NOTEBOOK NO. 107
ISSUED TO Kerri Cooper
ON April 1st 20 08
DEPARTMENT
RETURNED 20

SCIENTIFIC NOTEBOOK COMPANY
2831 LAWRENCE AVENUE
STEVENSVILLE, MICHIGAN 49127
(800) 537-3028 - http://www.snco.com

Table of Contents	Page

Abbreviations

BA: Bacillus anthracis (sterne)
BC: Bacillus cereus
BT: Bacillus thuringiencis
P&ID: Process & Instrumentation Diagram

Read & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

NCFPD Overall Project Plan

Abstract/Executive Summary: The proposed continuation of this project aims to optimize the cleaning process by comparison of existing methods with different cleaning agents (product and process specific) in conjunction with high-power ultrasound, to further facilitate removal of food residues to improve sanitizer/sterilant efficiency. Further steps will be taken to scale-up the application of the vaporized/gaseous sterilants (vaporized hydrogen peroxide, ozone, chlorine dioxide and formaldehyde) to eliminate remaining spores in food residues attached to processing surfaces after cleaning treatments.

Optimization of this potential non-invasive cleaning-sanitizing strategy for removal and inactivation of *B. anthracis* (Sterne strain) could provide a less destructive alternative for decontamination of food processing equipment in the event of a biological threat in food processing facilities. This application could potentially save both time for remediation and offset the potential financial impact caused by the loss of critical capital items.

Primary Goal: To validate the effects of various cleaning and liquid/gaseous sanitizing protocols for decontamination of food processing equipment and facilities with spores of several potential surrogate *Bacillus* species including *B. anthracis* Sterne strains.

Objectives: To compare the effects of various vaporized and gaseous sterilants to sanitize cleaned surfaces in scaled-up, simulated but near-real world situations.

Introduction and Highlights: In a previous NCFPD-funded project, we investigated: (1) the formation of "biofilms" consisting of *Bacillus* spores (*B. cereus* ATCC 21281 and *B. thuringiensis* ATCC 33680) embedded in complex food matrices on different food contact surfaces and the ability of cleaning regimens to remove spores in food (Xie *et al.*, 2007); and (2) the effects of liquid and gaseous/vaporized sanitizers (or sterilants) to inactivate spores on clean and non-clean surfaces (Oh *et al.*, 2007).

Sanitizing technologies to be included in this phase of the study include: vaporized hydrogen peroxide (Heckert *et al.*, 1997; Rogers *et al.*, 2005), ozone (Kim *et al.*, 1999; Aydogan and Gurol, 2006), chlorine dioxide (Kreske *et al.*, 2006b; Ryu and Beuchat, 2005), and paraformaldehyde (Ackland *et al.*, 1980). Although ozone has been used as an anti-fungal fumigant applied to stored cereal crops (Allen *et al.*, 2003; Wu *et al.*, 2006), its use for facility fumigation has been rarely reported. Pan *et al.* (1992) reported that application of 600ppm ozone for 6 hours might be effective for routine sterilization of cages, bedding, clothing, and other materials in laboratory animal facilities. Khadre and Yousef (2001) compared the sporicidal actions of hydrogen peroxide and ozone, and found the former to be less effective against *Bacillus* spores even at 10,000x higher concentration.

Sanitizer challenge

All gaseous sterilants will be prepared and applied to simulated food contact surfaces according to manufacturer's instructions. Various concentrations and contact/application times will be assessed to determine appropriate end points and limits for detection (as described below). The following gaseous sterilants, or their equivalents, will be considered: Ozone (American Air Liquide - proprietary on-site generation)

References

Peter J. Slade, Ph.D. Year 4 Project Proposal for: Validation of Methods for Decontamination of Food Processing Equipment and Facilities Deliberately Contaminated with *Bacillus* Spores (2007).

Funding: National Center for Food Safety and Protection (NCFPD)

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kun C. Cao	Date 4/13/09
		Recorded by:	

Project Description

In 2001 a terrorist attack of distributing anthrax through the mail brought attention to the country that the use of spores as a weapon was feasible and the realization that steps have not been added in response to these possibilities. The food industry as a result began taking great strides in order to put methods in place that would allow for fast and easy response if any of these attacks were to ever affect their facilities. Anthrax which is the bacterial spore *Bacillus anthracis* can cause serious harm and even be deadly upon exposure. In case of any attacks within the food industry it is important to be prepared with steps such as a cleaning and sanitization process to quickly and efficiently eliminate the anthrax from any surfaces that it may come in contact with the spores. In order to simulate the contact surfaces, coupons made of stainless steel 316, glazed tile, Teflon, polypropylene, and rubber will be used as representatives of food contact surfaces. Due to *Bacillus anthracis'* extremely virulent nature, surrogate strains will be used in this study that may be similar to *Bacillus anthracis*. These strains are *Bacillus cereus* ATCC 21281, *Bacillus thuringiencis* ATCC 33680 , and *Bacillus anthracis* (Sterne strain). Food matrices that will be used are pancake mix, peanut butter, infant formula, vegetable oil, and a sucrose solution.

The use of ozone as a sanitizer for inactivation of *Bacillus* spores has been explored using different surfaces, but few studies have explored how it affects spores embedded in different food matrices. This is important because in most real world applications food matrices come in contact with many of the surfaces in the facilities and have the potential to become hardened and difficult to remove. Therefore a determination of the parameters, such as the contact time and ozone concentration required to inactivate the spores embedded in the food are very important in sanitizing. In order to determine this, an ozone delivery system must be designed to safely deliver and destroy the ozone.

Due to ozone's instability and potential deadly affect if inhaled at a high dosage, it is important to construct a process to safely deliver and destroy the ozone. A system must be built for controlled delivery and destruction of the ozone to the spores inoculated on the coupons.

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Ozone System Binder Table of Contents

The following information describes the information found in the Ozone Delivery System Binder. This binder contains all information concerning the design, equipment, safety procedu and validation of the system.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cook	Date
		Recorded by:	4/3/09

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

Ozone System Design

Page	Title	Date
19	Changes for Draft 5	5/9/08
20	Ozone Design System Draft 6	5/9/08
21	Personal Notes from Conference Call	5/9/08
22	Changes for Draft 6	5/9/08
23	Ozone Design System Draft 7	5/9/08
24	Ozone System Shutdown SOP - Pg 1	5/9/08
25	Ozone System Shutdown SOP - Pg 2	5/9/08
26	Ozone System Startup - Pg 1	5/9/08
27	Ozone System Startup - Pg. 2	5/9/08
28	Ozone System Startup - Pg. 3	5/9/08
29	Changes for Draft 7	5/14/08
30	Ozone Design System Draft 8	5/14/08
31	Conference Call w/ Air-Liquide Notes	5/14/08
32	Conference Call w/ Air-Liquide Notes	6/9/08
33	Meeting with Todd Diel	6/9/08
34	General Safety Procedures	6/9/08
35-40	Training w/ Air-Liquide Notes	6/9/08
41-43	HAZOP Training	6/9/08

To Page No. ____

Witnessed & Understood by me, _____ Date _____

Invented by: Kim C. Cooper

Recorded by: _____

Date 4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Ozone System Design

Page	Title	Date
~~44~~	Calculations	6/9/08
45	Corrections and Annotations - Draft 8	6/9/08
46	Changes from Draft 8	6/9/08
47	Ozone Design System - Draft 9, Version 1	6/12/08
48	Changes from Draft 9, Version 1	6/24/08
49	Ozone Generator P+ID	6/24/08
50	H1 Series Ozone Analyzer P+ID	6/24/08
51	Ozone Design System - Draft 9, Version 2	6/24/08
52	Changes from Draft 9, Version ~~2~~	7/28/08 KC
53	Ozone Design System - Draft 9, Version 3	7/28/08
54	Changes from Draft 9, Version 3	9/25/08
55	Ozone Delivery System - Draft 10, Version 1	9/25/08
56	Changes from Draft 10	12/2/08
57	Ozone Delivery System - Draft 11	12/2/08
58 - 65	Ozone System Startup + Shutdown SOP	4/3/09
~~66 - 69~~	~~Ozone~~ KC Disconnecting the O_2 + N_2 Cylinders KC	~~4/3/09~~
66 - 69	Emergency Shutdown Procedures	4/3/09
70 - 71	Disconnecting the Oxygen + Nitrogen Cylinders	4/3/09

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cha	Date 4/3/09
		Recorded by:	

Ozone System Design

Page	Title	Date
72-74	Ozone System General Safety Procedures	4/3/09
75-78	Setting Needle Valves for Ozone System	4/3/09
79-82	What is HAZOP	4/3/09
83	Ozone Delivery System HAZOP	4/3/09
84-102	NCFST Ozone Delivery System HAZOP	4/3/09

Equipment + Parts

Page	Title	Date
1	Equipment List	5/9/08
2	Equipment List	5/9/08
3	Equipment List	5/9/08
4	Equipment List	5/9/08
5	Equipment List	5/9/08
6	Equipment List Updated 5/14/08	5/14/08
7	Equipment List Updated 5/14/08	5/14/08
8	Equipment List Updated 5/14/08	5/14/08
9	Equipment List Updated 5/14/08	5/14/08
10	Equipment List Updated 5/14/08	5/14/08

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Validation

Page	Title	Date
1	Nitrogen Flush & Pressure Validations	10/14/08
2	Calculating O_2 amount present after O_3 production	10/14/08
3	Purging the Ozone Generator	10/17/08
4	Purging Ozone Generator Instructions	10/17/08
5	Starting Ozone Delivery System	11/2/08
6	Troubleshooting the Ozone Generator	11/2/08
7	Troubleshooting the Ozone Generator (II)	11/2/08
8	Submitted Email for Ozone Generator from Ozonia	11/5/08
9	Ozone Generator's Required Electrical Power	11/5/08
10	Ozone Generator Analysis	11/24/08
11-15	Introduction to Transformers	11/24/08
16	Second Attempt to Start Generator	12/3/08
17	Ozone Generator Parameters	12/15/08
18	Testing Voltage for 100% PSU on Generator	12/15/08
19	Determination of Parameters for Various O_3 Conc.	12/15/08
20	Verification of O_3 Concentration Parameters for Titration	1/20/09
21-27	Ozone Titration Method	1/22/09
28	Ozone Titration	1/22/09

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Validation

Page	Title	Date
29-33	Ozone Gas Titrations Raw Data Run 1-6	4/3/09
34-36	Ozone Gas Titrations Calculations Run 1-6	4/3/09
37-43	Ozone Design System Status Update	4/3/09
44	Ozone Gas Titrations Raw Data Run 7	4/3/09
45	Ozone Gas Titrations Raw Data Run8	4/3/09
46	Ozone Gas Titration Calculations Run78	4/3/09
47-52	Calculating Parameters for the Ozone Generator to Achieve Ozone Concentrations	4/3/09

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE Ozone System Startup + Shutdown Procedure

From Page No. ____

Throughout the experiment refer to this ~~procedure~~ procedure for operating the ozone system.

Ozone System Startup + Shutdown Procedure

1.0 Purpose and Scope

1.1 To safely startup and shutdown the ozone system for delivery and destruction to the anaerobic reaction tank

2.0 Responsibility

2.1 The scientific personnel that carries out this procedure

3.0 Hazards and Safety Considerations

3.1 Ozone is toxic to humans and should be contained and destroyed effectively

3.2 Oxygen can be combustible if in contact with organic materials, all parts are oxygen cleaned

3.3 Oxygen analyzer should be used to ensure less than 23% is being released into the atmosphere

4.0 Equipment and Supplies

- Oxygen tank
- Nitrogen tank
- Ozone Generator (Ozonia/CFS-1A)
- Buffer tank (10gal pressure vessel)
- Ozone Analyzer (IN USA/H1)
- Ambient Ozone Analyzer (IN USA/IN2000L2-LC)
- Anaerobic Reaction Tank (Schütt Labortechnik/stainless steel)
- MnO_2 Destruction tank
- Humidity tank, excess water tank, and destruction tank (Ace Glass/500ml Gas washing bottle)
- Stainless Steel piping (Valex 316L, Specification 301, ¼ in)
- Ball Valves (Swagelok/SS-42GS4-SC11)
- Needle Valves (Swagelok/SS-4MG-SL-SC11)
- Pressure Release Valve (Swagelok/SS-RL3S4/SC11)
- Pressure Gauge (Blue Ribbon Sales & Service Corp/BR4001-4LD, oxygen cleaned)
- PVDF Tubing (McMaster-Carr/5390K342, 3-A sanitary tubing)
- Butterfly Valve (McMaster-Carr/4682K74, ½ in connections)
- Check Valve (Swagelok/SS-4C-1-SC11)
- Flowmeter (Mcmaster-Carr/8051K17)
- Nylon Tubing (McMaster-Carr/5112K653, 3/8in OD)
- Oxygen Detector (McMaster-Carr/18995T14)
- Vaneometer

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

5.0 Operational Parameters

- Humidity tank volume: 250ml
- KI Destruction Tank volume: 300mL KI solution (40g/L)
  - 12g KI / 250ml H_2O

6.0 Operational Procedure

6.1 Turn on the following:

6.1.1 Fume Hood

a. Refer to vaneometer to ensure that air is flowing upward into the fume hood

6.1.2 Ambient ozone analyzer

6.1.3 In-line ozone analyzer

6.2 Ensure needle valves are set correctly according to SOP: MC-SA-SOP005-V01

6.3 Calculating the Volume of Gas in the O_2 and N_2 Cylinders

6.3.1 Determine pressure within the cylinder by referring to the gauge connected to the cylinder

6.3.2 Calculate the volume of the cylinder using this ratio by solving for x:

$$\frac{2600\,psi}{300\,ft^3} = \frac{\text{gauge pressure (psi)}}{\text{x ft}^3}$$

6.3.3 Record the gas cylinder volumes in the log book located in Lab 105

6.3.4 If volume is not sufficient for experimental run then refer to the disconnecting O_2 and N_2 cylinders SOP (MC-SA-105-SOP003-V01)

***This should be done before every experimental run**

6.4 Flushing the Anaerobic Reaction Tank

6.4.1 Ensure the following valves are closed

a. PR 1-2 and BV 1-11

6.4.2 Open BV6

6.4.3 Open BV5

a. Switch the orange lever to the open position

6.4.4 Set PR2 to 10 psi

a. Flow rate: 5 L/min

6.4.5 Allow nitrogen to flow through the Anaerobic Reaction Tank into the KI Destruction Tank

6.4.6 Verify flush time with ambient ozone analyzer by placing tubing perpendicular to the exit port of the KI Destruction Tank

a. Ozone concentration should be zero

6.4.7 Stop nitrogen flow by setting PR2 to zero

essed & understood by me,	Date	Invented by: Kerri C. Cooper	Date
		Recorded by:	4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

6.4.8 Close valve BV5
- a. Switch the orange lever to the shut position

6.4.9 Close valve BV6

6.5 Setting Parameters for the Ozone Generator

6.5.1 Turn water supply on
- a. Constraints:
  - Flow: 1.5 L/min
  - Pressure: not to exceed 50psi
  - Butterfly valve open to 40°

6.5.2 Switch on (MAIN-ON) ozone generator and wait until the screen turns green
- a. Screen should read: MAINS ON→ SYSTEM CHECK

6.5.3 Select desired language (English, German, Latin, French, Italian, Spanish)
- a. Continually press SET POINT until desired language is displayed

6.5.4 Wait 5 seconds to allow the ozone generator to set the language

6.5.5 Ensure SET POINT is set to LOCAL
- a. Screen should read 0% LOCAL, PSU OFF

6.5.6 Open oxygen cylinder

6.5.7 Set PR1 to 50psi by turning the regulator knob

6.5.8 Open valves (located under table)
- a. BV2
- b. BV1

6.5.9 Set pressure on the generator to 1.5bar using the PCV 201 dial
- a. Refer to PI 201 pressure gauge

6.5.10 Turn power supply on by switching PSU ON (located on ozone generator)
- a. Screen should read PSU ON

6.5.11 Set gas flow by adjusting the HCV 201 dial located on the front of the generator

6.5.12 Adjust pressure and gas flow to desired parameters

6.5.13 Increase %PSU by pressing the SET POINT button.

6.6 Checking MnO_2 Effectiveness

6.6.1 Ensure BV2 is open

6.6.2 Follow ozone generator startup procedure

6.6.3 Place tubing connected to the ambient ozone analyzer (Model IN2000L2-LC) perpendicular to flow to the exit port of the MnO_2 destruction column
- a. The analyzer should read 0.05ppm or less

6.6.4 Record concentration in the log book

6.6.5 Using temperature probe determine temperature of the gas at the exit port
- a. **Do not touch column – it may be hot**

6.6.6 Record information in the log book

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date
		Recorded by:	4/3/09

6.6.7 If concentration exceeds 0.05ppm immediately switch ozone generator MAINS OFF. If the concentration is below 0.05ppm continue with the following steps

a. DO NOT OPEN COLUMN → MnO_2 requires special handling

6.6.7..1 Notify Ed Steiner (NCFST Director of Facilities and Pilot Plant) and Vaushi Rasanayagam (Air-Liquide)

6.6.8 Open BV7 and allow ozone to flow into the Buffer Tank
6.6.9 Close BV2
6.6.10 Pressurize tank to 5 psi
6.6.11 Open BV2
6.6.12 Close BV7

6.7 Checking KI Destruct Tank Effectiveness

6.7.1 Open BV6
6.7.2 Open BV4
6.7.3 Open BV10
6.7.4 Open BV8
6.7.5 Allow ozone to flow through the KI Destruction Tank
6.7.6 Place tubing connected to the ambient ozone analyzer (Model IN2000L2-LC) at the exit port of the KI destruction tank

a. The analyzer should read 0.05ppm

6.7.7 Close BV8
6.7.8 Close BV10
6.7.9 Close BV4
6.7.10 Close BV6
6.7.11 Record effluent ozone concentration in log book
6.7.12 If ozone limits exceed 0.05ppm follow the next steps otherwise skip to step 6.7.18
6.7.13 Remove KI Destruction Tank from the system
6.7.14 Dispose of the KI according to safety procedures
6.7.15 Refill with new KI solution
6.7.16 Reattach KI Destruction Tank to system
6.7.17 Follow the Flushing the Anaerobic Reaction Tank procedure

6.8 Achieving Desired Concentration

6.8.1 Open BV3
6.8.2 Open BV11
6.8.3 Open BV8
6.8.4 Open BV7
6.8.5 Close BV2

nessed & Understood by me,	Date	Invented by: Yuri C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

6.8.6 Allow ozone to flow through the H1 analyzer at 0.5L/min until desired concentration is achieved
- a. Adjust %PSU and flow rates as needed
- b. Monitor the flow rate on the analyzer to ensure it is exactly 0.5L/min. You may have to adjust NV2 and BV3
- c. Monitor G1 on the buffer tank to make sure it remains zero

6.8.7 Close valve BV8
6.8.8 Close valve BV11
6.8.9 Close valve BV3
6.8.10 Pressurize buffer tank to 5psi
- a. Refer to G1

6.8.11 Open valve BV2
6.8.12 Close valve BV7

6.9 Stopping Gas Flow

6.9.1 Reduce power supply to 0% LOCAL by pressing the down button for SET POINT
6.9.2 Turn off ozone generator power supply by switching to PSU OFF
- a. Screen should indicate: PSU OFF and PURGING time
- b. The screen background should be orange

6.9.3 Allow for the equipment to purge the system with the feedgas and remove the residual ozone in the generator
- a. This process runs for 90s

6.9.4 Once this is completed the screen will turn green and read: PSU OFF and POWER 00000kw
6.9.5 Set ozone generator pressure to zero
- a. Adjust by turning PCV 201 dial

6.9.6 Stop oxygen flow into the ozone generator
- a. Set PR1 to zero
- b. Close valve BV1

6.9.7 Close valve BV2

6.10 Experimental Run

6.10.1 Once desired concentration is achieved open valve BV6
6.10.2 Open valve BV4
6.10.3 Open valve BV10
6.10.4 Open valve BV8
6.10.5 Allow ozone to flow through the anaerobic tank
6.10.6 Close valve BV6
6.10.7 Pressurize anaerobic tank to 1.4psi (0.1bar)
- a. Refer to G2

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kami C. Cooper	Date
		Recorded by:	4/3/09

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

6.10.8 Close valve BV8
6.10.9 Close valve BV10
6.10.10 Close valve BV4
6.10.11 Perform experimental run
6.10.12 Refer to Step 6.4 for flushing the Anaerobic Reaction Tank
6.10.13 Remove samples replace lid securely

6.11 Flushing the System

6.11.1 Open valves
- a. BV3
- b. BV8

6.11.2 Depressurize buffer tank by allowing the ozone to flow out of the tank
- a. G1 should read 0psi before continuing

6.11.3 Set PR2 to 25psi
6.11.4 Open valves
- a. BV11
- b.BV9

6.11.5 Allow nitrogen to flush through the system
6.11.6 Monitor the H1 in-line analyzer until the %wt reads 0%
6.11.7 Close valves
- a. BV8
- b. BV11
- c. BV3

6.11.8 Pressurize buffer tank to 10psi
6.11.9 Close BV9
6.11.10 Open valves (in the following order)
- a. BV6
- b. BV4
- c.BV10
- d.BV8

6.11.11 Allow nitrogen to flush through system
- a. Monitor ozone concentration released from the KI column using the ambient ozone analyzer ensuring that it does not exceed 0.05ppm
- b. If the concentration does not exceeds 0.05ppm continue with Step 6.11.17

6.11.12 If concentration exceeds 0.05ppm immediately close the following valves:
- a. BV8
- b. BV10
- c.BV4
- d.BV6

6.11.13 Remove both KI destruct columns from the system
6.11.14 Transfer the KI waste into the designated waste container

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kevin C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

6.11.15 Fill the bottles with new KI solution and then continue with Step 6.11.18
6.11.16 Open BV3
6.11.17 Close valves (in the following order)
- a.BV10
- b.BV4

6.11.18 Allow remainder of nitrogen to flow out of tank
- a. Make sure G1 reads 0psi

6.11.19 Close
- a. BV8
- b. BV3

6.11.20 Set PR2 to 10psi
- a. Flow rate: 5 L/min

6.11.21 Open valve BV5 to remove humidity in the anaerobic reaction tank
- a. Switch orange lever to open

6.11.22 Allow nitrogen to flush through the tank for at least 1.5min
6.11.23 Set PR2 to zero
6.11.24 Close valve BV5
- a. Switch orange lever to shut position

6.11.25 Allow Anaerobic Reaction Tank pressure to reach 0psi
- a. Refer to G2

6.11.26 Close valve BV6

6.12 If performing another experimental run return to Step 6.5.5. If the experimentation is done for the day, continue with step 6.13

6.13 <u>Shut Down Procedure (once all experimental runs are completed)</u>

6.13.1 Switch MAINS OFF
6.13.2 Turn off water supply
- a. Close butterfly valve

6.13.3 Close gas cylinders by turning the valve
- a. Nitrogen
- b. Oxygen

6.13.4 Turn off analyzers
- a. H1 (in-line)
- a. Ambient analyzer

6.13.5 Turn off fume hood

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cook	Date
		Recorded by:	4/3/09

Project No. _____

Book No.

