NOTEBOOK NO. 106
ISSUED TO Kerri Cooper
ON April 1st 20 08
DEPARTMENT
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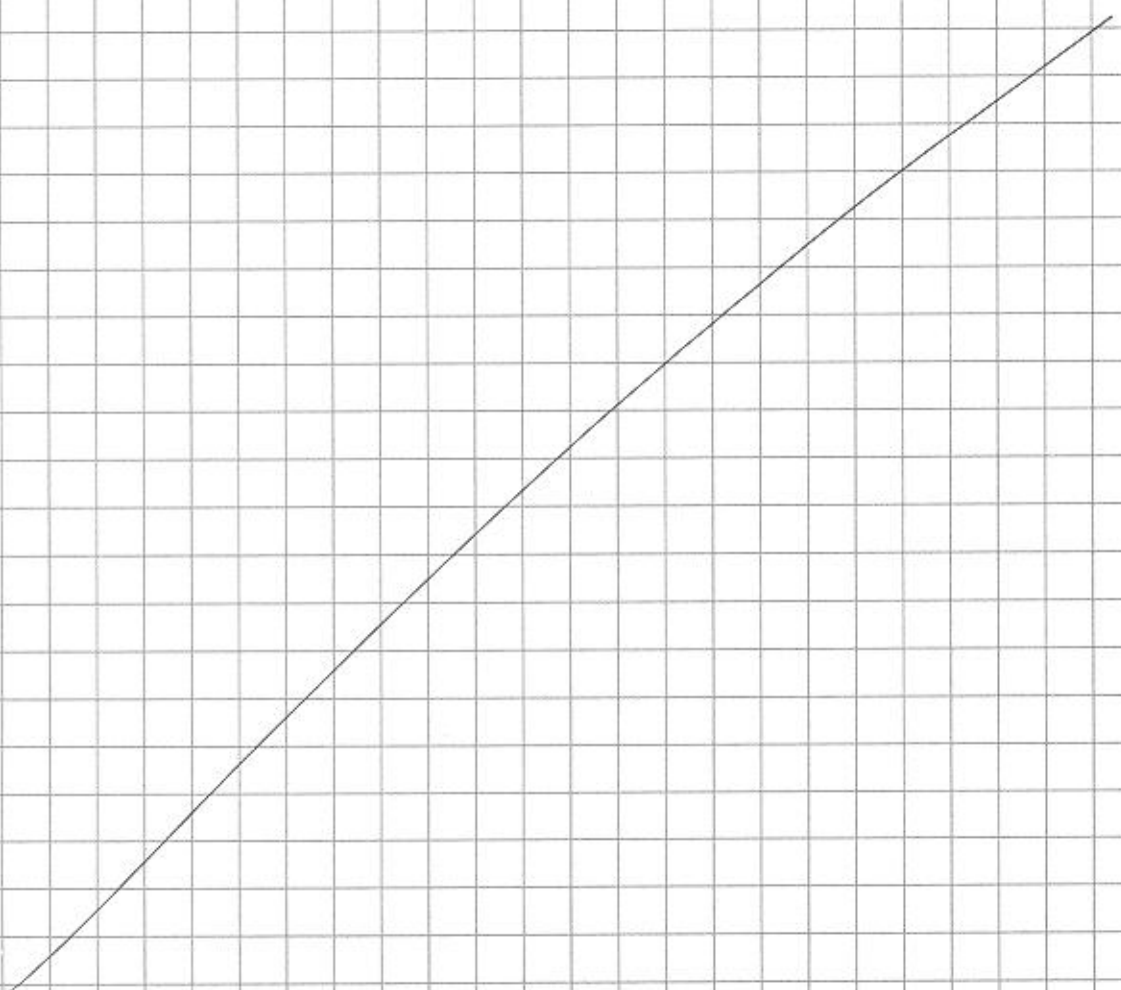
Abbreviations



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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)

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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.

