one to twenty, or five per cent.

(b) Introduces the appropriate challenge microorganisms or inoculum as follows:

(i) Using a microbial suspension to seed the test waste load with the sufficient number of challenge microorganisms determined in accordance with paragraph (A)(3) of this rule.

(ii) Using a carrier system to introduce one carrier with the appropriate inoculum for each ten pounds of infectious waste in the test waste load with a minimum of three carriers in each test waste load, with all carriers distributed evenly throughout the waste load.

(6) Enumeration procedures to ensure the applicant enumerates either the initial inoculum in the

stock suspension or a representative sample of carriers as follows:

(a) For the stock suspension, the following:

(i) Enumerate all initial stock suspensions of bacterial spores and control suspensions immediately prior to introduction into the test waste load.

(ii) Inoculate the test waste load immediately prior to introduction into the treatment unit.

(iii) Use the stock suspension number obtained from enumerating the stock suspensions in accordance with paragraph (A)(3) of this rule to determine the theoretical challenge and the adjusted theoretical challenge for each test run in accordance with the appendix to this rule.

(b) For a carrier system, the following:

(i) Verify the inoculum contained on a representative sample of carriers using the manufacturer's lot enumeration.

(ii) Determine the theoretical challenge for each microorganism and the adjusted theoretical challenge for each test run in accordance with the appendix to this rule.

(7) Procedures to ensure the applicant performs treatment test runs in accordance with the appendix to this rule to evaluate the treatment unit using bacterial spore suspensions or spore carriers that do the following:

(a) Use full-scale production units for all testing.

(b) Use a sufficient number of representative samples in each treatment test run by evaluating the following factors:

(i) The total treatment capacity.

(ii) Whether the alternative treatment technology is a batch or continuous treatment process.

(iii) The physical state of the processed infectious waste, such as loose or conglomerated.

(iv) The results of the recovery test run performed in accordance with paragraph (A)(3) of this rule.

[Comment: More processed waste samples should be collected from larger test loads to ensure that samples are representative. As a general guideline, Ohio EPA would recommend that at least nine samples be collected. The nine collected samples may be used to make three composite samples.]

(c) Use a minimum of three treatment test runs.

(d) Meet the performance standard for treatment specified in rule 3745-570-210 of the Administrative Code at the completion of all three test runs.

(8) A permanent record of the following observations or recordings:

(a) The date and time that each test waste load is placed into the treatment unit.

(b) The date and time that each sample is retrieved from the treatment unit.

(c) The applicable observed or recorded operational parameters at which the treatment unit was operated.

(9) Procedures to ensure the applicant manages the samples collected from each test run upon exit of the treatment unit in accordance with the following:

(a) Neutralizes, if applicable, all controls and samples immediately upon exiting the treatment unit using a documented or previously tested neutralizer that will not affect the viable number of bacterial spores being tested.

(b) Cools all samples and controls to ambient temperature upon exiting the treatment unit and prior to preparation of the dilutions.

[Comment: The use of a buffered diluent will satisfy the requirement of cooling and preparation of the dilutions. This requirement need not be a two-step process.]

(c) Prepares dilutions from each collected sample.

(B) Efficacy testing report. The applicant shall ensure the test manager responsible for conducting the efficacy testing prepares an efficacy testing report that presents the raw data and results gathered in accordance with the protocol as specified in paragraph (A) of this rule and at a minimum contains the following:

(1) An introduction describing the intent of the efficacy testing including the name, address, and telephone number of the laboratory and the name of the test manager.

(2) Testing parameters and all results based upon a protocol that complies with paragraph (A) of this rule.

(3) Sufficient information so that the reported results and procedures can be reproduced by an independent laboratory.

(4) A description of all materials and methods used to perform the efficacy testing, subsequent dilution of samples, and incubation of samples.

(5) All raw data including all individual microbial counts.

(6) Log_{10} reduction levels achieved for the bacterial spores obtained from the efficacy testing of the three test waste loads that achieved the performance standard for treatment specified in rule 3745-570-210 of the Administrative Code.

(7) An example of each calculation used to determine the log_{10} reduction levels using the formulas found in the appendix to this rule.

(8) Documentation of the ability of the treatment technology to achieve the performance standard for treatment as specified in rule 3745-570-210 of the Administrative Code.

(9) A statement, signed and certified in accordance with rule 3745-500-50 of the Administrative Code, documenting that the efficacy testing was conducted in compliance with an efficacy testing protocol that complies with this rule.