MOFFETT CENTER	Page 1 of 3
Title: **Determining the effect of Nitrogen Gas on spores dried on various food contact surfaces**	Author: Kerri C. Cooper
Document No.: MC-QA-105-SOP-012-V01	Effective Date: March 5, 2009

1.0 Purpose:
To determine the effect of nitrogen gas, if any, on the inactivation of dried *Bacillus* spores on various food contact surfaces.

2.0 Responsibility
The scientific personnel that carries out the experiment.

3.0 Hazards and Safety Considerations
- Operator must follow all safety instructions for the Ozone Delivery System
- *Spores of Bacillus anthracis (sterne) s*train will be used

4.0 Equipment and Supplies
- Sterile Conical tubes containing 10 glass beads (diameter 2mm)
- Sterile forceps
- Sterile de-ionized water (dH_2O)
- Tryptic soy agar (TSA)
- Buffered Peptone Water (BPW) (9mL/tube and 10mL/tube)
- Shaking water bath (Thermo Haake/SWB25)
- Incubator (Imperial III)
- *Bacillus anthracis* (sterne strain) suspension (solved in 50% ethanol)
- 10 mm coupons (stainless steel 316, Teflon, glazed tile)
- 1 N NaOH Solution
- Nitrogen Gas (99.9%)
- Bio-safety cabinet (BSC)
- Ozone Delivery System

5.0 Operational Procedures

5.1 Preparation of coupons:
- 5.1.1 Place 4 coupons of each type in a sterile petri dish
- 5.1.2 Load the coupons with 0.1mL of spores
- 5.1.3 Place the coupons in the refrigerator to dry for 4hrs

5.2 Analysis of Control Coupons (6 stainless steel coupons)
- 5.2.1 Place each coupon in a conical tube filled with 10 sterilized glass beads and 10mL of BPW
- 5.2.2 Vortex the sample for 1min
- 5.2.3 Heat shock the conical tubes in a water bath at 80°C for 20min
- 5.2.4 Remove conical tubes from water bath and place them in ice H_2O for 2min
- 5.2.5 Conduct microbiology analysis and plate on TSA

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kerri C Cooper	Date 4/3/09
		Recorded by:	

5.2.6 Incubate for 24hrs at 32°C

MOFFETT CENTER	Page 2 of 3
Title: **Nitrogen Gas Effects on *Bacillus anthracis* (sterne) strains inoculated on various coupon surfaces**	Author: Kerri C. Cooper
Document No.:	Effective Date: March 5, 2009

5.3 Analysis for Nitrogen Exposed Coupons

- 5.3.1 Place the coupons in the Anaerobic Reaction Tank (2 coupons for each coupon type)
- 5.3.1 Pressurize the tank with nitrogen to 0.15bar
- 5.3.1 Allow the coupons to be exposed to the nitrogen for 1hr
- 5.3.2 Remove coupons from the Anaerobic Reaction Tank
- 5.3.3 Place each coupon in 10mL of BPW with 10 sterilized glass beads
- 5.3.4 Vortex for 1min
- 5.3.5 Heat shock samples in water bath at 80°C for 20min
- 5.3.6 Remove from water bath and place in ice H_2O for 2min
- 5.3.7 Conduct microbiological analysis and plate on TSA
- 5.3.8 Incubate plates for 18-24hrs at 32°C

5.4 Day 2

- 5.4.1 Remove plates from incubator
- 5.4.2 Count colonies
- 5.4.3 Record data in lab notebook

6.0 References and Supporting Documents

1. Cooper, Kerri. Ozone Delivery System P&ID (Draft 11). December 2008.

2. Nitrogen Material Safety and Data Sheet (MSDS). < http://awisco.net/MSDS/Nitrogen.pdf>.

3. Oxygen Material Safety Data Sheet (MSDS). < http://www.rsbs.anu.edu.au/O2/pdf/Air%20Liquide%20MSDS%20O2.pdf>.

4. Ozone Material Safety Data Sheet (MSDS). <http://www.ozoneapplications.com/info/ozone_msds.htm>.

5. Ozone Safe Working Practices. Work SafeBC. <http://www.worksafebc.com/publications/health_and_safety/by_topic/assets/pdf/ozone_bk47.pdf>.

Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 4/3/09
		Recorded by:	

MOFFETT CENTER	Page 3 of 3
Title: **Nitrogen Gas Effects on *Bacillus anthracis* (stern) strains inoculated on various coupon surfaces**	Author: Kerri C. Cooper
Document No.:	Effective Date: March 5, 2009

6. Xie, P. 2007. The Effectiveness of Cleaning Procedures to Remove Food Matrices Containing Bacillus Spores on Food Contact Surfaces Before Sanitizer Application. *M.S thesis, IIT, Chicago.*

Media Recipes & SOP's
MC-QA-314-RCP08-V01
MC-QA-314-RCP012-V01
MC-SA-105-SOP001-V01
MC-SA-105-SOP002-V01

Witnessed & Understood by me,	Date	Invented by: Kerri C Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Refer to Pg. 17 for procedure → Raw Data Run1

Determination of Nitrogen Effect on Bacillus Spores
Inoculation Parameters: Bacillus anthracis (sterne) strain
Coupons: stainless steel 316, Teflon, Glazed tile Date: 3/9/09
Food matrix: sucrose Contact Time: 30min

Samples	3	4	5	6	7	8	9
C-GT-A					72	15	2
					62	10	3
C-GT-B					55	13	4
					69	11	4
C-TF-A					52	12	1
					55	11	2
C-TF-B					71	17	3
					71	12	1
C-SS-A					71	19	2
					65	21	1
C-SS-B					62	14	1
					49	12	1
GT-A	T	T	T	223	67	22	2
	T	T	T	251	72	10	1
GT-B	T	T	T	305	162	8	10
	T	T	T	282	115	29	1
TF-A	T	T	T	152	62	7	2
	T	T	T	142	50	11	1
TF-B	T	T	T	134	72	9	1
	T	T	T	121	69	10	1
SS-A	T	T	T	280	110	11	1
	T	T	T	285	82	11	0
SS-B	T	T	T	123	71	9	1
	T	T	T	151	62	8	1

To Page No. _

Witnessed & Understood by me,	Date	Invented by: Kimi C Cooper	Date 4/3/09
		Recorded by:	

Run 1 Calculations

Determination of Nitrogen Effect on Bacillus Spores
Inoculation Parameters: Bacillus anthracis (sterne) strain
Coupons: stainless steel 316, Teflon, Glazed tile
Food matrix: sucrose Contact Time: 30min

Samples	3	4	5	6	7	8	9	avg count
C-GT-A					72	15	2	4.42E+09
					62	10	3	
C-GT-B					55	13	4	5.82E+09
					69	11	4	
C-TF-A					52	12	1	3.19E+09
					55	11	2	
C-TF-B					71	17	3	4.16E+09
					71	12	1	
C-SS-A					71	19	2	4.18E+09
					65	21	1	
C-SS-B					62	14	1	2.86E+09
					49	12	1	
GT-A	T	T	T	223	67	22	2	4.03E+09
	T	T	T	251	72	10	1	
GT-B	T	T	T	305	162	8	0	4.03E+09
	T	T	T	282	115	29	1	
TF-A	T	T	T	152	62	7	2	3.11E+09
	T	T	T	142	50	11	1	
TF-B	T	T	T	134	72	9	1	2.78E+09
	T	T	T	121	69	10	1	
SS-A	T	T	T	280	110	11	1	2.35E+09
	T	T	T	285	82	11	0	
SS-B	T	T	T	123	71	9	1	2.65E+09
	T	T	T	151	62	8	1	

Samples	log (control)	log(samples)	log reduction
GT-A	9.65	9.61	0.04
GT-B	9.76	9.61	0.16
TF-A	9.50	9.49	0.01
TF-B	9.62	9.44	0.17
SS-A	9.62	9.37	0.25
SS-B	9.46	9.42	0.03

Summary

From the above data it can be concluded there is no apparent effect of Nitrogen on the spores. This experiment will be repeated for accuracy.

Witnessed & Understood by me,	Date	Invented by: Kui C Coope	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Refer to Page 17 for Procedure → Run 2 Raw Data

Determination of Nitrogen Effect on Bacillus Spores
Inoculation Parameters: Bacillus anthracis (sterne) strain
Coupons: stainless steel 316, Teflon, Glazed tile Date: 3/10/09
Food matrix: sucrose Contact Time: 30min

Samples	3	4	5	6	7	8	9
C-GT-A					60	11	2
					62	8	5
C-GT-B					57	12	3
					63	18	4
C-TF-A					54	9	0
					67	11	2
C-TF-B					64	10	1
					71	8	1
C-SS-A					75	24	0
					69	18	1
C-SS-B					77	12	1
					59	21	0
GT-A	T	T	T	315	62	18	2
	T	T	T	335	71	15	1
GT-B	T	T	T	252	138	6	0
	T	T	T	271	106	21	2
TF-A	T	T	T	134	57	6	1
	T	T	T	142	47	10	1
TF-B	T	T	T	115	63	12	1
	T	T	T	102	60	12	0
SS-A	T	T	T	265	105	7	0
	T	T	T	294	50	14	1
SS-B	T	T	T	115	69	9	0
	T	T	T	142	63	15	1

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Run 2 Calculations

Determination of Nitrogen Effect on Bacillus Spores
Inoculation Parameters: Bacillus anthracis (sterne) strain
Coupons: stainless steel 316, Teflon, Glazed tile
Food matrix: sucrose Contact Time: 30min

Samples	3	4	5	6	7	8	9	avg count
C-GT-A					60	11	2	5.06E+09
					62	8	5	
C-GT-B					57	12	3	5.60E+09
					63	18	4	
C-TF-A					54	9	0	2.61E+09
					67	11	2	
C-TF-B					64	10	1	2.58E+09
					71	8	1	
C-SS-A					75	24	0	2.86E+09
					69	18	1	
C-SS-B					77	12	1	2.83E+09
					59	21	0	
GT-A	T	T	T	315	62	18	2	4.14E+09
	T	T	T	335	71	15	1	
GT-B	T	T	T	252	138	6	0	3.83E+09
	T	T	T	271	106	21	2	
TF-A	T	T	T	134	57	6	1	2.46E+09
	T	T	T	142	47	10	1	
TF-B	T	T	T	115	63	12	1	2.42E+09
	T	T	T	102	60	12	0	
SS-A	T	T	T	265	105	7	0	2.60E+09
	T	T	T	294	50	14	1	
SS-B	T	T	T	115	64	9	0	2.36E+09
	T	T	T	142	43	15	1	

Samples	log (control)	log(samples)	log reduction
GT-A	9.70	9.62	0.09
GT-B	9.75	9.58	0.16
TF-A	9.42	9.39	0.03
TF-B	9.41	9.38	0.03
SS-A	9.46	9.42	0.04
SS-B	9.45	9.37	0.08

Summary

This data also shows no nitrogen effect on the spores. Therefore it is concluded that the nitrogen does not need to be taken into account in the following experiments.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	4/3/09

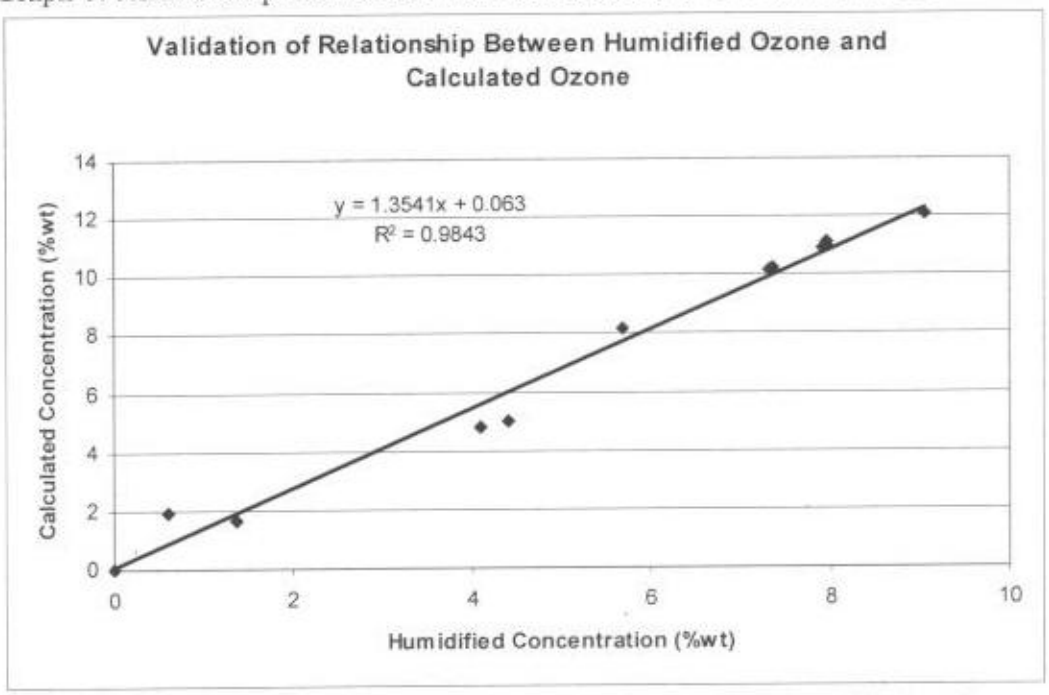
MOFFETT CENTER	Page 2 of 6
Title: **Calculating Parameters for the Ozone Generator to Achieve Ozone Concentrations**	Author: Kerri Cooper
Document No.:	Effective Date: March 2009

5.0 Operational Procedure

5.1 Determined the ozone concentration for the experimental run.

a. Knowing the concentration that should be achieved for the experimental run use the following graph to determine the humidified concentration;

Graph 1: Relationship Between Humidified Ozone and Calculated Ozone

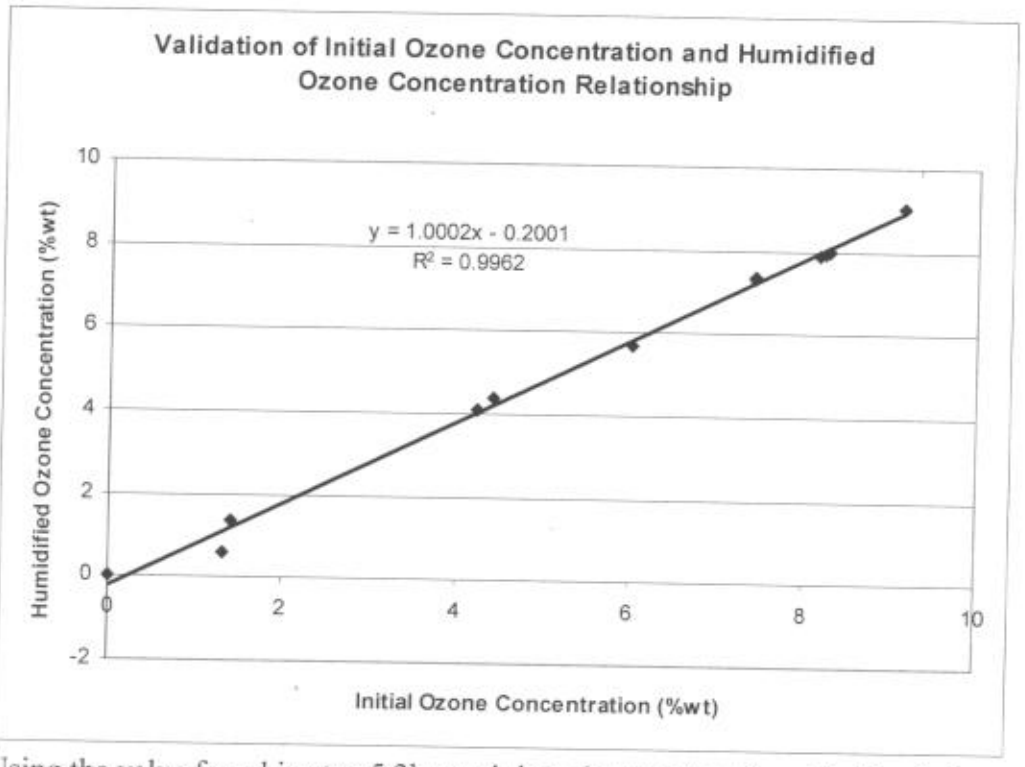


Using this the equation formulated from the graph: $y = 1.3541x + 0.063$, where x = humidified ozone concentration (%wt) and y = calculated concentration (%wt). The known concentration is the calculated concentration so put it in the equation and solve for the value of x.

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

b. Use the following graph to find the initial concentration of ozone.

Graph 2: Relationship Between the Initial Ozone Concentration and Humidified Ozone Concentration



Using the value found in step 5.3b, put it into the equation found in Graph 2: $y = 1.0002x - 0.2001$, where x = initial ozone concentration (%wt) and y = humidified ozone concentration (%wt). Solve for the value of x.

Note: This concentration is the concentration that will be used in the next steps and will be the value reading for the in-line ozone analyzer.

Witnessed & Understood by me,	Date	Invented by: Kristi C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

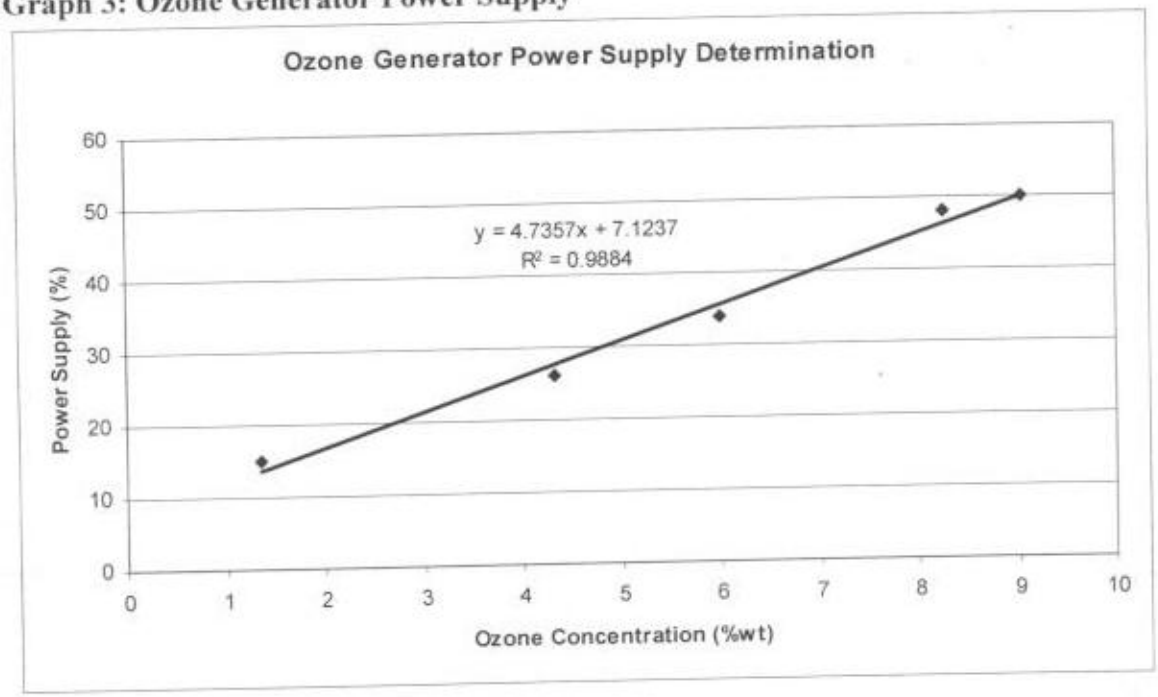
Book No. _____ TITLE _____

From Page No. ____

5.2 Determining the Electrical Power

5.2.1 Using **Graph 3** below, determine the electrical power setting (kW) on the ozone generator based upon the desired concentration

Graph 3: Ozone Generator Power Supply



Using the equation: $y = 4.7357x + 7.1237$, where x = ozone concentration (%wt) and y = power supply (%), solve for the value of y.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

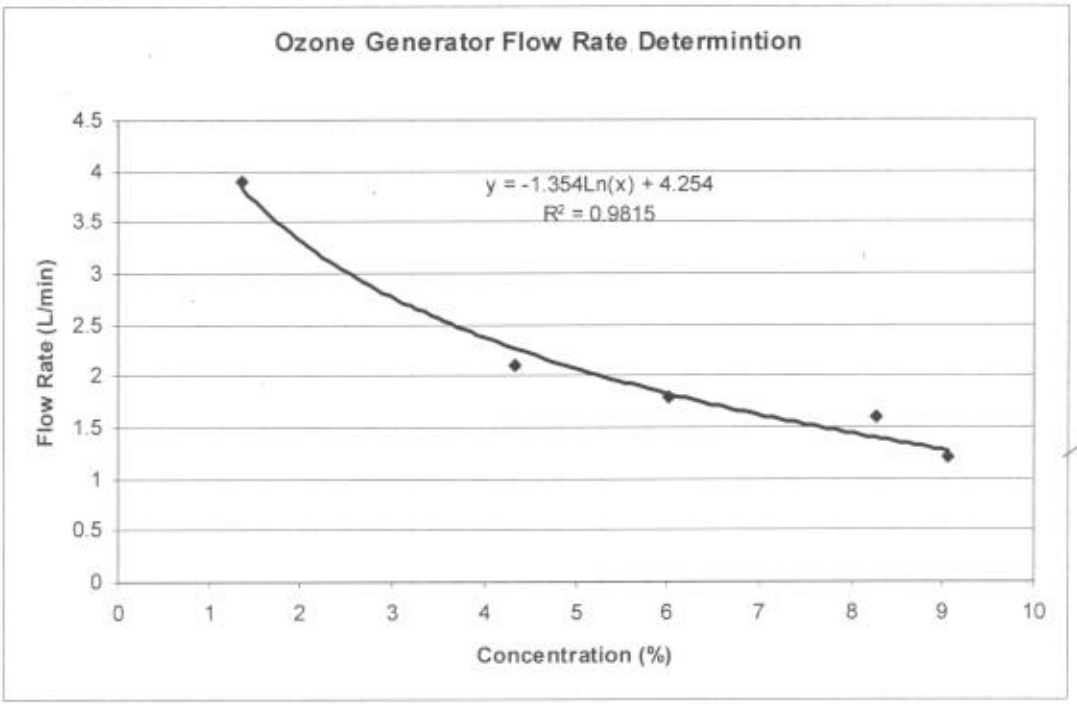
TITLE _____ Book No. _____

From Page No. ____

5.3 Determining the Ozone Generator's Flow Meter Scale

5.3.3 Using the already determined ozone concentration find the gas flow rate from the generator

Graph 4: Gas Flow Rate Determination



Using the equation from the graph above: $y = -1.354\ln(x) + 4.254$, where x = ozone concentration (%wt) and y = flow rate (L/min), solve for the value you y.