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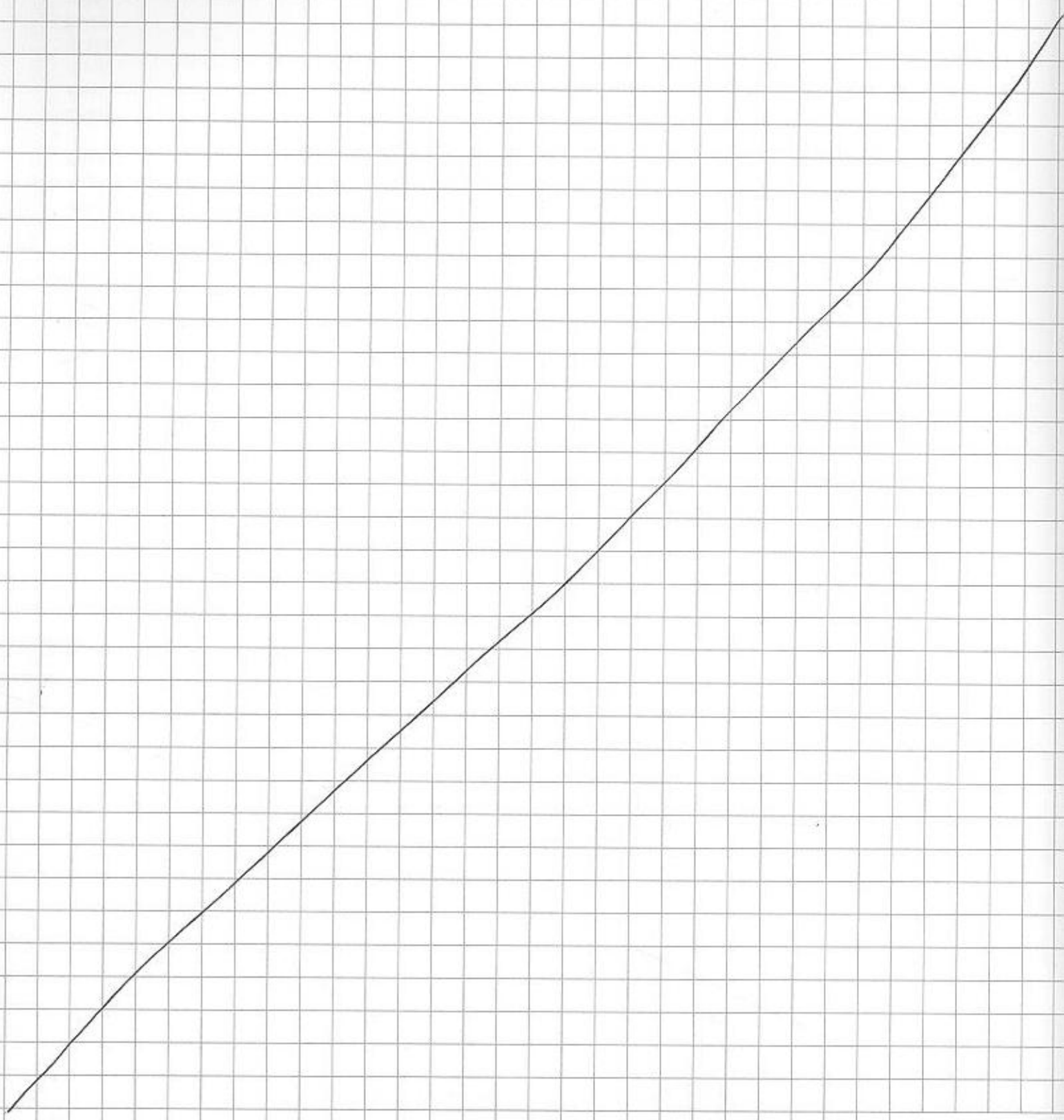
The information in this lab notebook follows the previous data collected in lab notebook number 107. For more information refer to that lab notebook.



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Witnessed & Understood by me,	Date	Invented by: Kuri C. Cooper	Date 5/20/09
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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

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5.2.6 Incubate for 24hrs at 32°C

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

6.0 References and Supporting Documents

1. Buranov, S.N., V.I. Karelin, V.D. Selemir, O.N. Vorobieva, A.S. Tsareva, V.V. Shkarin. "Sterilizing Effect of Ozone on Live Spores of Anthrax Bacillus." Pulsed Power Conference, 2005 IEEE. June. 1416-1417.

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

3. Oxygen Material Safety Data Sheet (MSDS).

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>.

