

WP# 28 Partial Exposure Testing of Efficacy on BI's with Chlorine Dioxide Gas – Sept 1, 2022

Background

The level of chlorine dioxide exposure necessary to ensure an effective sterilization is measured in ppm-hours. A sterilization run requires 720 ppm-hours of exposure in order to ensure a complete log 6 reduction in microbe, however runs as low as 300 ppm hours can also perform this level of kill. Typically, this exposure is performed in one continuous sitting, which means the item in need of sterilization is exposed to the chlorine dioxide gas and the item is not aerated until the total dosage of 720 ppm-hours is met. It can be asked, then, if it is necessary to perform the exposure in one sitting, or if performing the exposure in several sittings has the same effect. In other words, are four exposures of 100 ppm-hours equally as effective at ensuring a successful sterilization as one exposure at 400 ppm-hours? It is understood that multiple partial cycles for some sterilants do not equal the efficacy of a “full” cycle.

This study was developed to determine if partial exposures to chlorine dioxide are as effective as continuous exposures at the same total ppm-hours. Effectiveness was determined by exposing biological indicators (BI's) to chlorine dioxide in 100 ppm hour intervals and comparing to continuous exposures up to 400 ppm-hours. If the partial exposures demonstrated comparable effectiveness, they would be determined to be equivalent to a continuous exposure.

STUDY MATERIALS EQUIPMENT

- ClorDiSys Solutions, Inc Minidox-M Chlorine Dioxide gas generator
- Test Chamber - 17 cu ft (0.48 cu m) Isolator
- EBM PAPST 4600Z compact axial fan, 105.9 CFM, fan diameter 4.68”
- Incubator set to 57°C
- Biological Indicators: Noxillizer *Geobacillus stearothermophilus* (GS), Lot# CNS2201060, Population 1.6×10^6 per strip, Expiration: January 2023
- Growth Media: True Indicating growth media for *Geobacillus stearothermophilus*

TEST METHOD

Partial Exposures

30 biological indicators were placed inside the test chamber before the first run. The Minidox M was connected to the chamber. Four cycles were run with the following parameters:

- RH Setpoint of 65% with a condition time of 30 minutes.
- CD gas concentration setpoint of 1 mg/L

- Maximum ppm-hours of 100

The chamber was fully aerated at the end of each cycle. After cycles one and two, the biological indicators were removed from the chamber. They were returned to the chamber for the subsequent cycle after a period of at least 24 hours. After the third cycle, the biological indicators were removed from the chamber and 15 of them were dropped in growth media and placed in the incubator. The remaining 15 were returned to the chamber for the last cycle after another 24 hour period. They were removed from the chamber, dropped in growth media, and incubated at the conclusion of the final cycle.

The ppm hours of each run were recorded along with the accumulated ppm hours of the previous runs. Several days after the biological indicators were dropped, the growth media was examined and the number of biological indicators which were successfully killed was recorded. After one round of partial exposure testing was completed, a second round was performed with a new set of 30 biological indicators.

Control Exposure

15 biological indicators were placed inside of the chamber. The Minidox-M was connected to the chamber. Four cycles were completed with the same parameters as above, with the exception that the maximum ppm-hours for each control run were set to 100, 250, 300, and 400 ppm-hours.

At the end of each run, the biological indicators were removed from the chamber and dropped in growth media. The biological indicators from the 100 ppm-hour run were dropped after letting them sit for a period of four days to show that they were not being affected by residual chlorine dioxide. A new set of biological indicators was placed in the chamber for each run. Total ppm-hours were recorded for each run. Several days after the biological indicators were dropped, the growth media was examined and the number of biological indicators which were successfully killed was recorded.

RESULTS

The table below documents the results of the biological indicators after incubation.

Date of Run	X No Growth	X With Growth	Run Concentration (mg/L)	Rh Setpoint (%Rh)	Condition (Min)	Final PPM Hours	Accumulated PPM Hours
First Round							
5/24/2022	No Drop	No Drop	1.0	65	30	104.2	104.2
5/25/2022	No Drop	No Drop	1.0	65	30	107.3	211.5
5/26/2022	15	0	1.0	65	30	111.2	322.7
5/27/2022	15	0	1.0	65	30	97.6	420.3
Second Round							
6/7/2022	No Drop	No Drop	1.0	65	30	99.2	99.2
6/8/2022	No Drop	No Drop	1.0	65	30	102.7	201.9
6/9/2022	14	1	1.0	65	30	96.8	298.9
6/10/2022	15	0	1.0	65	30	99.4	398.1
Control Runs							
7/14/2022 (Dropped 7/18/2022)	0	15	1.0	65	30	100.8	X
7/15/2022	3	12	1.0	65	30	251.0	X
7/18/2022	15	0	1.0	65	30	303.3	X
7/19/2022	15	0	1.0	65	30	405.9	X

Conclusions

Based on the results of the experiment performed, it was proven that several partial exposures to chlorine dioxide is very nearly equivalent to a singular continuous exposure with the same total amount of ppm-hours. The results seemed to show that total kill happens somewhere between the 250 to 300 ppm-hour mark with the experimental setup used. The one biological indicator which exhibited growth in the second round of partial exposure tests at the 298.9 ppm-hour dosage may indicate that the partial exposures are very slightly less effective, however the partial exposure quickly becomes just as effective shortly thereafter. A control biological indicator which had no exposure was also dropped and exhibited growth, indicating the biological indicators used were viable.

It is worth noting that the biological indicators were stored in Tyvek pouches, preventing the influence of outside contamination. In order for partial chlorine dioxide exposure to be utilized in

a real world application, similar precautions would need to be taken to prevent outside contamination between cycles.

Summary

Based on the results of the experiment performed, it was proven that several partial exposures to chlorine dioxide is very nearly equivalent to a singular continuous exposure with the same total amount of ppm-hours.