5.5 Record all parameters in the Ozone Delivery System Startup Notebook

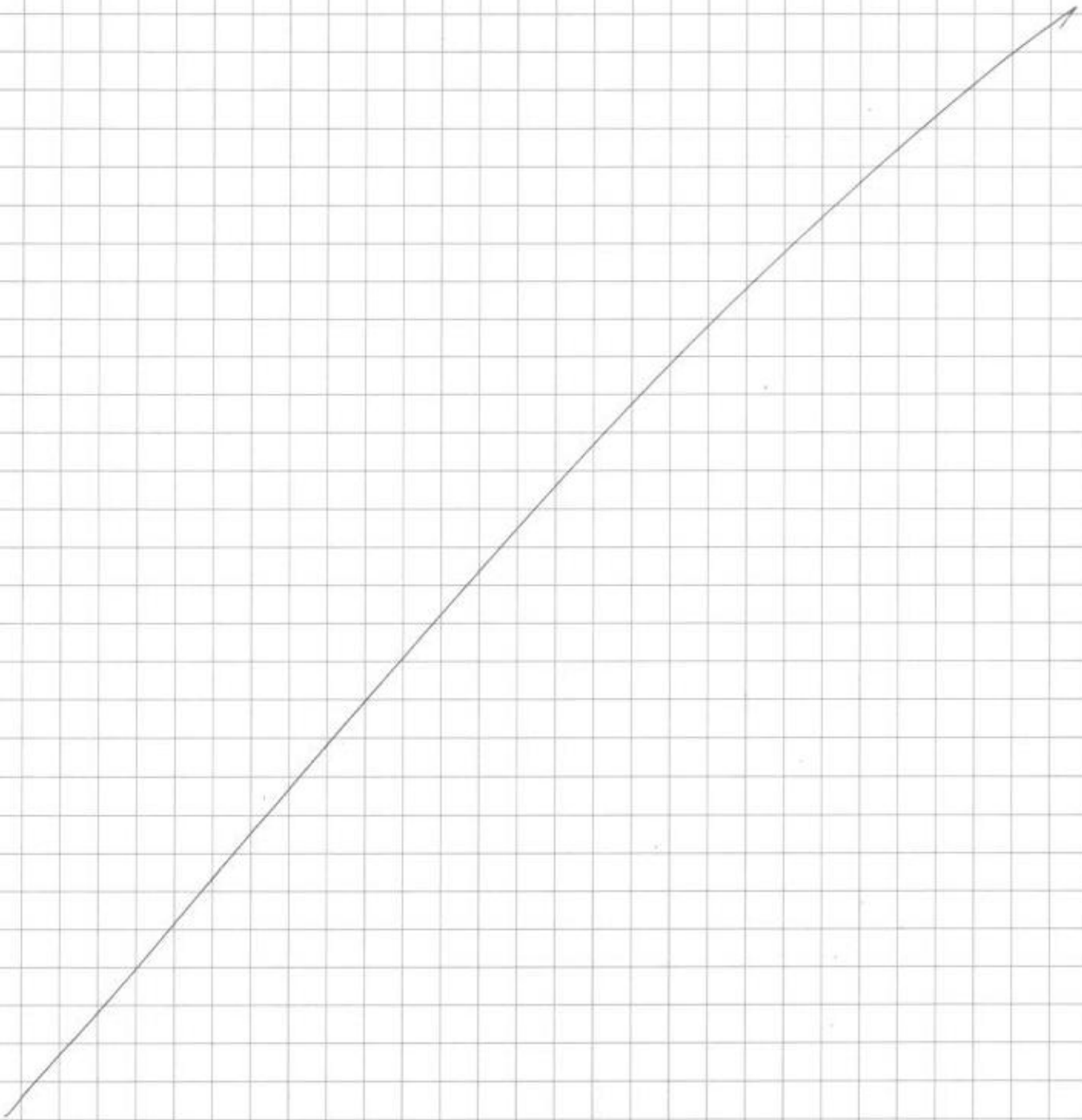
To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kevin C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____



To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Title: Optimizing Ozone Gas Treatment on spores: Various concentrations and contact times

1.0 Purpose:

To determine the most effective concentrations in the most efficient time that will sanitize stainless steel coupons spotted with *Bacillus* spores.

2.0 Responsibility

The scientific personnel that carries out the experiment.

3.0 Hazards and Safety Considerations

- Operator must follow all safety instructions for the Ozone Delivery System
- Spores of *Bacillus anthracis* (stern) strain were used

4.0 Equipment and Supplies

- Sterile Conical tubes containing 10 glass beads (diameter 2mm)
- Sterile forceps
- Sterile de-ionized water (dH_2O)
- Tryptic soy agar (TSA)
- Buffered Peptone Water (BPW) (9mL/tube and 10mL/tube)
- Shaking water bath (Thermo Haake/SWB25)
- Incubator (Imperial III)
- *Bacillus anthracis* (sterne strain) suspension (solved in 50% ethanol)
- 10 mm coupons (stainless steel 316)
- 1 N NaOH Solution
- Sanitizer (6% ozone gas)
- Bio-safety cabinet (BSC)
- Ozone Delivery System

5.0 Operational Procedures

5.1 Preparation of coupons:

5.1.1 Put 6 stainless steel coupons in a sterile petri dish

5.1.2 Load the coupons with 0.1mL of spores

5.1.3 Place the coupons in the refrigerator to dry for 4hrs

5.2 Analysis of Control Coupons (2 stainless steel coupons)

5.2.1 Place each coupon in a conical tube filled with 10 sterilized glass beads and 10mL of BPW

5.2.2 Vortex the sample for 1min

5.2.3 Heat shock the conical tubes in a water bath at 80°C for 20min

5.2.4 Remove conical tubes from water bath and place them in ice H_2O for 2min

5.2.5 Conduct microbiology analysis and plate on TSA

To Page No. ____

nessed & Understood by me,	Date	Invented by: Kim C Cook	Date
		Recorded by:	4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 29

5.3 Analysis for Ozone Treated Coupons (4 stainless steel coupons)

- 5.3.1 Place the coupons in the Anaerobic Reaction Tank (2 coupons for each concentration and each time limit)
- 5.3.2 Be sure to follow the flushing Anaerobic Reaction tank guidelines in SOP: MC-SA-105-SOP001-V01
- 5.3.3 Remove 2 coupons per concentration at 10min and 30min contact time
- 5.3.4 Place coupons in 10mL of BPW with 10 sterilized glass beads
- 5.3.5 Vortex for 1min
- 5.3.6 Heat shock samples in water bath at 80°C for 20min
- 5.3.7 Remove from water bath and place in ice H_2O for 2min
- 5.3.8 Conduct microbiological analysis and plate on TSA
- 5.3.9 Incubate plates for 18-24hrs at 32°C

5.4 Day 2

- 5.4.1 Remove plates from incubator
- 5.4.2 Count colonies
- 5.4.3 Record data in lab notebook

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date 4/3/09
		Recorded by:	

Raw Data - Run 1 (10min, 30min)
Refer to pg. 29 for procedure

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain Date: 3/25/09
Coupon Type: stainless steel 316 Sample: A 20
Ozone Concentration: 8%
Contact Times: 10min, 30min

Samples	3	4	5	6	7	8	9
C-A					73	10	0
					77	11	1
C-B					87	10	0
					75	7	2
10-A	T	T	T	262	54	6	0
	T	T	T	285	48	8	1
10-B	T	253	42	8	2	0	0
	T	221	66	5	0	0	0
30-A	T	107	66	45	11	4	1
	T	110	64	38	16	1	0
30-B	T	T	T	251	56	4	0
	T	T	T	262	41	6	1

C: Control

Witnessed & Understood by me, _____ Date _____ Invented by: Kui C. Cook Date 4/3/09
Recorded by: _____

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Run 1 Calculations

Determination of Ozone's Effect on Bacillus anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8%

Contact Times: 10, 30min

3	4	5	6	7	8	9	avg
				73	10	0	2.3
				77	11	1	
				87	10	0	2.6
				75	7	2	
T	T	T	262	54	6	0	1.9
T	T	T	285	48	8	1	
T	253	42	8	2	0	0	2.4
T	221	66	5	0	0	0	
T	107	66	45	11	4	1	9.3
T	110	64	38	16	1	0	
T	T	T	251	50	4	0	1.7
T	T	T	262	41	6	1	

›g (control)	log (samples)	log reduction
9.36	9.30	0.06
9.42	7.39	2.04
9.36	8.97	0.39
9.42	9.23	0.19

KC

Summary

Due to the variations for both the 30min & 10min contact time this experiment will be repeated.

To Page No

Witnessed & Understood by me, _____ Date _____

Invented by: Kimi C. Coogan

Recorded by: _____

Date 4/13/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Raw Data - Run 2

Refer to pg. 29 for procedure

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8%

Contact Times: 10min, 30min

Date: 3/26/09

Samples: A20 + A21

Samples	3	4	5	6	7	8	9
C-A					67	8	1
					87	9	1
C-B					107	16	1
					105	14	1
10-A	T	T	240	34	2	0	0
	T	T	254	26	2	1	0
10-B	T	T	204	47	6	0	0
	T	T	213	30	3	1	0
30-A	T	265	43	1	0	0	0
	T	280	46	0	0	0	0
30-B	T	T	305	52	1	0	0
	T	T	297	46	2	0	0

C: Control

To Page No. ____

Witnessed & Understood by me, _____ Date

Invented by: Kimi C. Cooper

Recorded by:

Date 4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Run 2 Calculations

Determination of Ozone's Effect on Bacillus anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8%

Contact Times: 10, 30min

Samples	3	4	5	6	7	8	9	avg
C-A					67	8	1	2.62
					87	9	1	
C-B					107	10	1	1.28
					105	14	1	
10-A	T	T	240	34	2	0	0	1.25
	T	T	254	26	2	1	0	
10-B	T	T	204	47	6	0	0	1.41
	T	T	213	30	3	1	0	
30-A	T	265	43	1	0	0	0	7.68
	T	280	46	0	0	0	0	
30-B	T	T	305	52	1	0	0	9.41
	T	T	297	46	2	0	0	

Samples	log (control)	log (samples)	log reduction
10-A	9.42	8.10	1.32
10-B	10.11	8.15	1.96
30-A	9.42	6.89	2.53
30-B	10.11	7.97	2.13

C: Control

T: Too numerous to count

KC

Summary

The data above shows that for the 10min contact time ~~only~~ EC an average log_{10} reducti of 1.5 can be obtained. An average reduction of 2.25 log_{10} reduction can be achieved wi a 30min contact time. Since the concentration of 8% is the maximum for the current syst the contact time will be increased to 1hr to determine if a greater log reduction can be achieved.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

LE _____ Book No. _____

n Page No. ____

Raw Data - Run ~~1~~ ~~3~~ 1

Refer to page 29 for procedure

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8%

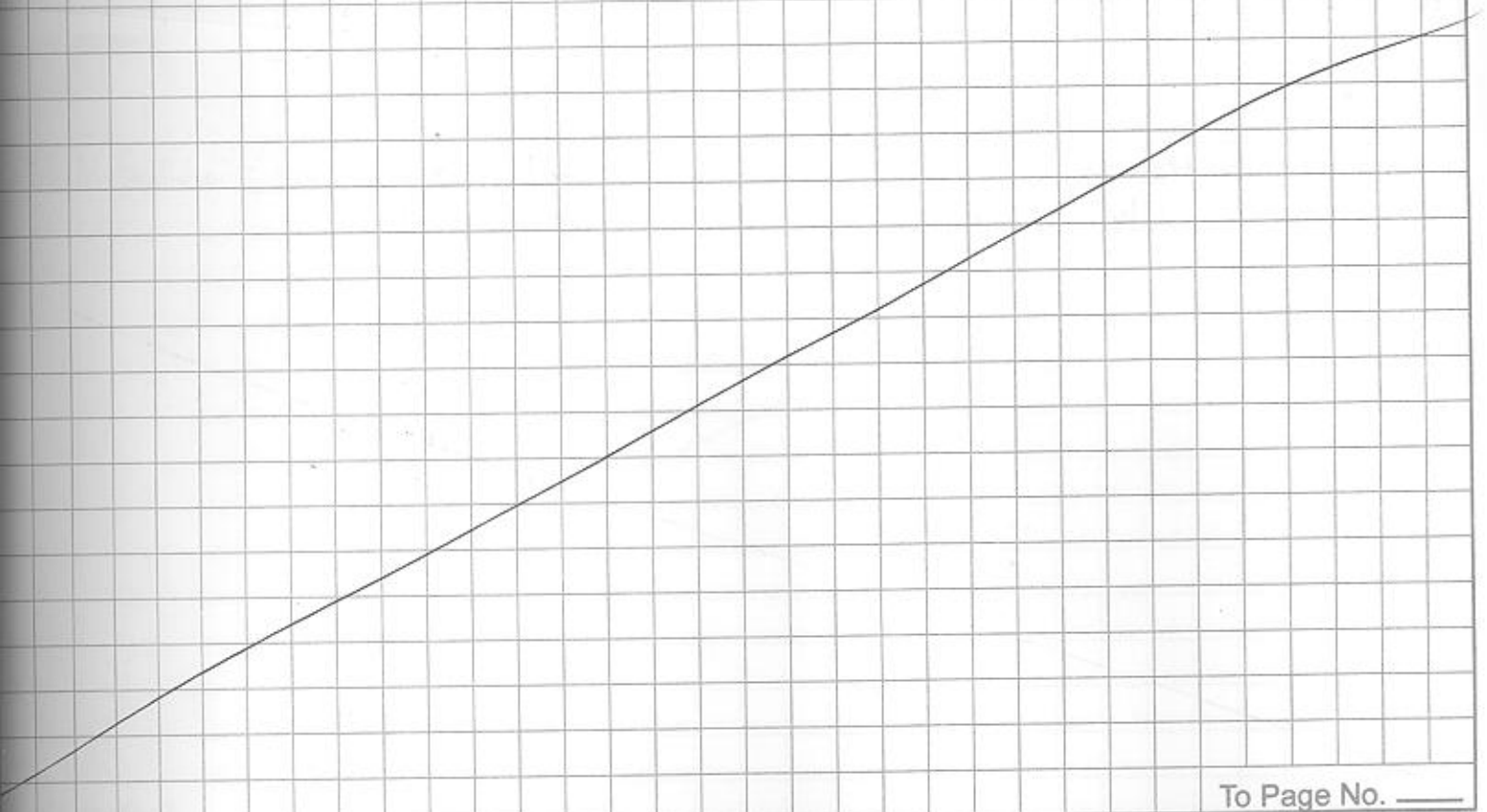
Contact Times: 1hr

Date: 3/27/09

Sample: A21

Samples	3	4	5	6	7	8	9
C-A					82	12	0
					96	15	1
C-B					109	16	0
					108	11	0
A	T	T	195	31	7	0	0
	T	T	143	24	1	0	0
B	17	3	0	0	0	0	0
	22	2	0	0	0	0	0

C:Control



To Page No. ____

nessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

KC

~~Run 3 Calculations~~ Run 1 Calculations

Procedure: See pg. 47

Determination of Ozone's Effect on Bacillus anthracis (sterne)								
Coupon Type: stainless steel 316								
Ozone Concentration: 8%								
Contact Times: 1hr								
Samples	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**avg**
C-A					82	12	0	2.74
					96	15	1	
C-B					109	16	0	2.94
					108	11	1	
A	T	T	195	31	7	0	0	8.44
	T	T	143	24	1	0	0	
B	17	3	0	0	0	0	0	4.45
	22	2	0	0	0	0	0	

Samples	log (control)	log (samples)	log reduction
A	9.44	7.93	1.51
B	9.47	4.65	4.82

C: Control
T: Too numerous to count

KC

Summary

The data for the 1hr contact time is showing too great of a variance. Therefore this experiment will be repeated and the coupons will be increased to 4.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Raw Data - Run 2

Procedure: See pg. 47

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 1hr

Date: 3/31/09

Sample: A20 + A21

Samples	2	3	4	5	6	7	8	9
C-A					~~96~~ KC	96	9	3
					~~101~~ KC	101	11	2
C-B						99	11	4
						86	10	1
C-C						112	7	2
						120	15	2
C-D						111	5	1
						109	9	3
A	T	223	59	3	0	0	0	0
	T	212	54	4	1	0	0	0
B	T	215	58	7	0	0	0	0
	T	297	46	2	0	0	0	0
C	T	T	212	33	0	0	0	0
	T	T	166	38	0	0	0	0
D	7	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0

C: Control

KC

To Page No. ____

Witnessed & Understood by me,

Date

Invented by: Kristi C Cooper

Recorded by:

Date 4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Calculated Data - Run 2
See pg. 48 for Procedure

Determination of Ozone's Effect on Bacillus anthracis (sterne)
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated)
Contact Times: 1hr

Samples	2	3	4	5	6	7	8	9	avg count
C-A						96	9	3	4.49E+09
						101	11	2	
C-B						99	11	4	4.48E+09
						86	10	1	
C-C						112	7	2	4.26E+09
						120	15	2	
C-D						111	5	1	3.80E+09
						109	9	3	
A	T	223	59	3	0	0.	0	0	1.63E+06
	T	212	54	4	1	0	0	0	
B	T	215	58	7	0	0	0	0	1.23E+06
	T	297	46	2	0	0	0	0	
C	T	T	212	33	0	0	0	0	5.44E+06
	T	T	166	38	0	0	0	0	
D	7	0	0	0	0	0	0	0	6.50E+02
	6	0	0	0	0	0	0	0	

Samples	log (control)	log (samples)	log reduction
A	9.65	6.21	3.44
B	9.65	6.09	3.56
C	9.63	6.74	2.89
D	9.58	2.81	6.77

C: Control
T: Too numerous to count

KC

Summary
Although Sample D had a much larger $\log_{10}$ reduction, the other samples (A, B, C) produced an average of about ~~3~~ 10^{-3}. This indicates that the contact time needs to be increased. Therefore the next experiment will use a 90min contact time.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Doog	Date 4/3/09
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Raw Data - Run 1

Procedure - See pg. 47

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 90min

Date: 4/1/09

Sample A21

Samples	3	4	5	6	7	8	9
C-A						14	2
						5	0
C-B						17	0
						10	0
C-C						13	1
						15	1
C-D						75	14
						80	7
A	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0
	1	0	0	0	0	0	0

C: Control

KC

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Calculated Data - Run 2 1 KC

Procedure: look forward to pg. 48

Determination of Ozone's Effect on Bacillus anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 90min

Samples	3	4	5	6	7	8	9	avg count
C-A						14	2	1.95E+09
						5	0	
C-B						17	0	1.35E+09
						10	0	
C-C						13	1	2.40E+09
						15	1	
C-D						75	14	1.83E+10
						80	7	
A	0	0	0	0	0	0	0	0.00E+00
	0	0	0	0	0	0	0	
B	0	0	0	0	0	0	0	0.00E+00
	0	0	0	0	0	0	0	
C	0	0	0	0	0	0	0	0.00E+00
	0	0	0	0	0	0	0	
D	0	0	0	0	0	0	0	5.00E+01
	1	0	0	0	0	0	0	

Samples	log (control)	log (samples)	log reduction
A	9.29	#NUM!	#NUM!
B	9.13	#NUM!	#NUM!
C	9.38	#NUM!	#NUM!
D	10.26	1.70	8.56