6. Rice, R.G. "Ozone and Anthrax-Knowns and Unknowns." Ozone Science & Engineering. 2002. 24: 151-158

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

Procedures: Refer to pg. 5

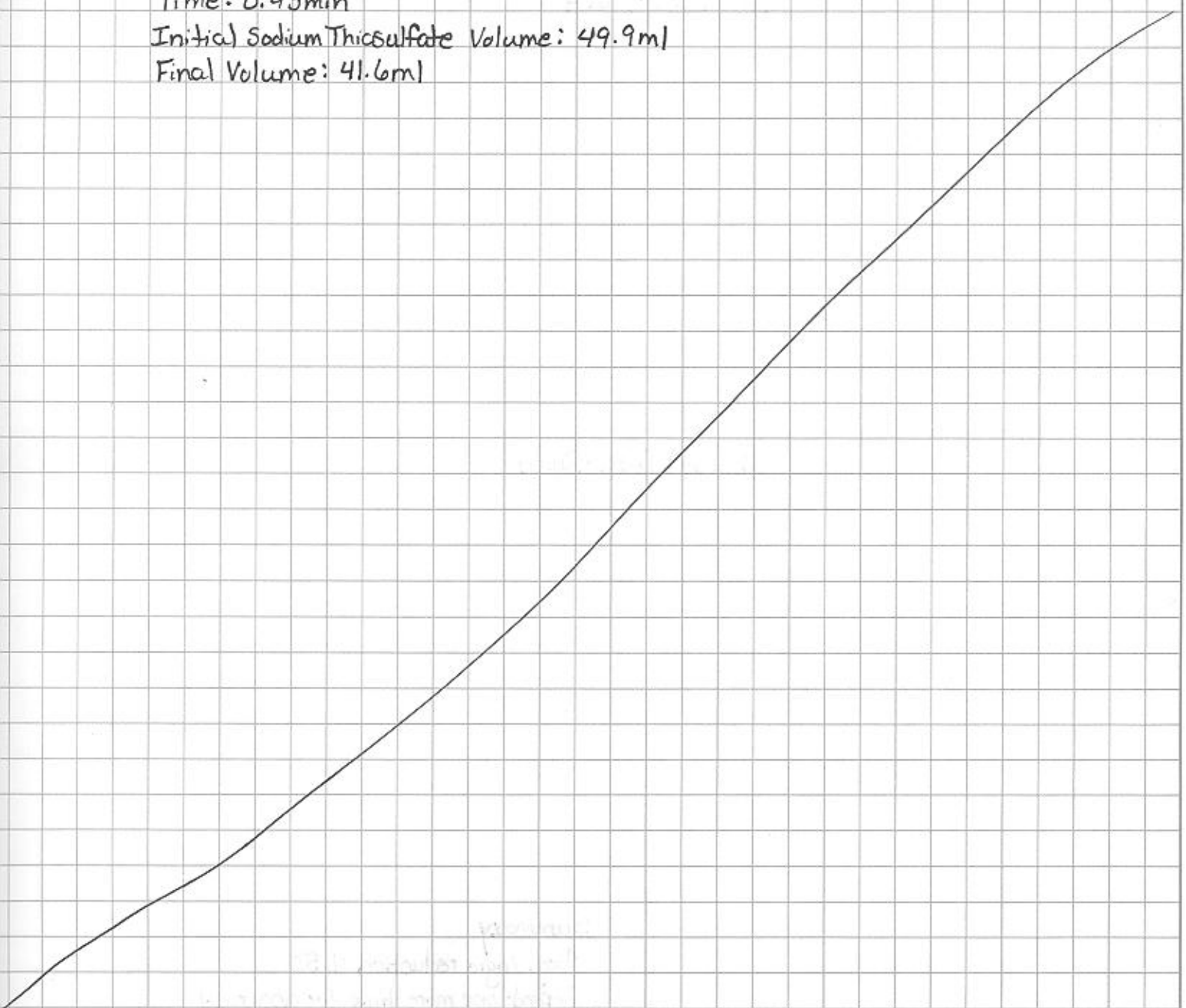
Titration Data

Ozone Flow Rate: 2.0L/min

Time: 0.43min

Initial Sodium Thiosulfate Volume: 49.9ml

Final Volume: 41.6ml



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KC Titration Calculation

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	49.9	41.6	1.38E-03	4.15E-04	4.15E-04	163	6.76E-02	7.80

Raw Data - Run 4

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: 5/21/09

KC

Samples	2	3	4	5	6	7	8	9
Control							3	2
							7	0
A	94	8	0	0				
	54	6	0	0				
B	109	14	2	0				
	107	11	1	0				
C	185	78	5	0				
	233	KC 79 49	6	0				
D	110	9	1	0				
	154	7	1	0				

Calculated Data - Run 4

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

KC

Samples	2	3	4	5	6	7	8	9	avg count
Control							3	2	1.50E+09
							7	0	
A	94	8	0	0					1.44E+04
	54	6	0	0					
B	109	14	2	0					3.83E+04
	107	11	1	0					
C	185	78	5	0					1.39E+05
	233	49	6	0					
D	110	9	1	0					3.12E+04
	154	7	1	0					

Samples	log (control)	log (samples)	log reduction
A	9.18	4.16	5.02
B	9.18	4.58	4.59
C	9.18	5.14	4.03
D	9.18	KC 4.49	4.68

Summary

- Avg. Log10 reduction 4.58
- Repeat one more time for accuracy
- O_3 concentration (calculated) 7.80%