C: Control

T: Too numerous to count

KC

Summary

The 90min contact time produced ~~8~~ KC 7 > 8 log10 reduction for all samples. In order to ensure some counts were not missing the 10^2 will be plated.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuri C. Cap	Date 4/3/09
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

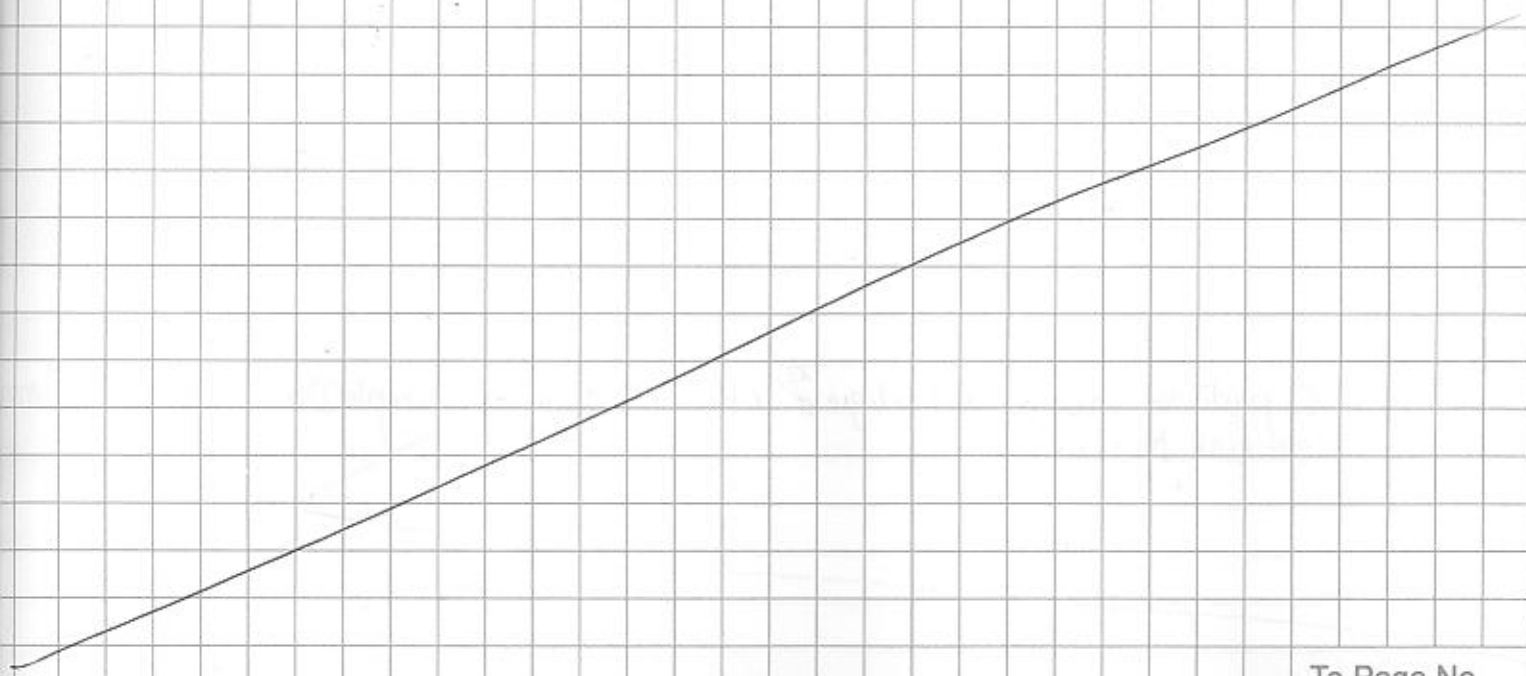
Run 1 - Raw Data
Procedure: Refer to pg. 52

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated)
Constraints: 45min O3 flow time, 20min nitrogen flush

Date: 4/1/09
Sample: A21

Samples	3	4	5	6	7	8	9
C-A						14	2
						5	0
C-B						17	0
						10	0
C-C						13	1
						15	1
C-D						75	14
						80	7
A	T	~~295~~ 295	84	10	0	0	
	T	T	282	81	3	0	
B	T	T	T	64	3	0	
	T	T	T	40	6	0	
C	T	T	211	20	0	0	
	T	T	187	22	0	0	
D	T	221	84	9	0	0	
	T	212	85	1	0	0	

C: Control



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C Coop	Date 4/3/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Run 1 - Calculated Data

Procedure: *Look forward to pg. 53

Determination of Ozone's Effect on *Bacillus* anthracis (sterne)
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated)
Constraints: 45min O_3 flow time, 20min nitrogen flush

Samples	3	4	5	6	7	8	9	avg count
C-A						14	2	1.95E+09
						5	0	
C-B						17	0	1.35E+09
						10	0	
C-C						13	1	2.40E+09
						15	1	
C-D						75	14	1.83E+10
						80	7	
A	T	295	84	10	0	0		8.03E+07
	T	T	282	81	3	0		
B	T	T	T	64	3	0		9.40E+07
	T	T	T	40	6	0		
C	T	T	211	20	0	0		4.09E+07
	T	T	187	22	0	0		
D	T	221	84	9	0	0		1.56E+07
	T	212	85	1	0	0		

Samples	log (control)	log (samples)	log reduction
A	9.29	7.90	1.39
B	9.13	7.97	1.16
C	9.38	7.61	1.77
D	10.26	7.19	3.07

C: Control
T: Too numerous to count

KC

Summary

Samples A, B, C produced about a 1.5 $\log_{10}$ % reduction. Due to sample D's 3 $\log_{10}$ reduction this experiment will be repeated.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/3/09
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Raw Data - Run 2

Procedure: Look forward to pg. 47

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

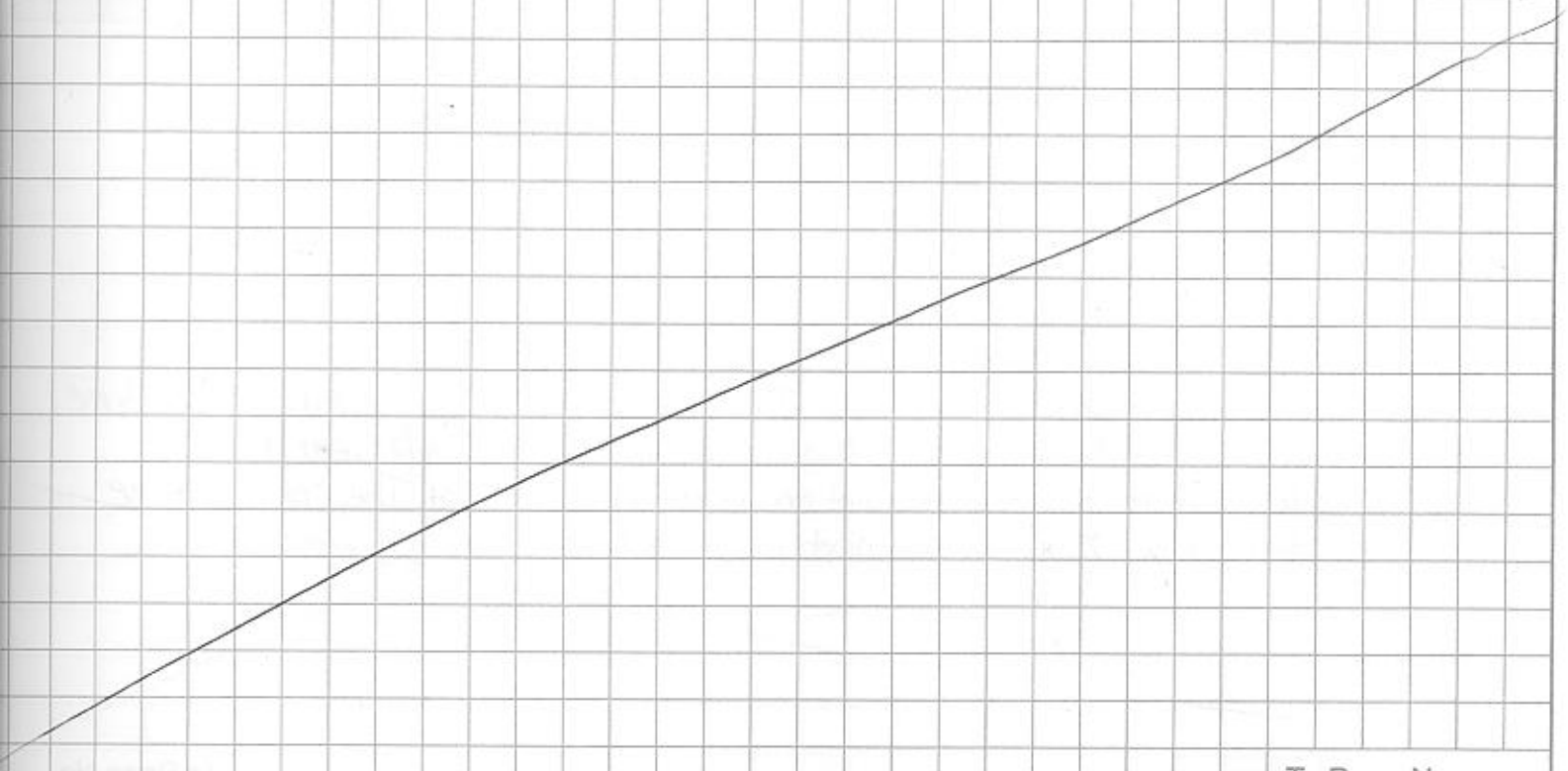
Contact Times: 90min

Date: 4/2/09

Samples: A21 & A22

Samples	2	3	4	5	6	7	8	9
C-A							10	1
							10	0
C-B							11	3
							19	1
C-C							9	0
							6	1
C-D							16	1
							12	1
A	0+2+1	0						
	0	0						
B	0	0						
	0	0						
C	0	0						
	0	0						
D	0+2+4	0						
	2+0+0	0						

C: Control



To Page No. ____

Witnessed & Understood by me,

Date

Invented by: Kuri C. Cooper

Recorded by:

Date 4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Calculated Data - Run 2

Procedure: Look forward to pg 47

Determination of Ozone's Effect on Bacillus anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 90min

Samples	2	3	4	5	6	7	8	9	avg count
C-A							10	1	1.50E+09
							10	0	
C-B							11	3	3.50E+09
							19	1	
C-C							9	0	1.25E+09
							6	1	
C-D							16	1	2.40E+09
							12	1	
A	0+2+1	0							1.50E+02
	0	0							
B	0	0							0.00E+00
	0	0							
C	0	0							0.00E+00
	0	0							
D	0+2+4	0							4.00E+02
	2+0+0	0							

Samples	log (control)	log (sample)	log reduction
A	9.18	2.18	7.00
B	9.54	#NUM!	#NUM!
C	9.10	#NUM!	#NUM!
D	9.38	2.60	6.78

C: Control

T: Too numerous to count

KC

Summary

The data is showing approximately a 7 log$_{10}$ reduction for the 90min contact time. Due to the results for samples B & C this contact time is not sufficient for experimentation because there are not enough colonies to count. The contact time will be decreased to 75min in the next experiment.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Thuri C. Cooper	Date
		Recorded by:	4/3/09

Project No. _____

ITLE _____ Book No. _____

rom Page No. ____

Run 2 - Raw Data

Procedure: See pg. 52

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

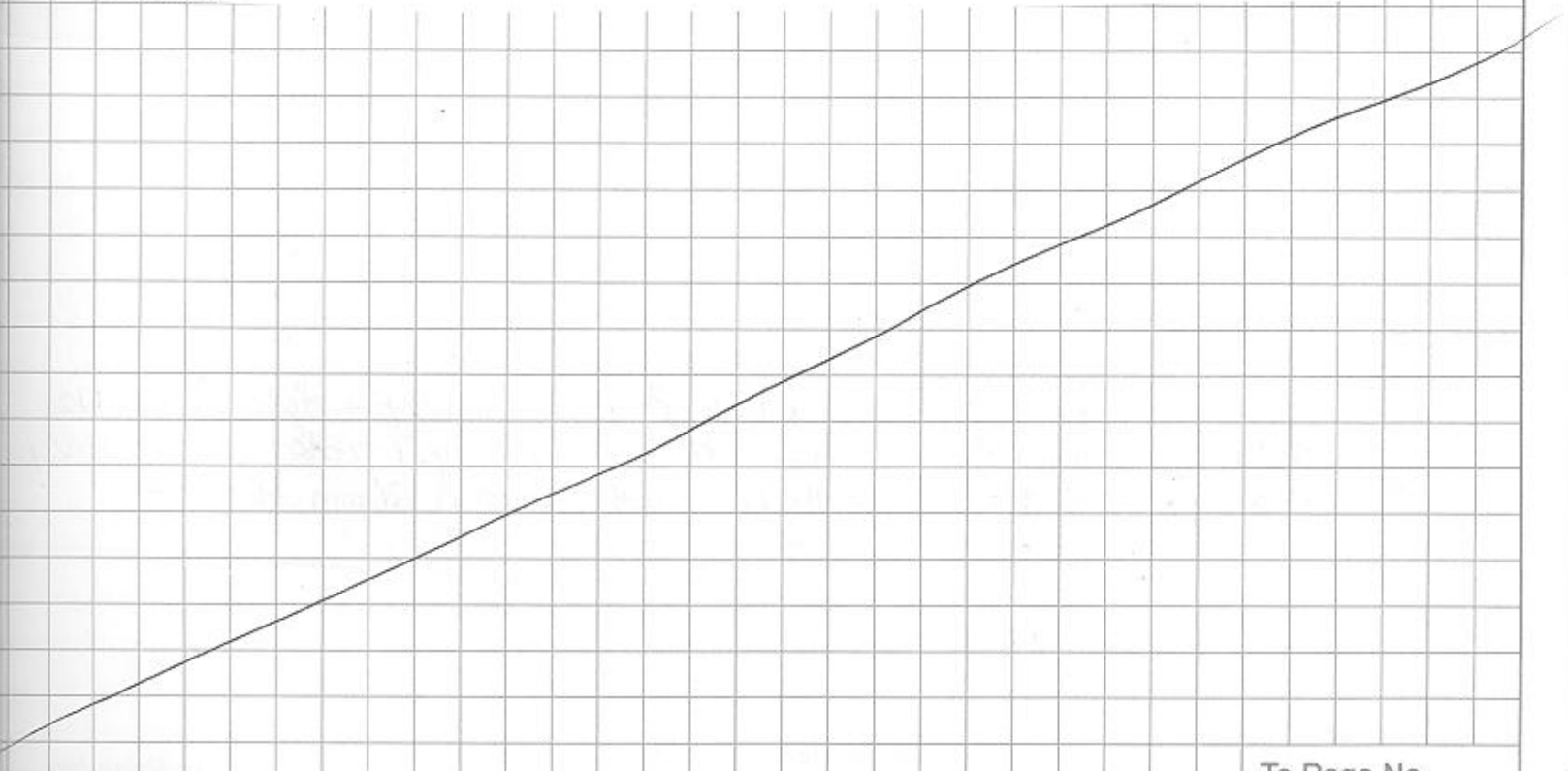
Constraints: 45min O3 flow time, 20min nitrogen flush

Date: 4/2/09

Sample: A22

Samples	3	4	5	6	7	8	9
C-A						10	1
						10	0
C-B						11	3
						19	1
C-C						9	0
						6	1
C-D						16	1
						12	1
A		171	36	5	0		
		148	44	2	1		
B		187	35	5	1		
		138	24	4	1		
C		149	29	0	0		
		202	40	2	0		
D		280	100	13	0		
		224	75	6	0		

C: Control



To Page No. ____

Witnessed & Understood by me,

Date

Invented by: Kim C. Cooper

Recorded by:

Date 4/3/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Run 2- Calculated Data

Procedure: Look forward to pg. 53

Determination of Ozone's Effect on *Bacillus* anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Constraints: 45min O_3 flow time, 20min nitrogen flush

Date: 4/2/09

Sample: A22

Samples	4	5	6	7	8	9	avg count
C-A					10	1	1.50E+09
					10	0	
C-B					11	3	3.50E+09
					19	1	
C-C					9	0	1.25E+09
					6	1	
C-D					16	1	2.40E+09
					12	1	
A	171	36	5	0			1.41E+07
	148	44	2	1			
B	187	35	5	1			1.89E+07
	138	24	4	1			
C	149	29	0	0			6.21E+06
	202	40	2	0			
D	280	100	13	0			2.08E+07
	224	75	6	0			

Samples	log (control)	log (samples)	log reduction
A	9.18	7.15	2.03
B	9.54	7.28	2.27
C	9.10	6.79	2.30
D	9.38	7.32	2.06

C: Control

T: Too numerous to count

KC

Summary

The $\log_{10}$ reduction for this data is a low 10^2 cfu/ml. Since the data on pg. 42 gave only a 1.5 $\log_{10}$ reduction, this experiment will be repeated for accuracy. There is also a 20min delay between the O_3 flow through and 20min flush of nitrogen.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date
		Recorded by:	4/3/09

Project No. _____
TITLE _____ Book No. _____

From Page No.

MOFFETT CENTER	Page 1 of 3
Title: Optimizing Ozone Gas Treatment on spores: Varying concentrations and contact times	Author: Kerri C. Cooper
Document No.: MC-QA-105-SOP114-V01	Effective Date: March 2009

1.0 Purpose:
To determine the most effective concentrations in the most efficient time that will sanitize stainless steel coupons spotted with *Bacillus* spores.

2.0 Responsibility
The scientific personnel that carries out the experiment.

3.0 Hazards and Safety Considerations
- Operator must follow all safety instructions for the Ozone Delivery System
- Spores of *Bacillus anthracis* (stern) strain were used

4.0 Equipment and Supplies
- Sterile Conical tubes containing 10 glass beads (diameter 2mm)
- Sterile forceps
- Sterile de-ionized water (dH_2O)
- Tryptic soy agar (TSA)
- Buffered Peptone Water (BPW) (9mL/tube and 10mL/tube)
- Shaking water bath (Thermo Haake/SWB25)
- Incubator (Imperial III)
- *Bacillus anthracis* (sterne strain) suspension (solved in 50% ethanol)
- 10 mm coupons (stainless steel 316)
- 1 N NaOH Solution
- Sanitizer (8% calculated ozone gas)
- Bio-safety cabinet (BSC)
- Ozone Delivery System

5.0 Operational Procedures

5.1 Preparation of coupons:
- 5.1.1 Place stainless steel coupons in a sterile petri dish
- 5.1.2 Load the coupons with 0.1mL of spores
- 5.1.3 Place the coupons in the refrigerator to dry for 4hrs

5.2 Analysis of Control Coupons
- 5.2.1 Place each coupon in a conical tube filled with 10 sterilized glass beads and 10mL of BPW
- 5.2.2 Vortex the sample for 1min
- 5.2.3 Heat shock the conical tubes in a water bath at 80°C for 20min
- 5.2.4 Remove conical tubes from water bath and place them in ice H_2O for 2min
- 5.2.5 Conduct microbiology analysis and plate on TSA

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 4/5/09
		Recorded by:	

MOFFETT CENTER	Page 2 of 3
Title: Ozone Gas Treatment at Various concentrations and contact times on *Bacillus anthracs* (stern) strains inoculated on stainless steel 316 coupons	Author: Kerri C. Cooper
Document No.:	Effective Date: March 5, 2009

5.3 Day 1: Analysis for Ozone Treated Coupons

- 5.3.1 Place the coupons in the Anaerobic Reaction Tank
- 5.3.1 Be sure to follow the flushing Anaerobic Reaction tank guidelines in SOP: MC-SA-105-SOP001-V01
- 5.3.2 Remove coupons after the predetermined contact time
- 5.3.3 Place coupons in 10mL of BPW with 10 sterilized glass beads
- 5.3.4 Vortex for 1min
- 5.3.5 Heat shock samples in water bath at 80°C for 20min
- 5.3.6 Remove from water bath and place in ice H_2O for 2min
- 5.3.7 Conduct microbiological analysis and plate on TSA
- 5.3.8 Incubate plates for 18-24hrs at 32°C

5.4 Day 2

- 5.4.1 Remove plates from incubator
- 5.4.2 Count colonies
- 5.4.3 Record data in lab notebook