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Date: 5/21/09

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

Procedure: Refer to pg. 5

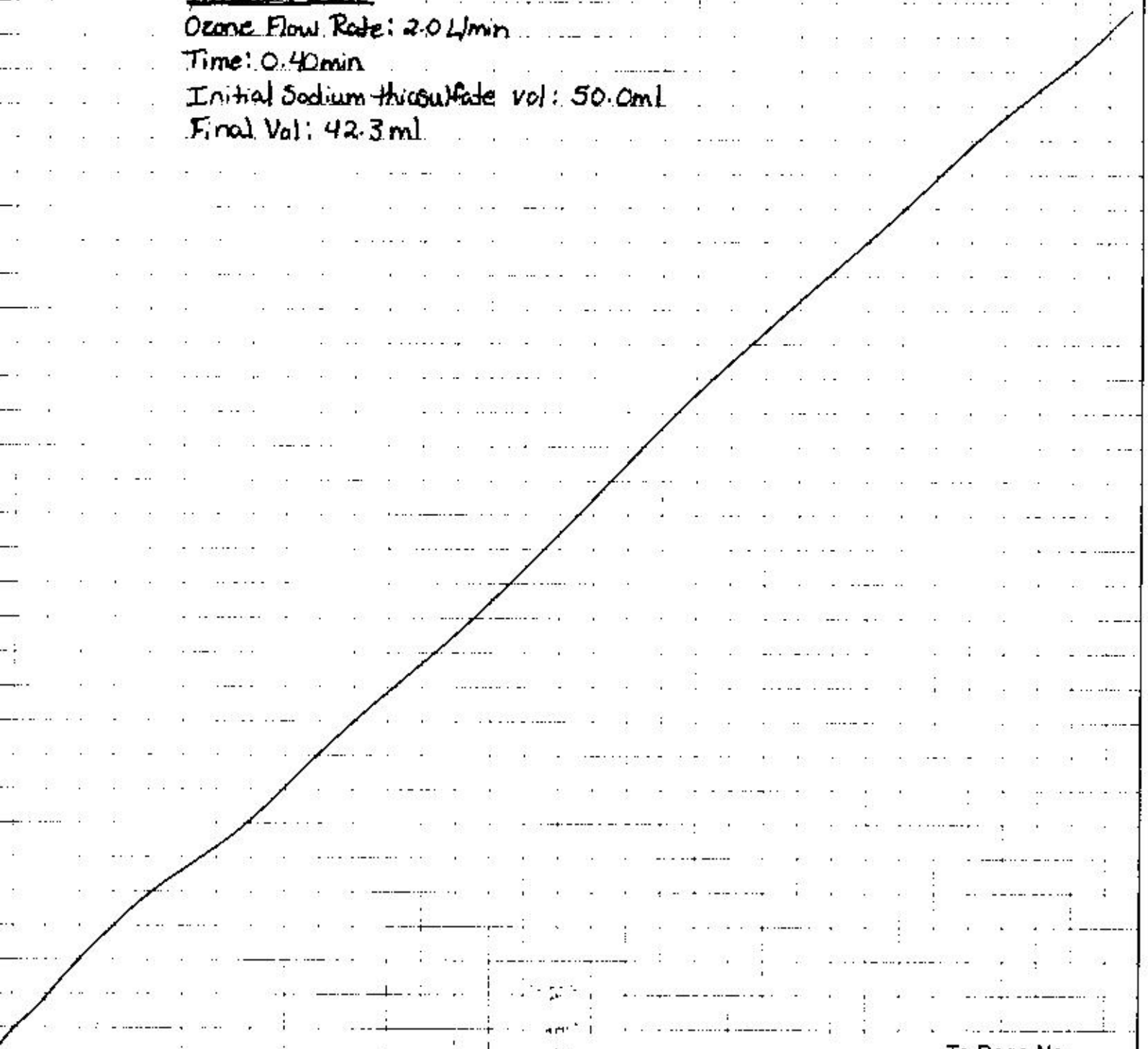
Titration Data

Ozone Flow Rate: 2.0 L/min

Time: 0.40min

Initial Sodium thiosulfate vol: 50.0ml

Final Vol: 42.3ml



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ZC Titration Data (calculated)

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	0.40	50.0	42.3	1.28E-03	3.85E-04	3.85E-04	163	6.27E-02	7.84

Raw Data - Run 5

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: 5/22/09

Samples	2	3	4	5	6	7	8	9
Control								
				ZC				
A	104	29	3	0				
	114	37	1	6				
B	273	68	8	0				
	257	66	5	0				
C	221	83	8	1				
	212	63	6	2				
D	157	18	1	6				
	141	10	1	0				

Calculated Data - Run 5

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 cou
Control							7	6	4.80E+0
							9	2	
A	104	29	3	0					6.39E+0
	114	37	1	0					
B	273	68	8	0					1.59E+0
	257	66	5	0					
C	221	83	8	1					3.15E+0
	212	63	6	2					
D	157	18	1	0					3.89E+0
	141	10	1	0					

Samples	log (control)	log (samples)	log reduction
A	9.68	4.81	4.88
B	9.68	5.20	4.48
C	9.68	5.50	4.18
D	9.68	4.59	5.09

Summary

- Avg. $\log_{10}$ reduction is 4.66
- The O_3 concentration is 7.84% wt
- The log reductions for 8% concentration with 90mi contact time ranges from 4.36 - 4.66