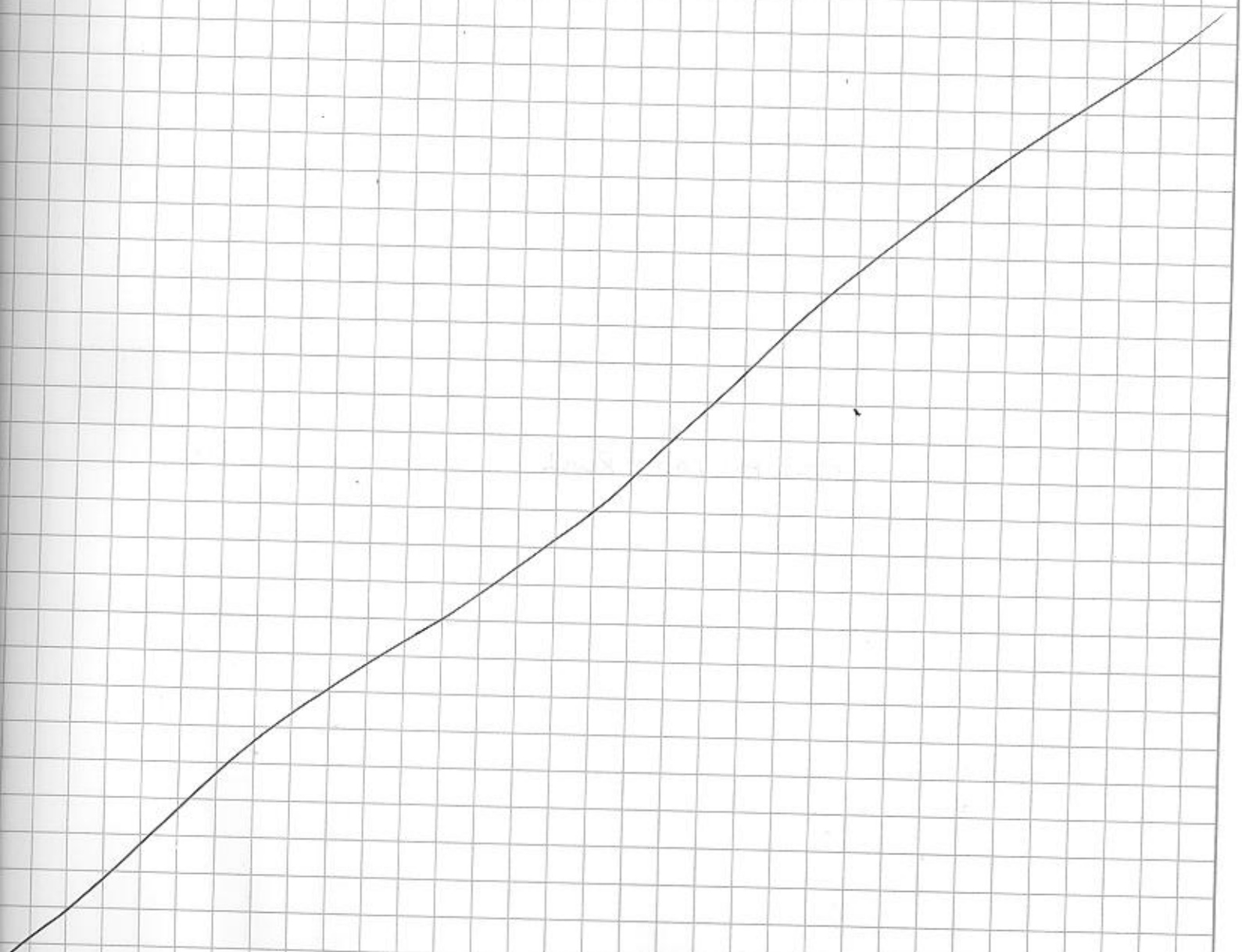
Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper Recorded by:	Date 4/5/06

Day 1: Determination of Ozone's Effect on Bacillus anthracis (sterne) strain - Run 1
Date: 4/2/09

~~Actual~~ KC Analyzer Reading: 6.24 %. ~~→ Calculated Concentration~~ KC

Humidified Concentration: $y = 1.0002(6.24) - 0.2001$
$= 6.04\%$ wt

Calculated Concentration: $y = 1.3541(6.04) + 0.063$
$= 8.24\%$ wt



To Page No. ____

Project No. _____

Book No. _____ TITLE _____

From Page No. 49

Raw Data - Run 1

Procedure: See pg. 47

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 75min

Date: 4/3/06

Samples: A22 + A23

Samples	2	3	4	5	6	7	8	9
C-A							17	0
							11	0
C-B							5	2
							5	0
C-C							11	1
							7	2
C-D							21	0
							17	5
A		127	20					
		206	31					
B		100	15					
		73	16					
C		0	0					
		0	0					
D		12	2					
		3	0					

C: Control

KC

Calculated Data - Run 1

KC

Determination of Ozone's Effect on Bacillus anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 75min

Samples	3	4	5	6	7	8	9	avg count
C-A						17	0	1.40E+09
						11	0	
C-B						5	2	1.50E+09
						5	0	
C-C						11	1	2.40E+09
						7	2	
C-D						21	0	4.40E+09
						17	5	
A	127	20	2	0	0			5.72E+05
	206	31	1	0	0			
B	100	15	0	0	0			2.92E+05
	73	16	1	0	0			
C	0	0	0	0	0			0.00E+00
	0	0	0	0	0			
D	12	2	0	0	0			6.75E+04
	3	0	1	0	0			

Witnessed & understood by:

Kuni C-Coopa

Recorded by:

4/5/09

Project No. _____

TITLE _____ Book No. _____

From Page No. 50

Calculated Data - Run 1 continued

Samples	log (control)	log (samples)	log reduction
A	9.15	5.76	3.39
B	9.18	5.46	3.71
C	9.38	#NUM!	#NUM!
D	9.64	4.83	4.81

C: Control
T: Too numerous to count

KC

Summary

- The ~~avgerage~~ KC average log reduction for all ~~the~~ KC samples A, B, and D is: 3.97 $\log_{10}$
- Sample C produced a > 6 $\log_{10}$ reduction
- This placement of the coupons in the chamber has not been monitored for KC ~~et~~ possible variations in ~~Log~~ KC samples. This will be done in the next experiment.
- The 75min contact time will be repeated to determine ~~if there a~~ KC the actual log reduction for this contact time.

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date 4/5/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page

MOFFETT CENTER	Page 1 of 2
Title: **Determining the Minimum Log Reduction the Ozone Delivery System Can Achieve**	Author: Kerri Cooper
Document No.:	Effective Date: April 2009

1.0 Purpose:
To determine the most effective concentrations in the most efficient time that will sanitize stainless steel coupons spotted with *Bacillus* spores.

2.0 Responsibility
The scientific personnel that carries out the experiment.

3.0 Hazards and Safety Considerations
- Operator must follow all safety instructions for the Ozone Delivery System
- Spores of *Bacillus anthracis* (stern) strain were used

4.0 Equipment and Supplies
- Sterile Conical tubes containing 10 glass beads (diameter 2mm)
- Sterile forceps
- Sterile de-ionized water (dH_2O)
- Tryptic soy agar (TSA)
- Buffered Peptone Water (BPW) (9mL/tube and 10mL/tube)
- Shaking water bath (Thermo Haake/SWB25)
- Incubator (Imperial III)
- *Bacillus anthracis* (sterne strain) suspension (solved in 50% ethanol)
- 10 mm coupons (stainless steel 316)
- 1 N NaOH Solution
- Sanitizer (8% calculated ozone gas)
- Bio-safety cabinet (BSC)
- Ozone Delivery System

5.0 Operational Procedures
Day 1:

5.1 Preparation of coupons:
- 5.1.1 Place stainless steel coupons in a sterile petri dish
- 5.1.2 Load the coupons with 0.1mL of spores
- 5.1.3 Place the coupons in the refrigerator to dry for 4hrs

5.1 Analysis of Control Coupons
- 5.1.1 Place each coupon in a conical tube filled with 10 sterilized glass beads and 10mL of BPW
- 5.2.2 Vortex the sample for 1min
- 5.2.3 Heat shock the conical tubes in a water bath at 80°C for 20min
- 5.2.4 Remove conical tubes from water bath and place them in ice H_2O for 2min

Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 4/6/09
		Recorded by:	

Project No. _____
TITLE _____ Book No. _____
From Page No. 52

MOFFETT CENTER	Page 2 of 2
Title: **Determining the Minimum Log Reduction the Ozone Delivery System Can Achieve**	Author: Kerri Cooper
Document No.:	Effective Date: April 2009

5.2.5 Conduct microbiology analysis and plate on TSA
5.2.6 Incubate for 24hrs at 32°C

5.3 Analysis for Ozone Treated Coupons
5.3.1 Place the coupons in the Anaerobic Reaction Tank
5.3.1 Following the SOP: MC-SA-105-SOP001-V01 allow the ozone to flow through the system for 45min
5.3.1 Stop the ozone flow and follow the flushing the system step in SOP: MC-SA-105-SOP001-V01
5.3.2 Remove coupons from the anaerobic tank
5.3.3 Place coupons in 10mL of BPW with 10 sterilized glass beads
5.3.4 Vortex for 1min
5.3.5 Heat shock samples in water bath at 80°C for 20min
5.3.6 Remove from water bath and place in ice H_2O for 2min
5.3.7 Conduct microbiological analysis and plate on TSA
5.3.8 Incubate plates for 18-24hrs at 32°C

Day 2
5.4 Remove plates from incubator
5.5 Count colonies
5.6 Record data in lab notebook

Day 1 → Date: 4/2/09
Analyzer Reading: 6.24 %wt
Calculated Concentration: 8.24 %wt

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date
		Recorded by:	4/5/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 53

Determining Minimum Log Reduction of the Ozone Delivery System for 8% wt concentration.

Raw Data - Run 3

See pg. 52 for procedure

Determination of Ozone's Effect on *Bacillus anthracis* (sterne) strain

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Constraints: 45min O3 flow time, 20min nitrogen flush

Date: 4/3/06

Samples: A22+A23

Samples	3	4	5	6	7	8	9
C-A						17	0
						11	0
C-B						5	2
						5	0
C-C						11	1
						7	2
C-D						21	0
						17	5
A	T KC	T	205	28			
	T KC	T	228	23			
B	T KC	T	339	42			
	T KC	T	210	46			
C	T KC	T	221	68			
		T	229	88			
D		T	330	50			
		T	321	64			

KC

Calculated Data - Run 3

Determination of Ozone's Effect on *Bacillus* anthracis (sterne)

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Constraints: 45min O_3 flow time, 20min nitrogen flush

Samples	4	5	6	7	8	9	avg count
C-A					17	0	1.40E+09
					11	0	
C-B					5	2	1.50E+09
					5	0	
C-C					11	1	2.40E+09
					7	2	
C-D					21	0	4.40E+09
					17	5	
A	T	205	28				4.72E+07
	T	228	23				
B	T	339	42				1.43E+08
	T	210	46				
C	T	221	68				1.01E+08
	T	229	88				
D	T	330	50				8.96E+07
	T	321	64				

To Page No. _____

Recorded by: Kimi C. Cooper

4/5/09

Project No. _____

TITLE _____ Book No. _____

From Page No. 54

Calculated Data - Run 3 continued

Samples	log (control)	log (samples)	log reduction
A	9.15	7.67	1.47
B	9.18	8.16	1.02
C	9.38	8.00	1.38
D	9.64	7.95	1.69

C: Control
T: Too numerous to count. KC

Summary

- The average log reduction for all the samples is 1.39 $\log_{10}$
- This data is similar to the data run on pg. 42 excluding sample D, but has about 1 $\log_{10}$ difference from the experiment ~~add~~ performed on pg. 46
- The experiment will be performed again for accuracy of the $\log_{10}$ reduction.

To Page No. ____

Witnessed & Understood by me, _____ Date _____

Invented by: Kim C. Cooper

Recorded by: _____

Date 4/15/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Summary of the Minimum Log Reduction

When trying to determine the effect of ozone on the Bacillus spores, there are many aspects that must be taken into account. One of the details is to determine if the ozone that flows through the system for a specified amount of time to achieve the desired concentration in the Anaerobic Tank and the nitrogen flushing for 20min to remove the ozone in the system after the experiment effects the spores. For the current $8\%_{wt}$ concentration being used for the experiments, a 45min ozone flow through time is required. The initial experiment for determining the basic effects of the gas was to allow the ozone to flow through for 45min and then remove the ozone immediately from the system by flushing with the nitrogen. Unfortunately, the design of the system does not allow for an immediate ozone removal due to the time required to flush the Buffer tank and pressurize it with nitrogen before beginning the flushing of the Anaerobic Tank. This wait time is 20min. Due to the safety aspect of the system, the chamber cannot be opened during the experiment to remove samples after each step. Therefore, a minimum log reduction experiment has been setup. This experiment takes into account the calculated amount of time required for the ozone to flow through the system, the 20min wait time to flush the Buffer Tank, and the 20min nitrogen flushing time. KC

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kurt C. Cooper	Date 4/15/09
		Recorded by:	

Date: 4/6/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 75min Exposure Time - Run 2

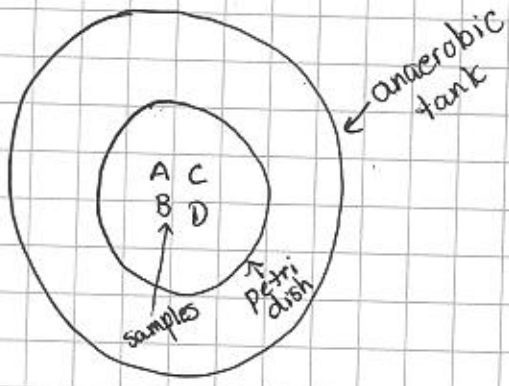
Procedure: Refer to pg. 47

Day 1:

Analyzer Reading: 6.24%

Calculated Concentration: 8.24%

Coupon placement:



essed & Understood by me,	Date	Invented by: Kuri C. Cogan	Date
		Recorded by:	4/7/09

Project No. _____
Book No. _____ TITLE _____

From Page No. 57 Date: 4/7/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 75min Exposure Time - Day 2
Raw Data - Run 2

Procedure: See pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 75min exposure time
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated)
Contact Times: 75min
Date: 4/7/09
Sample: A24

Samples	2	3	4	5	6	7	8	9
C-A							25	6
							20	2
C-B								
C-C								
C-D								
A		0	0	0	0	0		
		0	0	0	0	0		
B		0	0	0	0	0		
		0	0	0	0	0		
C		0	0	0	0	0		
		0	0	0	0	0		
D		0	0	0	0	0		
		0	0	0	0	0		

C: Control

Summary

- Average $\log_{10}$ reduction is >10
- Possible reason for deviation in initial experiment on pg. 51: more water was added to the shaker bath to ensure it completely covered the liquid in the conicle tubes. This was done during the heat shock step. This may have allowed for elimination of more vegetative cells.
- The placement of the samples did not appear to make a difference in this experiment.
- The contact time will be reduced to 60min in the next experiment.

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date
		Recorded by:	4/7/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Date: 4/8/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with an 60min Exposure Time - Run 3

Procedure: Refer to pg. 47

Day 1

Analyzer Reading: 6.22%wt

Humdified Conc: $y = 1.0002(6.22) - 0.2001$
$= 6.02\%wt$

~~Calculated Reading:~~

★Calculated Conc: $y = 1.3541(6.02) + 0.063$
$= 8.21\%wt$

Note

- The humidity tank stopper continued to pop during the O_3 flush through. The system was stopped and the problem was solved by releasing the pressure in the Excess Release Tank. Before and after each experiment always release the pressure in this tank.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min exposure time - Run 3

Procedure: Refer to pg. 48

Raw Data

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Date: 4/9/09

Samples: A24

Samples	2	3	4	5	6	7	8	9
C-A							11	2
~~KC~~ C-B							7	0
~~KC~~								
~~KC~~ C-C								
~~KC~~								
~~KC~~ C-D								
~~KC~~								
A		T	T	170	20	0		
		T	T	180	24	2		
B		T	T	120	35	3		
		T	T	115	29	4		
C		T	256	33	4	0		
		T	230	35	4	0		
D		T	215	67	5	0		
		T	320	69	3	1		

C: Control

KC

Calculated Data

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

KC

Samples	3	4	5	6	7	8	9	avg count
Control						11	2	1.90E+09
						7	0	
A	T	T	170	20	0			4.95E+07
	T	T	180	24	2			
B	T	T	120	35	3			7.88E+07
	T	T	115	29	4			
C	T	256	33	4	0			9.83E+06
	T	230	35	4	0			
D	T	215	67	5	0			1.85E+07
	T	320	69	3	1			

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date
		Recorded by:	4/9/09

Project No. _____

ITLE _____ Book No. _____

rom Page No. ____

Run 3 - Calculated Data Continued

Samples	log (control)	log (samples)	log reduction
A	9.28	7.69	1.58
B	9.28	7.90	1.38
C	9.28	6.99	2.29
D	9.28	7.27	2.01 KC

Summary

- The average log reduction for the samples is 1.8 $\log_{10}$
- This smaller log reduction compared to the previous runs could be due to the gas stopper popping on the humidity tank. This could mean the maximum concentration was not achieved in the Anaerobic Tank.
- This experiment will be repeated and ~~the~~ KC a 70min contact time will also be tested.

To Page No. ____

itnessed & Understood by me,

Date

Invented by: Kami C. Cooper

Recorded by:

Date 4/9/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Date: 4/9/09

Determination of the Minimum Log Reduction of the Ozone Delivery ~~Sty~~ System for 8% Concentration

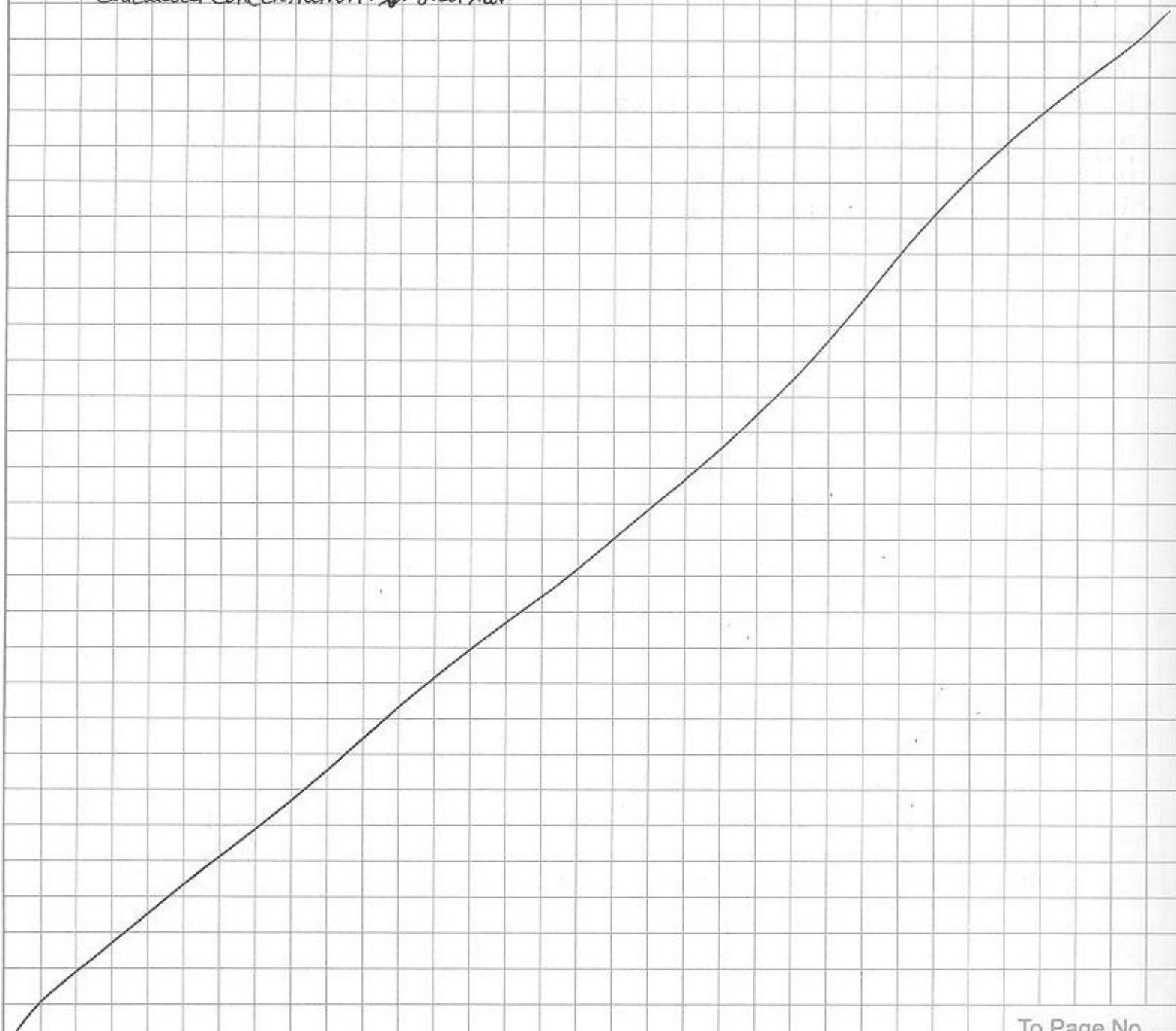
Procedure: Refer to pg. 52

Raw Data - Run 4

Day 1

Analyzer Reading: 6.22% wt

Calculated Concentration: ~~8~~ 8.21% wt



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Coaya	Date
		Recorded by:	4/10/09

Project No. _____
TITLE _____ Book No. _____
From Page No. ____

Raw Data - Run 4

Determination of the Minimum Log Reduction of the Ozone Delivery System for 8% Concentration
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated) Date: 4/10/09
Contact Times: 45min O_3 flow through time, 20min N_2 flush

Samples	2	3	4	5	6	7	8	9
C-A							11	2
							17	5
~~C-B~~								
~~C~~								
~~C-C~~								
~~C~~								
~~C-D~~								
~~C~~								
A	241	77	6					
	233	62	10					
B	187	49	3					
	201	52	1					
C	163	44	3					
	128	48	4					
D	172	61	2					
	115	60	2					

KC

Calculated Data - Run 4

Determination of the Minimum Log Reduction of the Ozone Delivery System for 8% Concentration
Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated)
Constraints: 45min O_3 flow through time, 20min nitrogen flush

KC

Samples	4	5	6	7	8	9	avg count
C-A					11	2	1.90E+09
				KC	~~7~~	~~0~~	
					17	5	
A	241	77	6				1.73E+07
	233	62	10				
B	187	49	3				8.99E+06
	201	52	1				
C	163	44	3				9.56E+06
	128	48	4				
D	172	61	2				9.49E+06
	115	60	2				

To Page No. ____

Witnessed & Understood by me, | Date | Invented by: Kevin C. Cooper | Date 4/10/09
Recorded by:

Project No. _____

Book No. _____ TITLE _____

From Page No. 63

Calculated Data - Continued

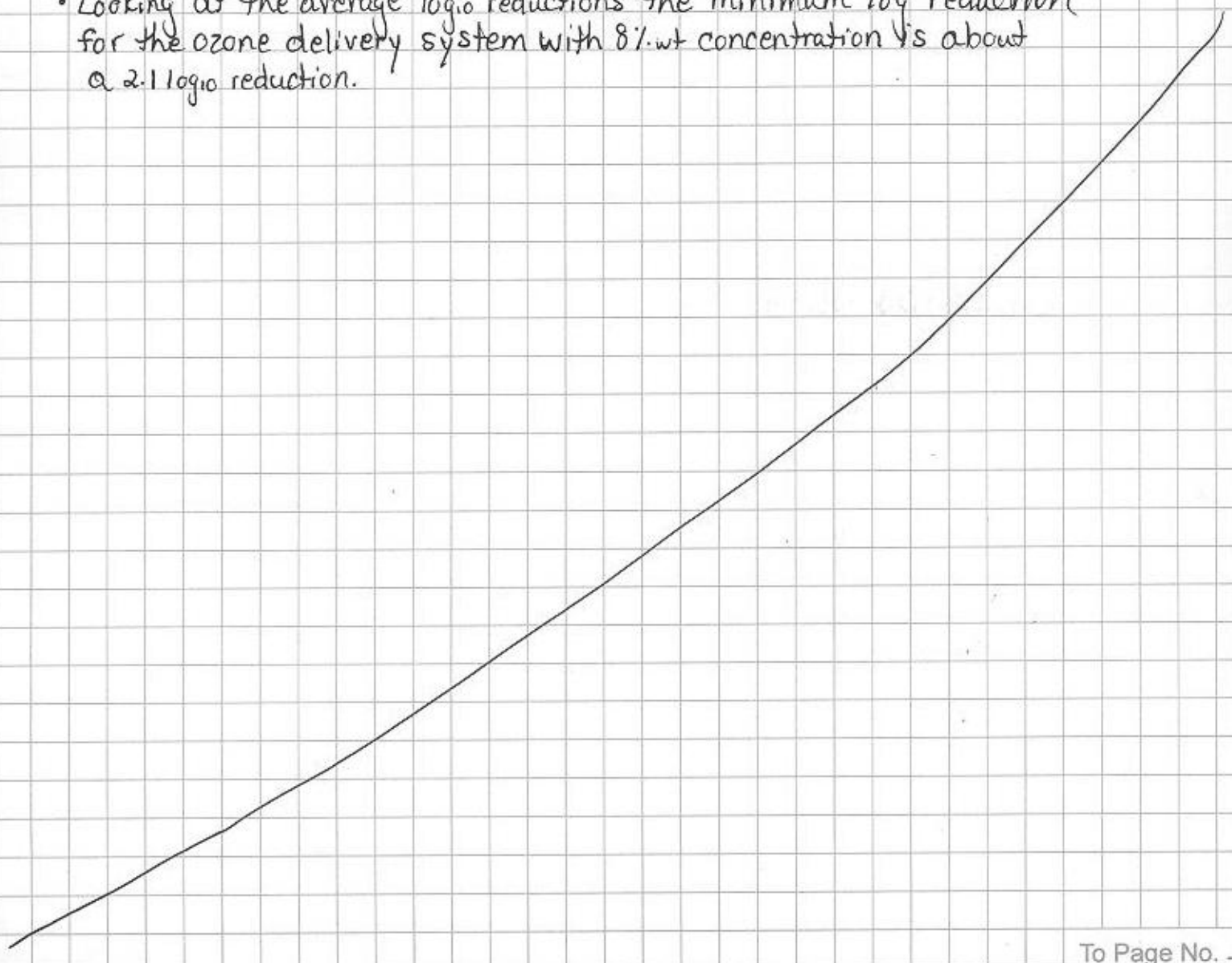
Date: 4/10/09

Samples	log (control)	log (samples)	log reduction
A	9.28	7.24	2.04
B	9.28	6.95	2.32
C	9.28	6.98	2.30
D	9.28	6.98	2.30

KC

Summary

- The average $\log_{10}$ reduction for the samples is 2.24 $\log_{10}$
- This data correlates to the data found on pg. 46
- Looking at the average $\log_{10}$ reductions the minimum log reduction for the ozone delivery system with 8% wt concentration is about a 2.1 $\log_{10}$ reduction.



To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Doop	Date 4/10/09
		Recorded by:	

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

Date: 4/10/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min + 70min Exposure Time

Procedure: Refer to pg. 47

Day 1

Ozone Concentration Analysis:

Analyzer Reading: 6.21%.wt

Humidified Concentration: $y = 1.0002(6.21) - 0.2001$
$= 6.01\%.wt$

Actual Ozone Concentration: $y = 1.3541(6.01) + 0.063$
$= 8.2\%.wt$

Note

· Added water to the water bath because the water level was too low to cover the liquid in the conical tubes.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kumi C. Coop	Date
		Recorded by:	4/18/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Raw Data - Refer to pg. 48 for procedure

Run 4

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Date: ~~4/10/09~~ KC 4/10/09

Samples	2	3	4	5	6	7	8	9
C-A							19	1
							12	2
~~KC C-B~~								
~~KC~~								
~~KC C-C~~								
~~KC~~								
~~KC C-D~~								
~~KC~~								
A		1	0	0	0	0		
		0	0	0	0	0		
B		3	0	0	0	0		
		1	0	0	0	0		
C		9	0	0	0	0		
		5	~~KC~~ 0	0	0	0		
D		0	0	0	0	0		
		0	0	0	0	0		

C: Control

KC

Raw Data Run 1

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Date: ~~4/10/09~~ KC 4/11/09

Samples	2	3	4	5	6	7	8	9
C-A							19	1
							12	2
~~KC C-B~~								
~~KC~~								
~~KC C-C~~								
~~KC~~								
~~KC C-D~~								
~~KC~~								
A		0	0	0	0	0		
		0	0	0	0	0		
B		215	36	4	0	0		
		292	35	6	2	0		
C		117	29	3	0	0		
		153	39	0	0	0		
D		33	1	0	0	0		
		16	1	0	0	0		

C: Control

To Page No. ____

Witnessed & Understood by me, _____ Date _____

Invented by: Kun C. Coop

Recorded by: _____

Date 4/18/09

Project No. _____

TITLE _____ Book No. _____

From Page No. 66

Calculated Data Date 4/11/09

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Samples	3	4	5	6	7	8	9	avg count
Control						19	1	3.05E+09
						12	2	
A	1	0	0	0	0			5.00E+02
	0	0	0	0	0			
B	3	0	0	0	0			2.00E+03
	1	0	0	0	0			
C	9	0	0	0	0			7.00E+03
	5	0	0	0	0			
D	0	0	0	0	0			0.00E+00
	0	0	0	0	0			

Samples	log (control)	log (samples)	log reduction
A	9.48	2.70	6.79
B	9.48	3.30	6.18
C	9.48	3.85	5.64
D	9.48	#NUM!	#NUM!

KC

Summary

- Samples A, B, C produced a $\log_{10}$ reduction ~~of~~ on average of 6.2
- Sample D produced a >6 $\log_{10}$ reduction
- The addition of the water in the waterbath may have caused a difference in log reduction from the previous experiments on pgs. 61 and 38 ~~d~~ KC by killing the vegetative cells. More experiments will have to be run to determine if this has occured.
- This experiment will be run again.
-

To Page No. _____

Witnessed & Understood by me, _____ Date _____

Invented by: Kimi C. Cooper

Recorded by: _____

Date 4/18/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 66 Date: 4/11/09

Calculated Data

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Samples	3	4	5	6	7	8	9	avg count
Control						19	1	3.05E+09
						12	2	
A	0	0	0	0	0			0.00E+00
	0	0	0	0	0			
B	215	36	4	0	0			2.11E+06
	292	35	6	2	0			
C	117	29	3	0	0			6.25E+05
	153	39	0	0	0			
D	33	1	0	0	0			3.45E+04
	16	1	0	0	0			

Samples	log (control)	log (samples)	log reduction
A	9.48	#NUM!	#NUM!
B	9.48	6.32	3.16
C	9.48	5.80	3.69
D	9.48	4.54	4.95

Summary

- The $\log_{10}$ reduction for Sample A > 6 log reduction
- Samples B, C, D produced an average of 3.93 $\log_{10}$ reduction
- This experiment will be repeated for accuracy

Witnessed & Understood by me,	Date	Invented by: Kimi C Cogar	Date
		Recorded by:	4/18/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Date: 4/15/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min + 70min Exposure Time

Day 1

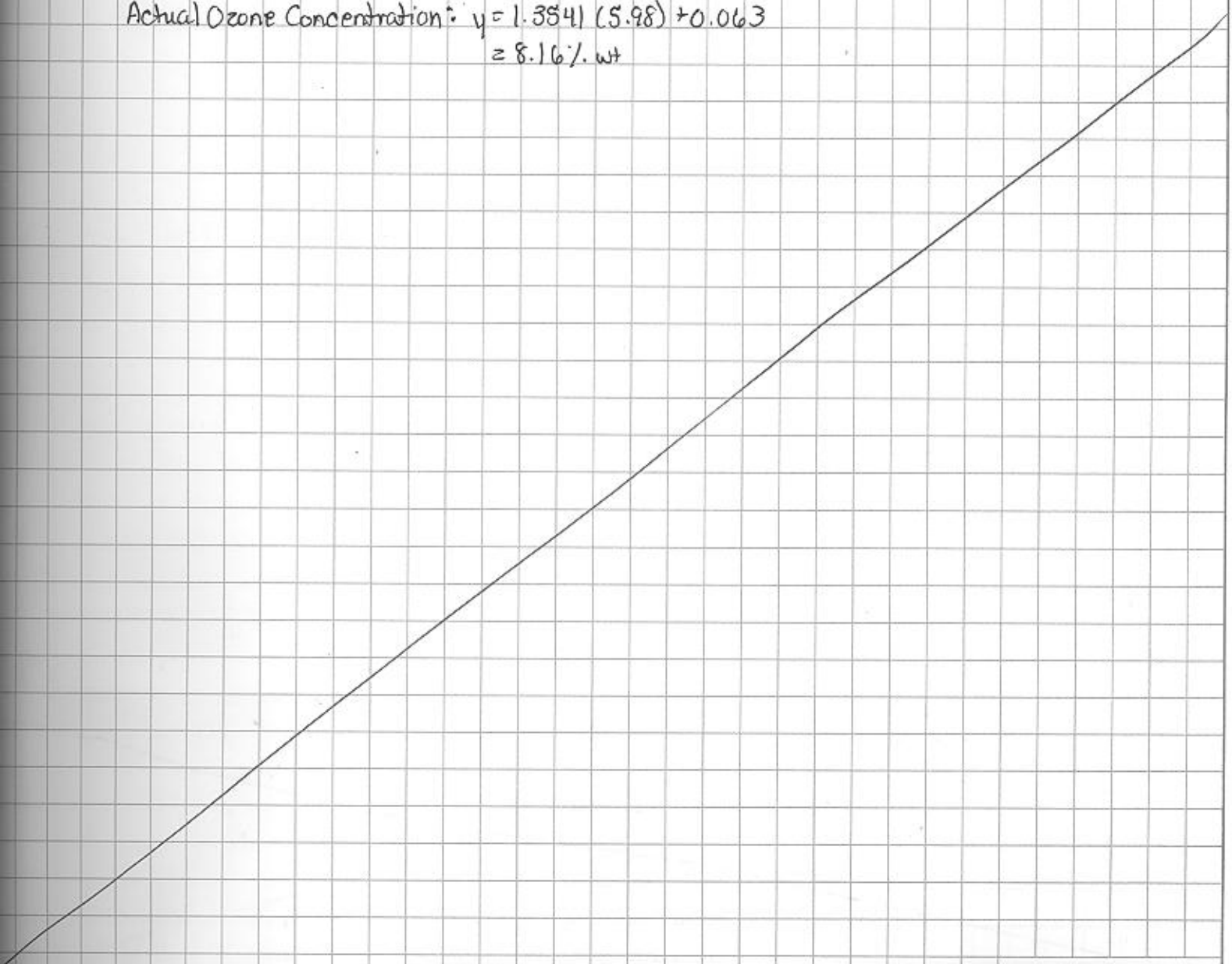
Analyzer Reading: 6.18% wt

Humidified Concentration: $y = 1.0002(6.18) - 0.2001$

$= 5.98\%$ wt

Actual Ozone Concentration: $y = 1.3541(5.98) + 0.063$

$= 8.16\%$ wt



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date
		Recorded by:	4/18/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 69

Date: 4/16/09

Raw Data

Run 5

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Date: 4/16/09

Sample: A26

Samples	3	4	5	6	7	8	9
C-A						18	5
						19	1
A	0	0	0	0	0		
	0	0	0	0	0		
B	0	0	0	0	0		
	0	0	0	0	0		
C	104	9	1	0	0		
	190	9	2	0	0		
D	0	0	0	0	0		
	0	0	0	0	0		

C: Control

KC

Run 2

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Date: 4/16/09

Sample: A26

Samples	3	4	5	6	7	8	9
C-A						18	5
						19	1
A	5	0	0	0	0		
	4	0	0	0	0		
B	8	0	0	0	0		
	5	0	0	0	0		
C	52	6	0	0	0		
	60	8	0	0	0		
D	1	0	0	0	0		
	0	0	0	0	0		

C: Control

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date
		Recorded by:	4/18/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Calculated Data Date: 4/16/09

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: ~~70min~~ KC 60min

Samples	3	4	5	6	7	8	9	avg count
Control						18	5	4.85E+09
						19	1	
A	0	0	0	0	0			0.00E+00
	0	0	0	0	0			
B	0	0	0	0	0			0.00E+00
	0	0	0	0	0			
C	104	9	1	0	0			3.87E+05
	190	9	2	0	0			
D	0	0	0	0	0			0.00E+00
	0	0	0	0	0			

Samples	log (control)	log (samples)	log reduction
A	9.69	#NUM!	#NUM!
B	9.69	#NUM!	#NUM!
C	9.69	5.59	4.10
D	9.69	#NUM!	#NUM!

KC

Summary

- Log Reduction for samples A, B, D $>10^6$
- Sample C ~~is~~ KC produced a $\log_{10}$ reduction of 4.10
- In this experiment I immediately performed the microbiology analysis after treatment as opposed to Run 4 on pg. 66 where the microbiology analysis was not done until after 1hr and the samples were stored in the refrigerator.
- This experiment will be repeated. and the 10^2 will be plated.

To Page No. ____

Witnessed & Understood by me, _____ Date _____

Invented by: Kimi C. Cooper

Recorded by: _____

Date 4/18/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 70

Date: 4/16/09

Calculated Data - Run 2

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Samples	3	4	5	6	7	8	9	avg count
Control						18	5	4.85E+09
						19	1	
A	5	0	0	0	0			4.50E+03
	4	0	0	0	0			
B	8	0	0	0	0			6.50E+03
	5	0	0	0	0			
C	52	6	0	0	0			1.26E+05
	60	8	0	0	0			
D	1	0	0	0	0			5.00E+02
	0	0	0	0	0			

Samples	log (control)	log (samples)	log reduction
A	9.69	3.65	6.03
B	9.69	3.81	5.87
C	9.69	5.10	4.59
D	9.69	2.70	6.99

KC

Summary

- The avg $\log_{10}$ reduction is 5.85
- Sample C showed a variation of a smaller $\log_{10}$ reduction
- Due to the great variations in the samples the 70min will not be repeated in order to determine the causes for the variations.

To Page No. _

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date
		Recorded by:	4/18/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Date: 4/16/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min Exposure Time

Run 6

Procedure: Refer to pg. 47

Day 1

Calculating Ozone Concentration → See pg. 24 for equations

Analyzer Reading: 6.18% wt

Humidied Concentration: $y = 1.0002(6.21) - 0.2001 (6.18\%) - 0.2001$

$= 6.02\%$ wt 5.99% wt

Actual Concentration: $y = 1.3541(5.99\%) + 0.063$

$= 8.18\%$

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/23/09
		Recorded by:	

Project No. _____
Book No. _____ TITLE _____

From Page No. ____

Raw Data - Run 6

Procedure: See pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316 Date: 4/17/09
Ozone Concentration: 8% (calculated)
Contact Times: 60min

Samples	KC 3 2	KC 4 3	KC 5 4	KC 6 5	KC 7 6	8	9
C-A						15	4
						11	2
A	2+1+0	1	0	0	0		
	0+1+1	0	0	0	0		
B	0+1+0	1	0	0	0		
	0	1	0	0	0		
C	122+118+172	85	0	1	0		
	167+148+63	71	45	1	0		
D	0	0	0	0	0		
	0	1	0	0	0		

Calculated Data - Run 6

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure tim

Coupon Type: stainless steel 316
Ozone Concentration: 8% (calculated) Date: 4/17/09
Contact Times: 60min

Samples	KC 3 2	4 3 KC	KC 5 4	KC 6 5	KC 7 6	8	9	avg c
Control						15	4	4.30
						11	2	
A	2+1+0	1	0	0	0			5.15
	0+1+1	0	0	0	0			
B	0+1+0	1	0	0	0			1.05
	0	1	0	0	0			
C	122+118+172	85	4	1	0			2.63
	167+148+63	71	5	1	0			
D	0	0	0	0	0			5.00
	0	1	0	0	0			

Samples	log (control)	log (samples)	log reduction
A	9.63	3.71	5.92
B	9.63	4.02	5.61
C	9.63	6.42	3.21
D	9.63	3.70	5.93

KC

Summary:
- ~~Avg. Count for~~ KC Average $\log_{10}$ reduction for the samples is 5.17
- This data resembles counts for Run 5 on pg. 71
- The Sample C varied from the other samples, this could be due to vegetative cells.

To Page

Witnessed & Understood by me, Date

Invented by: Kuri C. Cooper Date 4/23/09

Recorded by:

Project No. _____
TITLE _____ Book No. _____
From Page No. ____

Date: 4/~~21~~19/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min exposure time

Run 7

Procedure: Refer to pg. 47

Day 1

Calculating ozone concentration: See pg. 24

Analyzer Reading: 6.08% wt

Humidified Concentration: $y = 1.0002(6.08\%) - 0.2001$
$= 5.89\%$ wt

Actual Concentration: $y = 1.3541(5.89\%) + 0.063$
$= 8.04\%$

Note:
In previous experiments the microbiological analysis have been delayed after treatment and the samples were covered and placed in the refrigerator. To ensure there was no vegetation occurring during this time another sample was added and immediately plated after drying time with no heat shock step.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kim C. Cooper	Date 4/23/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Run 7 - Raw Data Date: 4/20/09

Procedure: See pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated) 4/20/09

Contact Times: 60min

Samples	2	3	4	5	6	7	8	9	10
Control - NHS							20	2	0
							11	2	0
Control - HS							13	2	
							15	3	
A	126+117+128	31	1	0					
	109+98+76	33	6	0					
B	33+54+56	14	1	0					
	36+51+53	8	5	0					
C	2+2+0	5	0	0					
	2+1+0	1	0	0					
D	T	T	89	12					
	T	T	92	3					

Note:
NHS: No Heat Shock
HS: Heat Shock

KC

Calculated Data - Run 7

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposu

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

KC

Samples	2	3	4	5	6	7	8	9
Control-NHS							20	2
							11	2
Control -HS							13	2
							15	3
A	371	31	1	0				
	283	33	6	0				
B	143	14	1	0				
	140	8	5	0				
C	4	5	0	0				
	3	1	0	0				
D	T	T	89	12				
	T	T	92	3				

Samples	log (control)	log (samples)	log reduction
A	9.59	5.00	4.59
B	9.59	4.74	4.84
C	9.59	3.53	6.06
D	9.59	5.22	4.37

Witnessed & Understood by me; Date Invented by: Kuni C. Cooper Date

Recorded by: 4/23/09

Project No. _____

TITLE _____ Book No. _____

From Page No. 76

Date: 4/20/09

Summary

- The average log_{10} reduction is 4.96 for all the samples
- Sample C is showing a greater log reduction of 6.06
- The ozone concentration is lower than the previous experiments that have been run. This may attribute to the lower log_{10} reductions.
- ~~The next experiment will be run to see if the concentration makes a difference.~~ KC
- The controls prove ~~there~~ KC no vegetation occurs when the samples are left in the refrigerator. They both produced a count of 10^9.
- The next experiments will examine the 40min + 50min contact times to determine if the time can be reduced. Also, the 60min contact time will be repeated at a concentration closer to the previous experiments.

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kuri C. Cooper	Date
		Recorded by:	4/23/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Date: 4/20/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min Exposure Time - Run 8

Procedure: Refer to pg. 47

Day 1

Analyzer Reading: 6.11% wt

Humidified Concentration: $y = 1.0002(6.11) - 0.2001$

$y = 5.92\%$ wt

Actual Concentration: $y = 1.3541(5.92) + 0.063$

$= 8.08\%$

Note

After the experiment was completed, the H-1 analyzer began to vary from 0.0% wt. In order to reset the analyzer nitrogen was flush through the system and auto-zeroed. *See manual for zero-calibration. Once the system was calibrated * an error message appeared reading: GAIN RATIO ERROR. See below for explanation.

GAIN RATIO ERROR

Warning condition

Cause: dirtying of the optical components. As the optical components get dirty, the instrument's zero may drift upwards. When a zero calibration is performed, a new zero will be established and the instrument will electronically compensate for the contamination. However, if the soiling of the optics exceed the dynamic range of the electronic compensation , then the "Gain Ratio Error" message will appear. See page 29, "ZEROING THE ANALYZER", for a complete discussion about zero calibration.

Course of Action: press the "E" key to clear the message. If the zero continues to drift, you will need to clean the optical components. Please refer to the chapter on Maintenance. Take steps to ensure that the sample gas is clean to avoid further soiling.

Reference: Models H1, H1-X, and H1-UH High Concentration Process Ozone Analyzers Operating Manual. Pg. 35 "Warning/Error Messages."

*The next step was to clean the cell optics.