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Witnessed & Understood by me,	Date	Invented by: Ruiz C. Cox	Date
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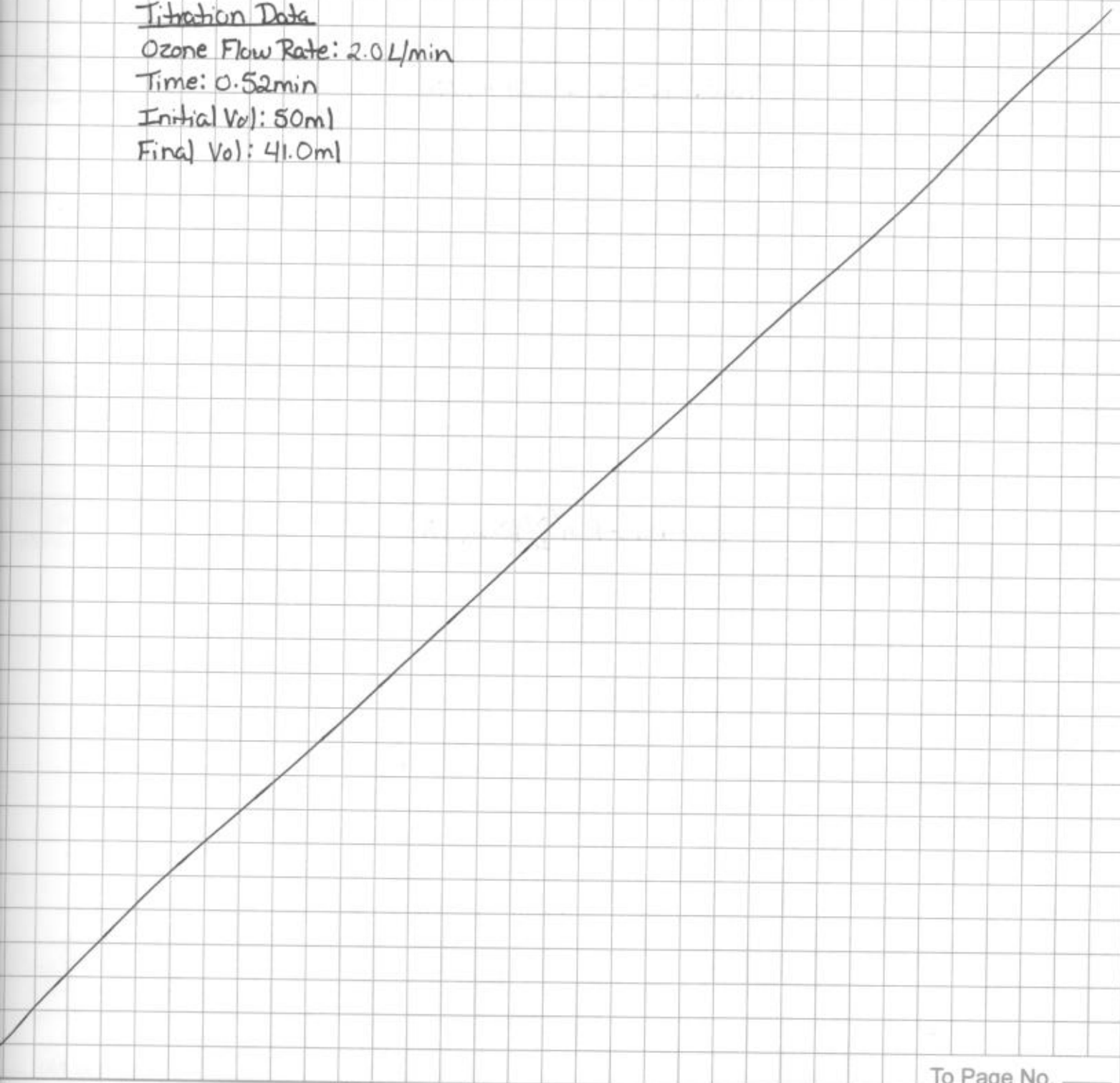
Date: 6/1/09

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

Procedure: Refer to pg. 5

Titration Data

Ozone Flow Rate: 2.0 L/min
Time: 0.52min
Initial Vol: 50ml
Final Vol: 41.0ml



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Calculated Ozone Conc.

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	% O3
2	0.52	50.0	41.0	1.50E-03	4.50E-04	4.50E-04	163	7.33E-02	7.09

Raw Data - Run 2 (120min, 7%)

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: ~~6/1/09~~ KC 6/2/09

Samples	2	3	4	5	6	7	8	9
Control							7	2
							11	1
A	90	13	0					
	95	15	1					
B	172	48	7					
	132	25	5					
C	21	5	0					
	24	3	1					
D	49	12	2					
	65	10	2					

Raw Data - Run 3 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 60min

Date: ~~6/1/09~~ KC 6/2/09

Samples	2	3	4	5	6	7	8	9
Control							7	2
							11	1
A				153	38	7	0	
				172	30	6	1	
B				T	142	46	1	
				T	140	37	0	
C				106	7	1	0	
				87	9	1	0	
D				83	6	1	0	
				102	12	1	0	

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Calculated Data - Run 2 (120, 7%)

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							7	2	2.40E+09
							11	1	
A	90	13	0						2.33E+04
	95	15	1						
B	172	48	7						8.49E+04
	132	25	5						
C	21	5	0						1.13E+04
	24	3	1						
D	49	12	2						3.67E+04
	65	10	2						

Samples	log (control)	log (samples)	log reduction
A	9.38	4.37	5.01
B	9.38	4.93	4.45
C	9.38	4.05	5.33
D	9.38	4.56	4.82

Summary

- Avg $\log_{10}$ reduction 4.90
- Data correlates to past experiment of 5.19
- Will repeat for third time
- O_3 concentration 7.09%

Calculated - Run 1 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 60min

Samples	3	4	5	6	7	8	9	avg count
Control						7	2	2.40E+09
						11	1	
A			153	38	7	0		1.65E+08
			172	30	6	1		
B			T	142	46	1		6.06E+08
			T	140	37	0		
C			106	7	1	0		2.77E+07
			87	9	1	0		
D			83	6	1	0		2.83E+07
			102	12	1	0		

Samples	log (control)	log (samples)	log reduction
A	9.38	8.22	1.16
B	9.38	8.78	0.60
C	9.38	7.44	1.94
D	9.38	7.45	1.93

1.406879

Summary

- Avg. $\log_{10}$ reduction 1.41
- Will repeat 2 more times for accuracy

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Date: 6/2/09

Determination of the Effect of 7% Ozone on Bacillus anthracis (Sterne) Strain for Various Contact Times

Procedure: Refer to pg. 5

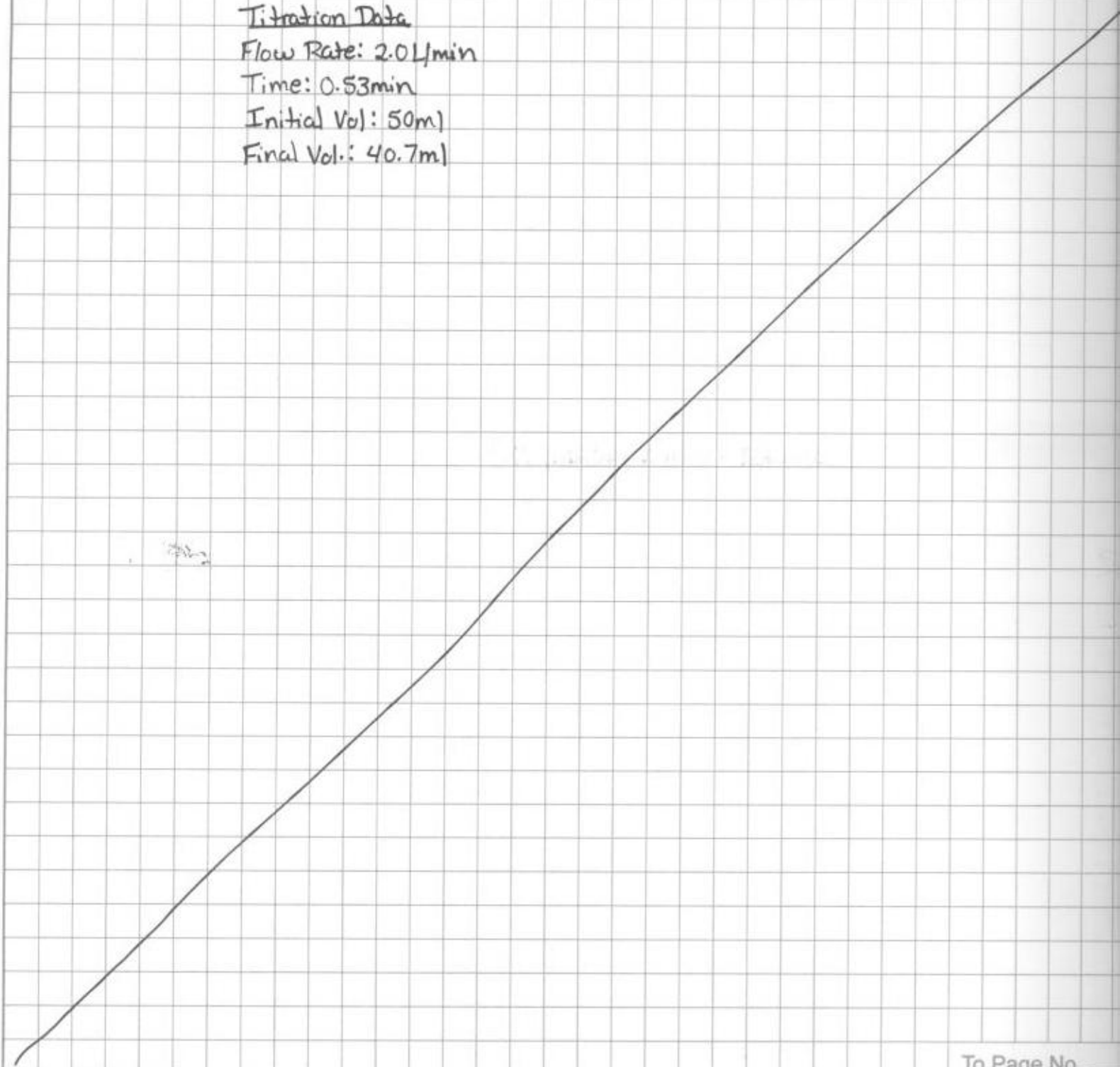
Titration Data

Flow Rate: 2.0 L/min

Time: 0.53 min

Initial Vol: 50 ml

Final Vol.: 40.7 ml



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Ozone Concentration Calculation

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	% O3
2	0.53	50.0	40.7	1.55E-03	4.65E-04	4.65E-04	163	7.58E-02	7.10

Raw Data - Run 2 (90min, 7%)

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: ~~6/2/09~~ 6/3/09

Samples	~~KC23~~	~~KC34~~	~~KC45~~	~~KC56~~	6	7	8	9
Control							22	1
							13	2
A	196	87	10	1				
	158	73	11	2				
B	T	173	29	4				
	T	182	41	3				
C	201	42	6	0				
	199	35	13	1				
D	168	67	5	0				
	142	86	4	1				

Raw Data - Run 2 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 60min

Date: ~~6/2/09~~ 6/3/09

Samples	2	3	4	5	6	7	8	9
Control							22	1
							13	2
A		T	T	212	62	5		
		T	T	183	66	5		
B		T	T	156	40	7		
		T	T	131	54	15		
C		T	T	119	27	2		
		T	T	113	27	1		
D		T	T	215	68	14		
		T	T	211	87	9		

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Invented by: Kuni C. Cooper

Recorded by: _____

Date 6/3/09

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Calculated Data - Run 2 (90min, 7%)

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								
Samples	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**avg count**
Control						22	1	3.25E+09
						13	2	
A	190	87	10	1				3.16E+06
	158	73	11	2				
B	T	173	29	4				8.78E+06
	T	182	41	3				
C	201	42	6	0				2.04E+06
	199	35	13	1				
D	168	67	5	0				1.87E+06
	142	86	4	1				