To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 4/23/09
		Recorded by:	

From Page No. 78

Cleaning the Cell Optics

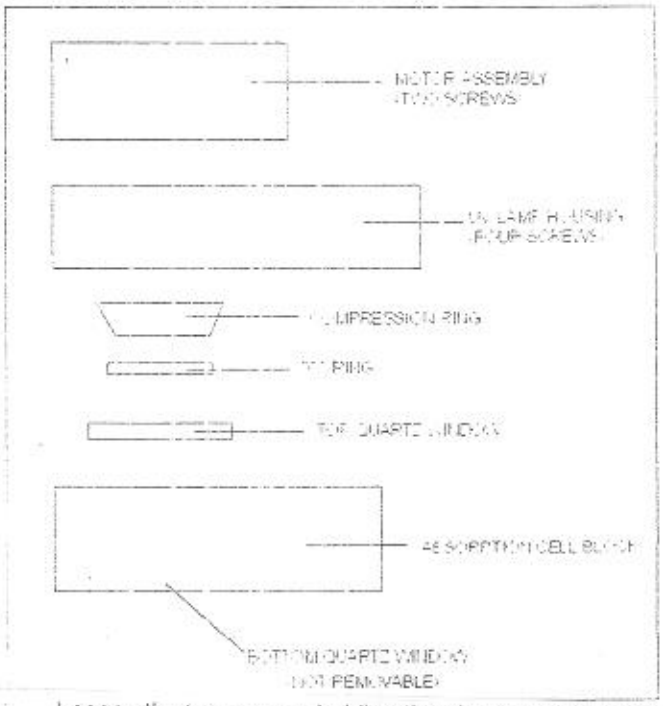
Date: 4/20/09

CAUTION: Handle optical components with care. Use powder less latex gloves and clean only with lint-free materials.

WARNING: This procedure violates the leak tightness of the instrument. Careless reassembly of the Optical Chamber could result in leaks of the gas sample. These leaks can cause damage to components inside the analyzer, pose a health hazard, and cause erroneous ozone measurements. You should leak-test the analyzer prior to resuming its normal use. IN USA, INC. assumes no responsibility and shall be held harmless for problems caused as a result of improper handling of the optical components. The optical chamber and all other gas-tight components of the Model H1 analyzer have been leak tested at the factory to 10^{-5} cc/sec using Helium.

The inside of the Optical Chamber can be cleaned by following the procedure below. Refer to Figure 8: Optical Chamber Assembly below for a description of the components.

Figure 8: Optical Chamber Assembly



1. Loosen the two screws holding the chopper motor assembly and remove the assembly. Make sure not to lose the spacers. Be careful not to loose any of the couplings on the motor's shaft.
2. Unplug the UV lamp from the UV lamp power supply board.
3. Loosen the four screws holding the UV lamp housing and remove the housing.
4. Remove the "O" ring, compression ring and top quartz window. Be careful not to chip or scratch the quartz. Clean this quartz window, using Isopropyl alcohol, and dry it us ng lint-free, clean soft cloth.
5. Clean the bottom quartz window the same way. Note that this window is not removable.
6.

Reference: Models H1, H1-X, and H1-UV High Concentration Process Design Ozone Analyzers Operating Manual. "Periodic Maintenance." Pgs 39-40

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kevin C. Crosse	Date 4/23/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. 79

Raw Data - Run 8

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Date: 4/21/09

Samples	2	3	4	5	6	7	8	9
Control							12	4
							15	1
A	T	213	26	0				
	T	146	23	2				
B	T	T	136	8				
	T	T	164	14				
C	T	T	144	15				
	T	T	128	18				
D	T	T	231	86				
	T	T	218	65				

KC

Calculated Data - Run 8

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Samples	2	3	4	5	6	7	8	9	av
Control							12	4	3.
							15	1	
A	T	213	26	0					5.
	T	146	23	2					
B	T	T	136	8					2.
	T	T	164	14					
C	T	T	144	15					3.
	T	T	128	18					
D	T	T	231	86					9.
	T	T	218	65					

Samples	log (control)	log (samples)	log reduction
A	9.59	5.72	3.87
B	9.59	6.41	3.17
C	9.59	6.48	3.11
D	9.59	6.99	2.59

KC

Summary

- Average $\log_{10}$ reduction is 3.19 for all the samples
- The change is log reduction from the previous experiments could be due to the lower concentration or the need to clean the optical lens on the analyzer.
- This experiment will be repeated with a concentration closer to previous.

To Page No

Witnessed & Understood by me,	Date	Invented by: Kuni C. Ogata	Date 4/23/09
		Recorded by:	

TITLE _____ Project No. _____ Book No. _____

From Page No. ____

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 40min + 60min exposure time.

Procedure: Refer to pg. 47

Day 1

Analyzer Reading: 6.19% wt

Humidified Concentration: $1.0002(6.19) - 0.2001 = y$

$y = 5.99\%$ wt

Actual Concentration: $y = 1.3541(5.99) + 0.063$

$= 8.18\%$ wt

Note

The 40min contact time experiment was completed and the 60min experiment was being started when the H-1 analyzer started varying in concentration between 5.37 - 6.02% wt. The analyzer would not stabalize for an acurate reading so the experiment was aborted. When the buffer tank and analyzer was flushed with nitrogen, an error message of: Negative Results was given. See below for possible explanation.

It is recommended that the UV Lamp be changed under the following conditions:

- After 12 month of continuous operation
- If after the warm-up period following a ZERO CALIBRATION the Analyzer displays the message: "Negative result".
- If the analyzer displays the message: "UV Lamp Error"

Reference: Models H1, H1-X, and H1-UH High Concentration Process Ozone Analyzers Operating Manual. "Periodic Maintenance" Pg. 37.

Result: A new UV lamp will be ordered.

To Page No. ____

nessed & Understood by me,	Date	Invented by: Kusi C. Coop	Date 4/23/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. 81

Raw Data - Run 1

Procedure: See pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 40min exposure

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 40min

Date: 4/22/09

Samples	3	4	5	6	7	8	9
Control					20 TNTC	20	1
					17 TNTC	17 3 TNTC	3
A	T	T	215	26	4		
	T	T	189	26	2		
B	T	T	217	42	7		
	T	T	193	44	24		
C	T	192	55	5	0		
	T	182	39	8	0		
D	T	T	T	140	15		
	T	T	T	156	20		

Calculated Data - Run 1

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 40min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 40min

Samples	3	4	5	6	7	8	9	avg count
Control						20	1	3.85E+09
						17	3	
A	T	T	215	26	4			7.62E+07
	T	T	189	26	2			
B	T	T	217	42	7			2.19E+08
	T	T	193	44	24			
C	T	192	55	5	0			1.31E+07
	T	182	39	8	0			
D	T	T	T	140	15			3.23E+08
	T	T	T	156	20			

Samples	log (control)	log (samples)	log reduction
A	9.59	7.88	1.70
B	9.59	8.34	1.25
C	9.59	7.12	2.47
D	9.59	8.51	1.08

KC

Summary

- Average log10 reduction for all samples: 1.63
- This average is below the minimum log reduction and could be due to the analyzer varia
- This experiment will be repeated with the new UV lamp.

To Page No. ___

Witnessed & Understood by me,	Date	Invented by: Kuni C.	Date 4/23/09
		Recorded by:	

Project No. _____

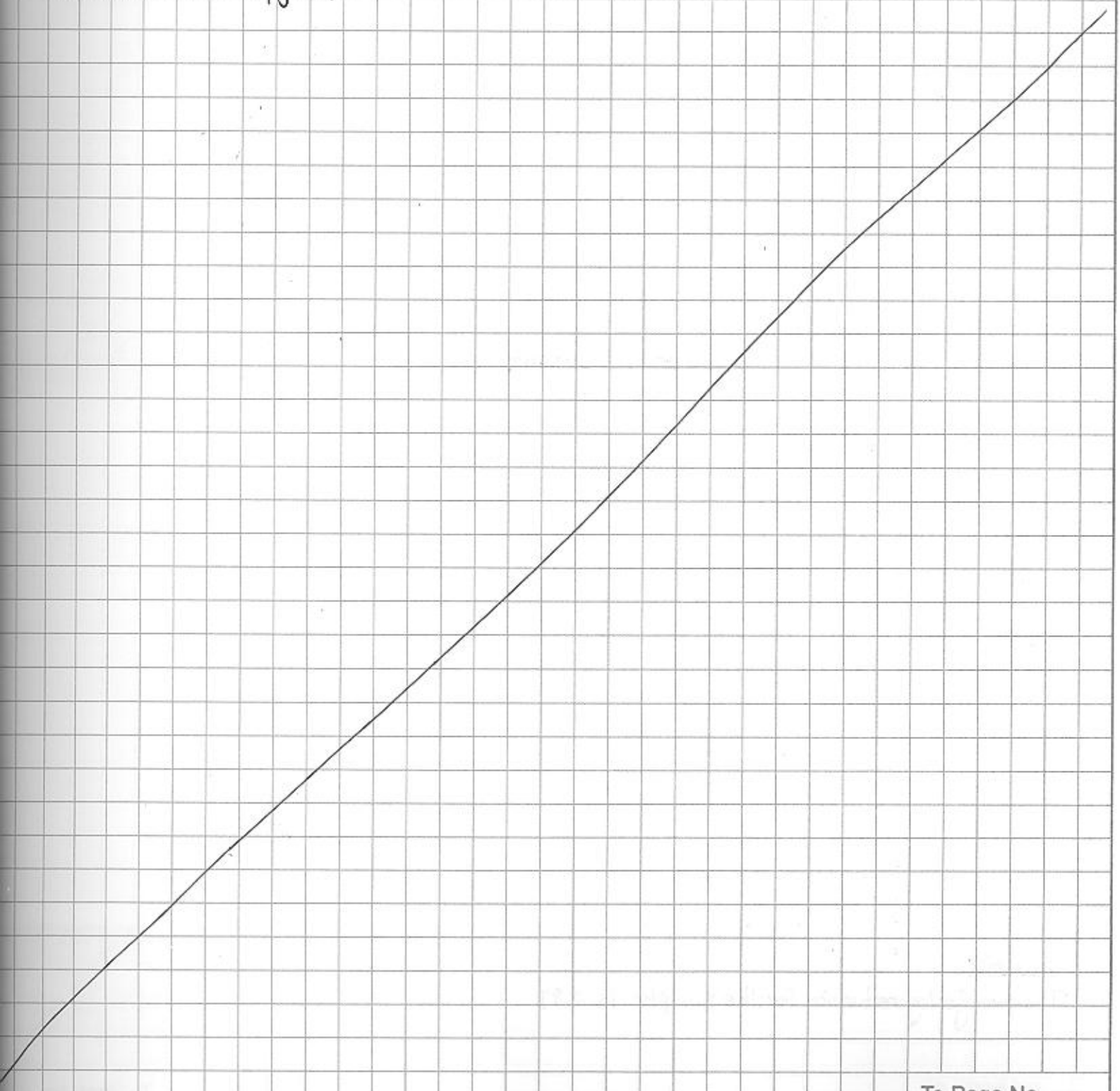
TITLE _____ Book No. _____

From Page No. ____

Date: 5/3/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 60min Exposure Time

Procedure: Refer to pg. 47



To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. 83

Raw Data - Run 9

Procedure pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Date: 5/4/09

Contact Times: 60min

Samples	2	3	4	5	6	7	8	9
Control							18	2
							17	0
A	T	T	T	58				
	T	T	T	62				
B	T	T	104	26				
	T	T	89	27				
C	T	T	89	22				
	T	T	77	22				
D	T	T	98	19				
	T	T	95	21				

Calculated Data - Run 9

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 60min

Samples	2	3	4	5	6	7	8	9	avg cou
Control							18	2	2.75E+0
							17	0	
A	T	T	T	58					6.00E+0
	T	T	T	62					
B	T	T	104	26					3.62E+0
	T	T	89	27					
C	T	T	89	22					3.03E+0
	T	T	77	22					
D	T	T	98	19					2.97E+0
	T	T	95	21					

Samples	log (control)	log (samples)	log reduction
A	9.44	6.78	2.66
B	9.44	6.56	2.88
C	9.44	6.48	2.96
D	9.44	6.47	2.97

Summary

The average log reduction for the samples is 2.87

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 5/4/09
		Recorded by:	

Project No. _____

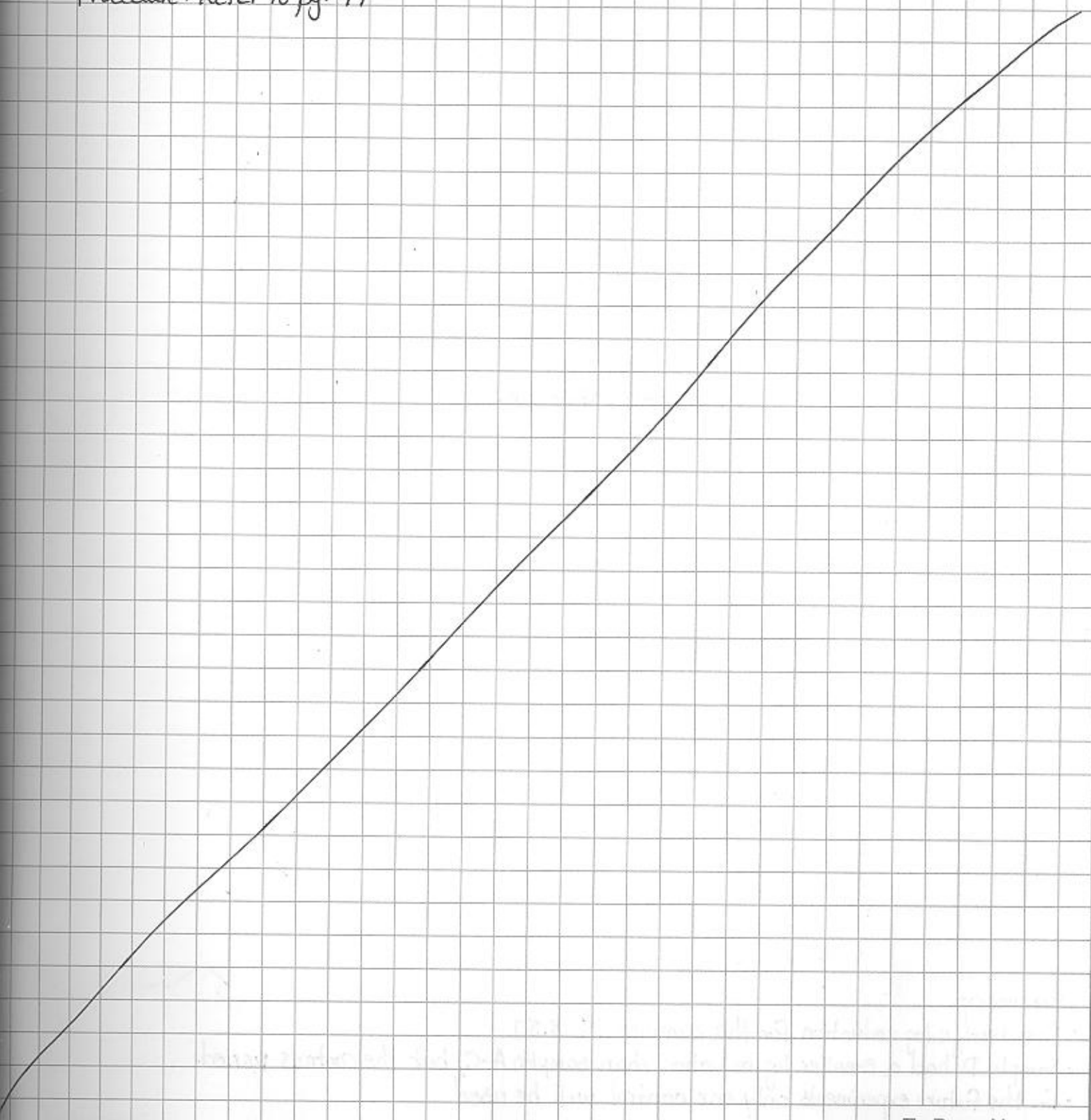
TITLE _____ Book No. _____

From Page No. ____

Date: 5/4/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 70min Exposure Time

Procedure: Refer to pg. 47



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Cao	Date 5/4/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. 85

Date: 5/5/09

Procedure: Refer to pg. 48

Raw Data: Run 3

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Date: 5/5/09

Samples	2	3	4	5	6	7	8	9
Control							21	2
							16	4
A	T	65	6	1				
	T	52	9	1				
B	T	T	123	18				
	T	T	160	22				
C	T	245	18	0				
	T	229	43	2				
D	T	T	221	34				
	T	T	187	38				

Calculated Data - Run 3

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 70min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 70min

Samples	2	3	4	5	6	7	8	9	avg count
Control							21	2	4.85E+09
							16	4	
A	T	65	6	1					2.34E+05
	T	52	9	1					
B	T	T	123	18					3.42E+06
	T	T	160	22					
C	T	245	18	0					6.42E+05
	T	229	43	2					
D	T	T	221	34					5.64E+06
	T	T	187	38					

Samples	log (control)	log (samples)	log reduction
A	9.69	5.37	4.32
B	9.69	6.53	3.15
C	9.69	5.81	3.88
D	9.69	6.75	2.93

KC

Summary

- The average log reduction for the samples is 3.57
- Sample D had a smaller log reduction than samples A-C, but the controls varied.
- In the future experiments only one control will be used

To Page No. _____

Witnessed & Understood by me,	Date	Invented by: Kimi C-Cage	Date 5/5/09
		Recorded by:	

Project No. _____

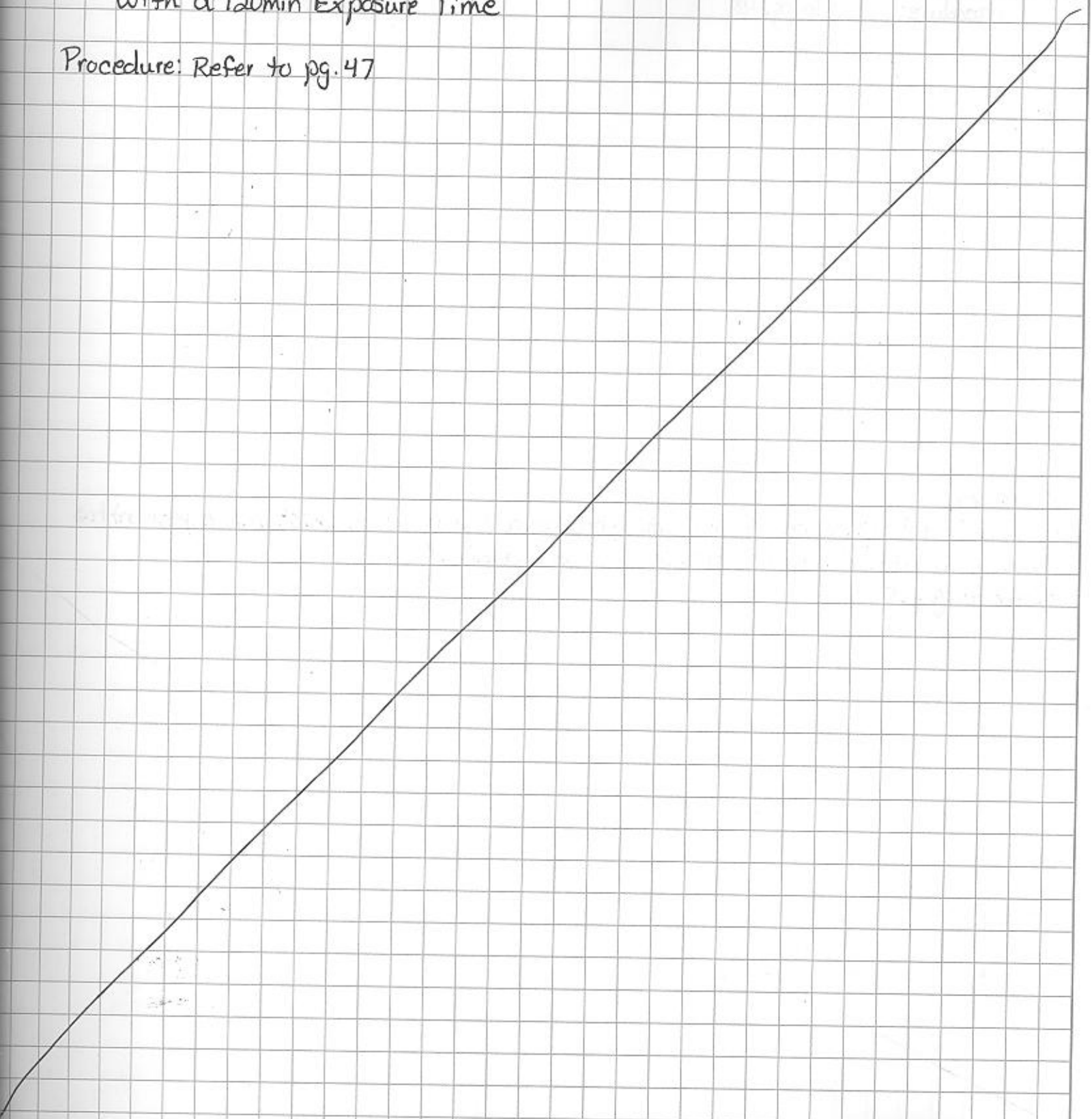
TITLE _____ Book No. _____

From Page No. ____

Date: 5/13/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 120min Exposure Time

Procedure: Refer to pg. 47



To Page No. ____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. 87

Raw Data - Run 3

Procedure: Refer to pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 120min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 120min

Date: 5/14/09

Samples	2	3	4	5	6	7	8	9
Control							17	1
A	0	0	0	0			23	1
	0	0	0	0				
B	0	0	0	0				
	0	0	0	0				
C	0	0	0	0				
	0	0	0	0				
D	0	0	0	0				
	0	0	0	0				