Samples	log (control)	log (samples)	log reduction
A	9.51	6.50	3.01
B	9.51	6.94	2.57
C	9.51	6.31	3.20
D	9.51	6.27	3.24

Summary KC

- Avg $\log_{10}$ ~~reduction~~ reduction: 3.01
- Will repeat for accuracy. Previous run produced 3.36 $\log_{10}$ reduction (Book #107, pg 93)

Calculated Data - Run 2 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time								
Coupon Type: stainless steel 316								
Ozone Concentration: 7% (calculated)								
Contact Times: 60min								
Samples	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**avg count**
Control						22	1	3.25E+09
						13	2	
A	T	T	212	62	5			1.09E+08
	T	T	183	66	5			
B	T	T	156	40	7			1.71E+08
	T	T	131	54	15			
C	T	T	119	27	2			5.36E+07
	T	T	113	27	1			
D	T	T	215	68	14			2.14E+08
	T	T	211	87	9			

Samples	log (control)	log (samples)	log reduction
A	9.51	8.04	1.47
B	9.51	8.23	1.28
C	9.51	7.73	1.78
D	9.51	8.33	1.18

1.429163

Summary

- Avg. log reduction 1.43
- Correlates with previous reduction of 1.41 (pg 13)
- O_3 concentration 7.10

To Page No

Witnessed & Understood by me,	Date	Invented by: Kimi C. Cooper	Date 6/3/09
		Recorded by:	

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Determination of 7% Ozone Concentration on Bacillus anthracis (sterne) Strain for Various Times

Procedure: Refer to pg. 5

Titration Data

Flow Rate: 2.0L/min

Time: 0.40min

Initial Vol: 50ml

Final Vol: ~~7.75~~ 43.0ml

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Witnessed & Understood by me,	Date	Invented by: / Recorded by:	Date
[signature]		[signature] Kimi C. Cooper	6/4/09

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Titration Calculation

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	% O3
2	0.40	50.0	43.0	1.17E-03	3.50E-04	3.50E-04	163	5.70E-02	7.13

Raw Data - Run 3 (90min, 7%)

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: ~~6/3/09~~ 6/4/09

Samples	2	3	4	5	6	7	8	9
Control							12	3
							12	2
A		T	112	35				
		T	117	12				
B		T	122	17				
		T	102	12				
C		T	158	13				
		T	152	8				
D		T	190	38				
		T	136	19				

Raw Data - Run 3 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time

Coupon Type: stainless steel 316

Ozone Concentration: 7% (calculated)

Contact Times: 60min

Date: ~~6/3/09~~ 6/4/09

Samples	2	3	4	5	6	7	8	9
Control							12	3
							12	2
A		T	T	172	86			
		T	T	187	52			
B		T	T	199	58			
		T	T	189	34			
C		T	T	315	115			
		T	T	283	43			
D		T	T	233	87			
		T	T	210	104			

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Witnessed & Understood by me,	Date	Invented by: Kui C. Cox	Date 6/4/09
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Calculated Data - Run 3 (90min, 7%)

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								
Samples	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**avg count**
Control						12	3	3.70E+09
						12	2	
A	T	112	35					3.50E+06
	T	117	12					
B	T	122	17					2.57E+06
	T	102	12					
C	T	158	13					2.60E+06
	T	152	8					
D	T	190	38					4.48E+06
	T	136	19					

Samples	log (control)	log (samples)	log reduction
A	9.57	6.54	3.02
B	9.57	6.41	3.16
C	9.57	6.41	3.15
D	9.57	6.65	2.92

3.063294

Summary
- Avg $\log_{10}$ reduction 3.06
- Correlates with previous data Pg. 16.
- O_3 concentration 7.13

Calculated Data - Run 3 (60min, 7%)

Determination of the Effect of 7% Ozone on *Bacillus anthracis* (sterne) Strain with a 60min exposure time								
Coupon Type: stainless steel 316								
Ozone Concentration: 7% (calculated)								
Contact Times: 60min								
Samples	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**avg count**
Control						12	3	3.70E+09
						12	2	
A	T	T	172	86				8.70E+07
	T	T	187	52				
B	T	T	199	58				6.54E+07
	T	T	189	34				
C	T	T	315	115				1.34E+08
	T	T	283	93				
D	T	T	233	87				1.18E+08
	T	T	210	104				

Samples	log (control)	log (samples)	log reduction
A	9.57	7.94	1.63
B	9.57	7.82	1.75
C	9.57	8.13	1.44
D	9.57	8.07	1.50