Summary

- The effect of ~~of~~ 8% O_3 on BA for 120min produces a $1 \log_{10}$ reduction $>10^6$. This experiment has been run and repeated 3 times producing the same data. ~~the~~
- Refer to pgs. 68

To Page No. _

Witnessed & Understood by me,	Date	Invented by: Kui C. Cao	Date 5/14/09
		Recorded by:	

Project No. _____

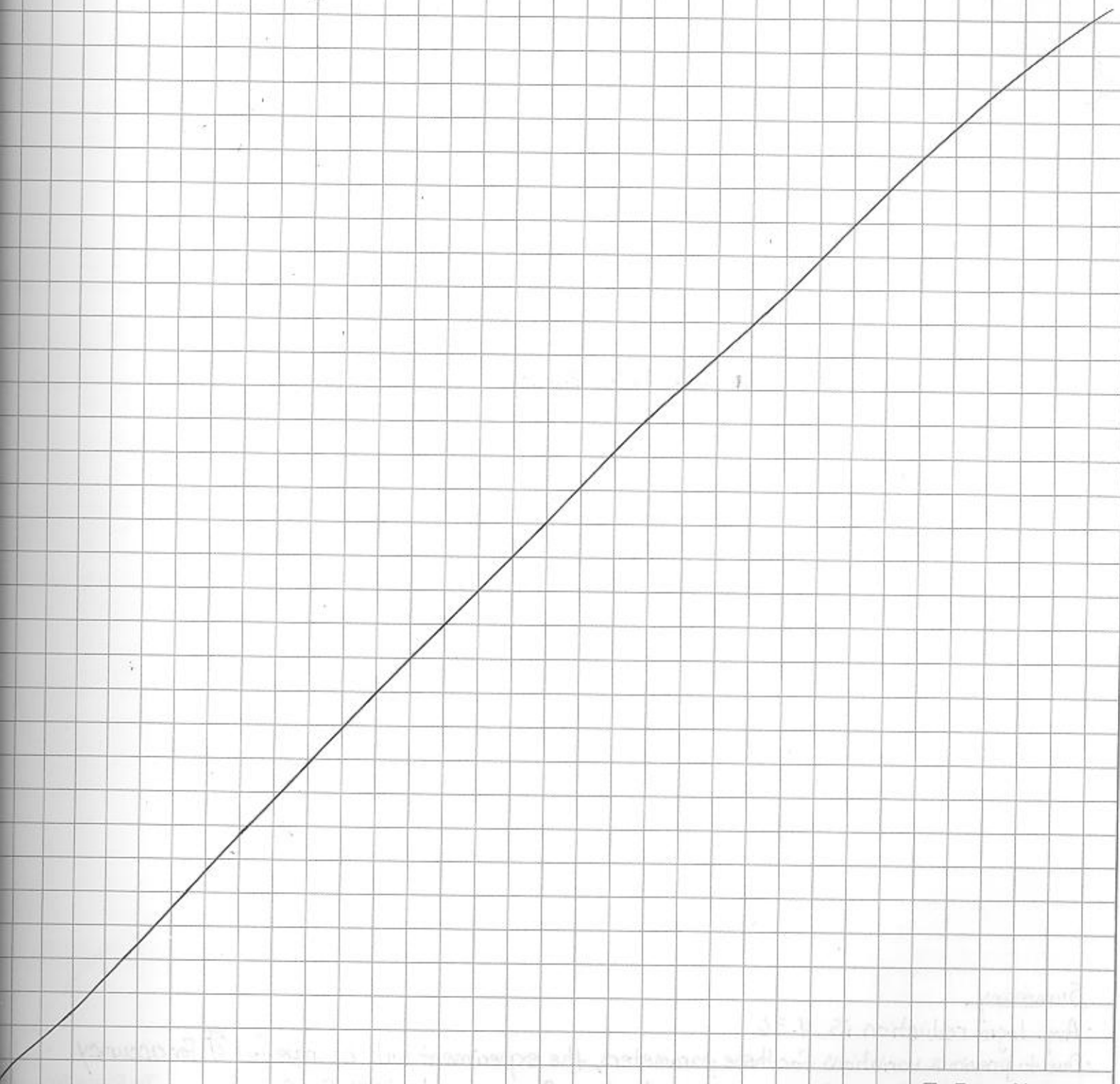
TITLE _____ Book No. _____

From Page No. ____

Date: 5/13/09

Determination of the Effect of 8% Ozone on Bacillus anthracis (sterne) Strain with a 90min Exposure Time

Procedure: Refer to pg. 47



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 5/14/09
		Recorded by:	

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Raw Data - Run3

Procedure: Refer to pg. 48

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 90min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 90min

Date: 5/14/09

Samples	2	3	4	5	6	7	8	9
Control							17	1
							23	1
A	111	11	1					
	115	12	2					
B	T	156	31					
	T	161	30					
C	151	22	2					
	163	21	2					
D	T	145	13					
	T	119	18					

Calculated Data - Run3

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 90min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 8% (calculated)

Contact Times: 90min

Samples	2	3	4	5	6	7	8	9	avg count
Control							17	1	3.00E+09
							23	1	
A	111	11	1						3.78E+04
	115	12	2						
B	T	156	31						4.64E+05
	T	161	30						
C	151	22	2						5.72E+04
	163	21	2						
D	T	145	13						2.87E+05
	T	119	18						

Samples	log (control)	log (samples)	log reduction
A	9.48	4.58	4.90
B	9.48	5.67	3.81
C	9.48	4.76	4.72
D	9.48	5.46	4.02

Summary

- Avg. log10 reduction is 4.36
- Due to previous variations for these parameters, the experiment will be repeated 4x for accuracy
- Titrations will be done in following experiments to confirm concentrations for O_3.

To Page No. ____

Witnessed & Understood by me, _____ Date _____

Invented by: Kuni C. Cag

Recorded by: _____

Date 5/14/09

Project No. _____

TITLE _____ Book No. _____

From Page No. ____

Date: 5/14/09

Determination of the Effect of 7% Ozone on Bacillus anthracis (sterne) Strain with a 90min Exposure Time

Procedures: Titrations. Refer to pg. 9

Ozone Parameters - Refer to pg. 47

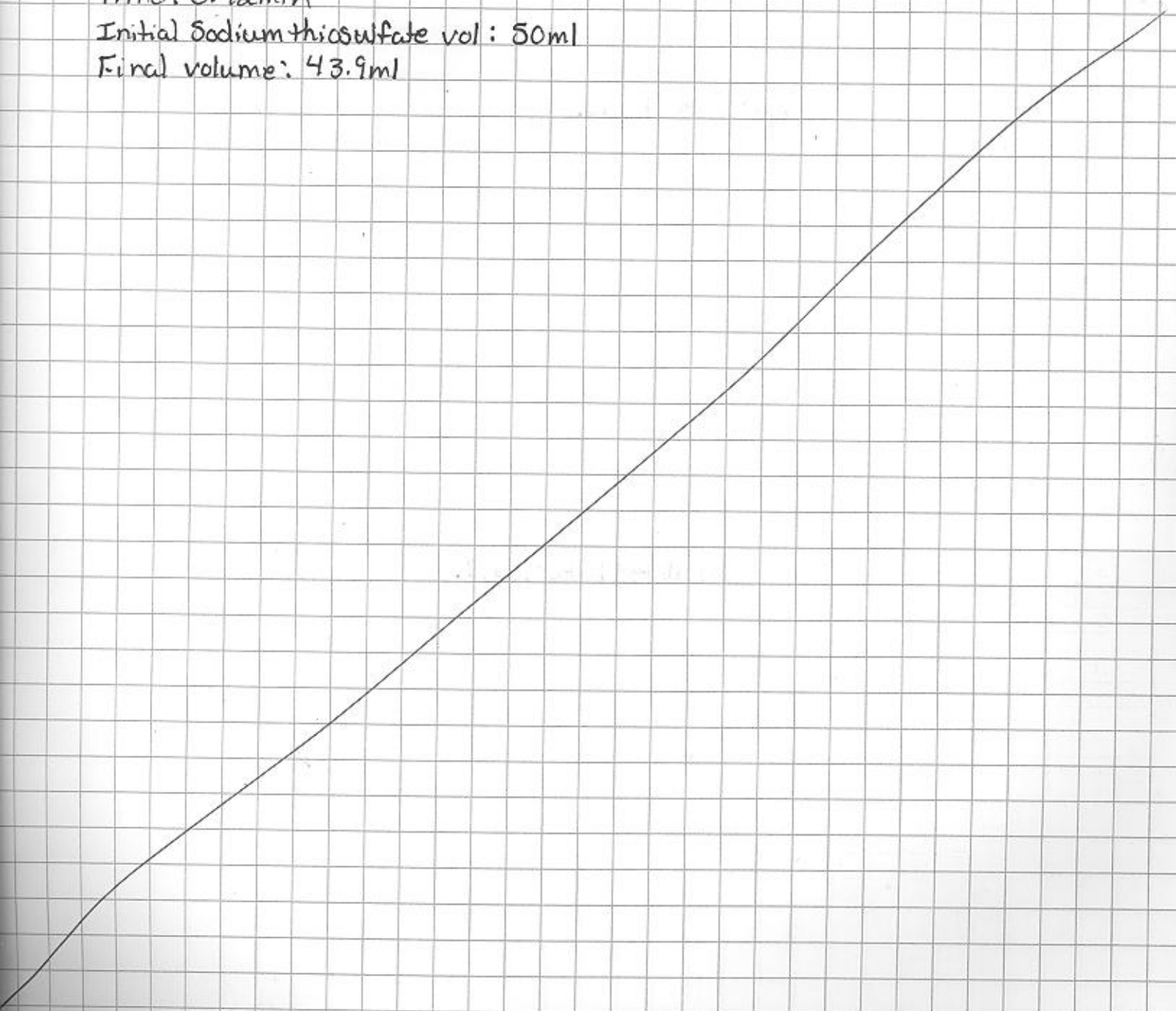
Titration Data

Ozone Flow Rate: 1.71 L/min

Time: 0.42min

Initial Sodium thiosulfate vol: 50ml

Final volume: 43.9ml



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper Recorded by:	Date 5/14/09

Project No. _____

Book No. _____ TITLE _____

From Page No. 91

Calculated Ozone Concentration

Procedure: Refer to pg. 9

KC

Analyzer Concentration (%wt)	Ozone Flow Rate (L/min)	Time (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	%
8.21	1.71	0.42	50.0	43.9	1.02E-03	3.05E-04	3.05E-04	163	4.97E-02	6.

Raw Data - Run 1

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 90min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 90min

Date: 5/15/09

Samples	2	3	4	5	6	7	8	9
Control							17	1
							23	1
A	T	111	11	1				
	T	115	12	2				
B	T	T	156	31				
	T	T	161	30				
C	T	151	22	2				
	T	163	21	2				
D	T	T	145	13				
	T	T	119	18				

KC

Calculated Data - Run 1

Determination of the Effect of 8% Ozone on *Bacillus anthracis* (sterne) Strain with a 90min exposure time

Coupon Type: stainless steel 316 KC

Ozone Concentration: ~~8% (calculated)~~ 7%

Contact Times: 90min

Samples	2	3	4	5	6	7	8	9	avg count
Control							17	1	3.00E+09
							23	1	
A	T	111	11	1					3.78E+05
	T	115	12	2					
B	T	T	156	31					4.64E+06
	T	T	161	30					
C	T	151	22	2					5.72E+05
	T	163	21	2					
D	T	T	145	13					2.87E+06
	T	T	119	18					

KC

To Page No. _____

Witnessed & Understood by me,	Date	Invented by:	Date
		Recorded by:	

Project No. _____

TITLE _____ Book No. _____

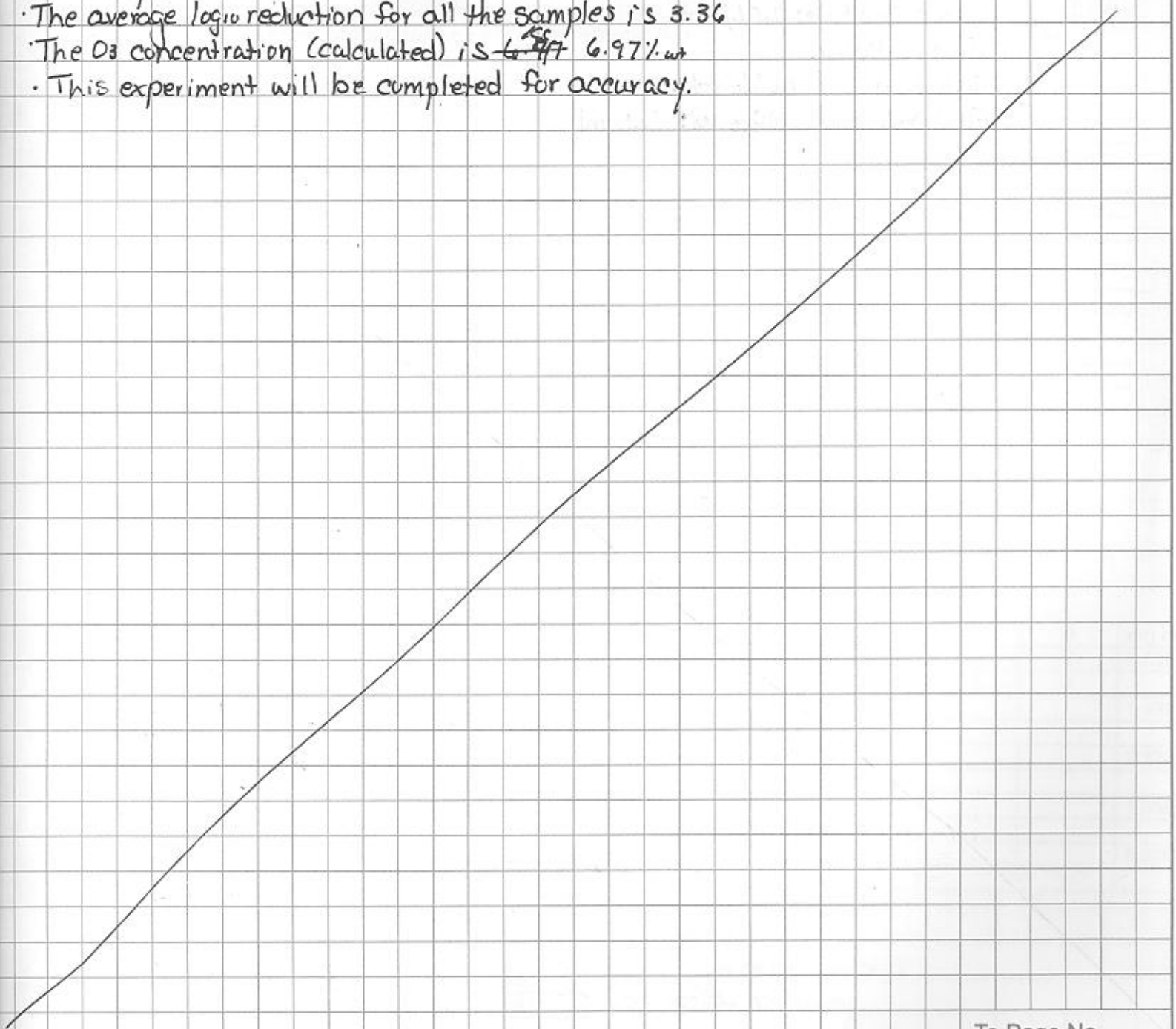
From Page No. 92

Samples	log (control)	log (samples)	log reduction
A	9.48	5.58	3.90
B	9.48	6.67	2.81
C	9.48	5.76	3.72
D	9.48	6.46	3.02

KC

Summary

- The average $\log_{10}$ reduction for all the samples is 3.36
- The O_3 concentration (calculated) is ~~6.97~~ 6.97% wt
- This experiment will be completed for accuracy.



To Page No. _____

Witnessed & Understood by me,

Date

Invented by: Kuni C. Ogawa

Recorded by:

Date 5/15/09

Project No. _____

Book No. _____ TITLE _____

From Page No. ____

Date: 5/19/09

Determination of 7% ~~Effec~~ Ozone Effect on Bacillus anthracis (sterne) Strain with a 120min Exposure Time

Procedures: Titration - Refer to pg 9
Ozone Parameters - Refer to pg. 47

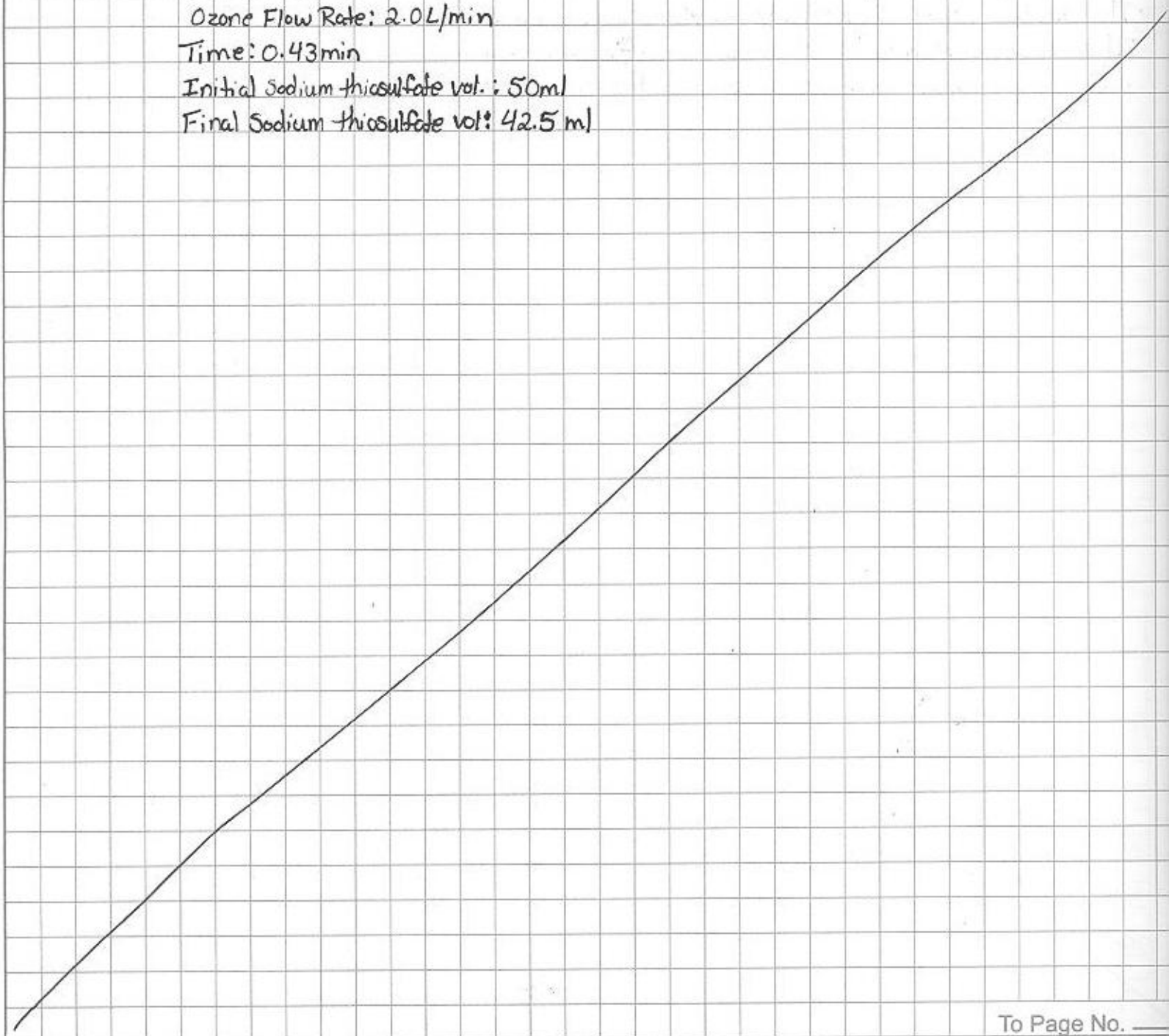
Titration Data

Ozone Flow Rate: 2.0L/min

Time: 0.43min

Initial Sodium thiosulfate vol.: 50ml

Final Sodium thiosulfate vol: 42.5 ml



To Page No. ____

Witnessed & Understood by me,	Date	Invented by: Kuni C. Ooaye Recorded by:	Date 5/20/09

Project No. _____

TITLE _____ Book No. _____

From Page No. 94

Calculated O_3 Concentration

Ozone Flow Rate (L/min)	Time (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I_2	mol I_2	mol O_3	Molar Volume O_3	Volume O_3	% O_3
2.0	0.43	50.0	42.5	1.25E-03	3.75E-04	3.75E-04	163	6.11E-02	7.05

Raw Data - Run 1

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 120min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 120min

Date: ~~5/19/09~~ 5/20/09 KC

Samples	2	3	4	5	6	7	8	9
Control								
A	370	66	7	0				
	247	61	4	0				
B	79	7	0	0				
	74	14	1	0				
C	247	42	3	0				
	283	44	4	0				
D	43	2	0	0				
	65	5	0	0				

Calculated Data - Run 1

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 120min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 120min

Samples	2	3	4	5	6	7	8	9	avg count
Control							18	6	6.55E+09
							13	4	
A	370	66	7	0					1.49E+05
	247	61	4	0					
B	79	7	0	0					2.32E+04
	74	14	1	0					
C	247	42	3	0					1.05E+05
	283	44	4	0					
D	43	2	0	0					8.90E+03
	65	5	0	0					

Samples	log (control)	log (samples)	log reduction
A	9.82	5.17	4.64
B	9.82	4.36	5.45
C	9.82	5.02	4.80
D	9.82	3.95	5.87

Summary

- Avg. log10 reduction 5.19
- Repeat for accuracy

To Page No. _____

Witnessed & Understood by me, _____ Date _____

Invented by: Kimi C. Dogo

Recorded by: _____

Date 5/20/09