1.580147

Summary
- Avg $\log_{10}$ reduction of 1.58
- Correlates to previous data Pg. 16

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Invented by: Kimi C. Cage

Recorded by: _____

Date 6/4/09

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Date: 6/4/09

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

Procedure: Refer to pg. 5

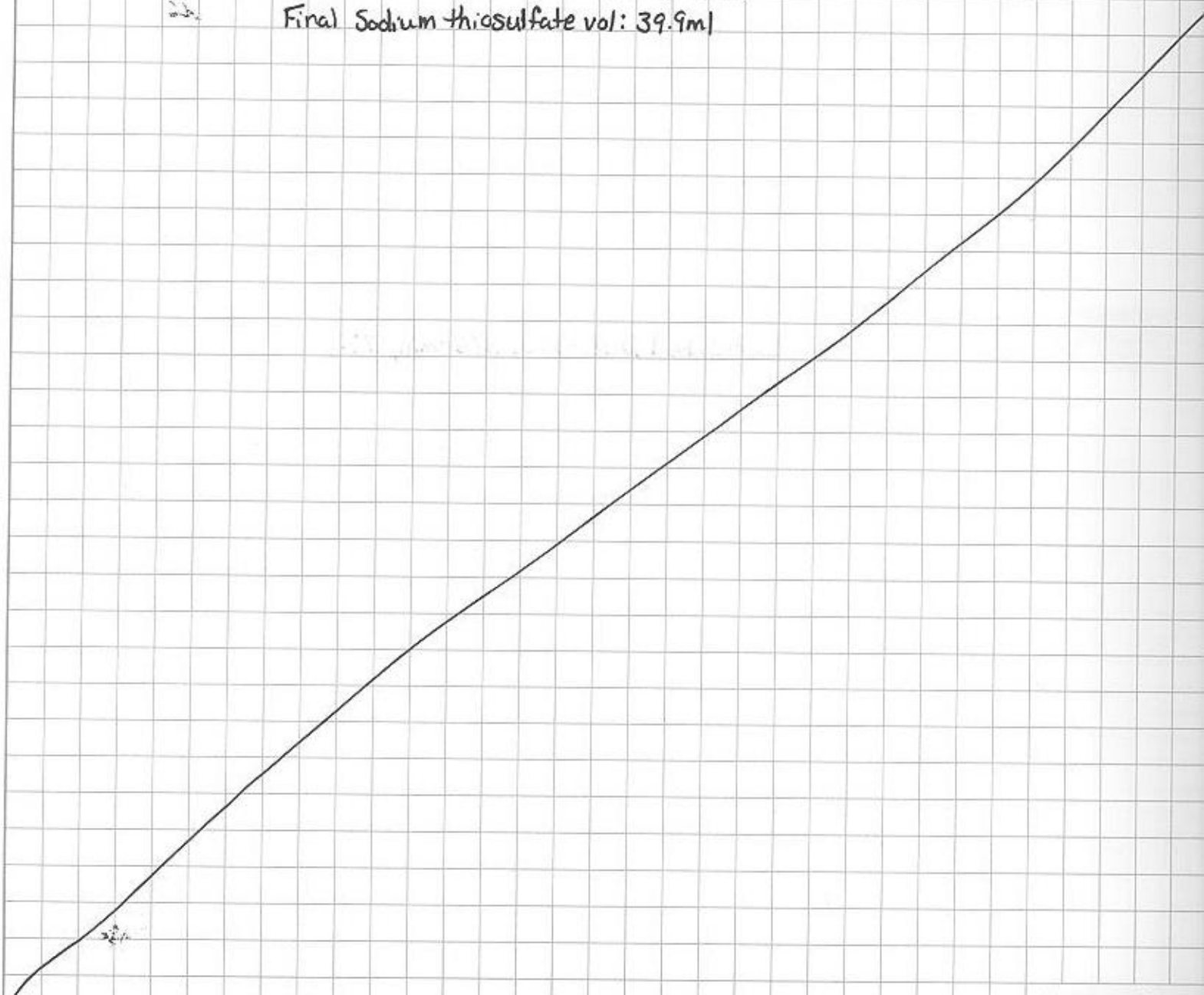
Titration Data

Flow Rate: 2.0 L/min

Time: 0.60min

Initial Sodium thiosulfate vol: 50.0ml

Final Sodium thiosulfate vol: 39.9ml



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Calculated Ozone Concentration

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	% O3
2	0.60	50.0	39.9	1.68E-03	5.05E-04	5.05E-04	163	8.23E-02	6.86

Raw Data - Run 3 (120min, 7%)

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

KC

Date: ~~6/4/09~~ 6/5/09

Samples	2	3	4	5	6	7	8	9
Control							7	2
							11	1
A	101	10	2					
	115	8	0					
B	198	63	5					
	146	32	3					
C	42	8	2					
	51	7	1					
D	83	22	6					
	91	31	9					

Calculated Data - Run 3 (120min, 7%)

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

KC

Samples	2	3	4	5	6	7	8	9	avg count
Control							7	2	2.40E+09
							11	1	
A	101	10	2						2.98E+04
	115	8	0						
B	198	63	5						1.05E+05
	146	32	3						
C	42	8	2						2.72E+04
	51	7	1						
D	83	22	6						1.10E+05
	91	31	9						

Samples	log (control)	log (samples)	log reduction
A	9.38	4.47	4.91
B	9.38	5.02	4.36
C	9.38	4.43	4.95
D	9.38	5.04	4.34

Summary

- Avg. $\log_{10}$ reduction is 4.64
- This correlates to the previous runs
- O_3 concentration is 6.86

Witnessed & Understood by me,

Date

Invented by: Kimi C. Cage

Recorded by:

Date 6/5/09

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MOFFETT CENTER	Page 1 of 3
Title: **Determining the Effect of 7% Ozone on *Bacillus* spores Embedded in Sucrose on Various Coupon Surfaces**	Author: Kerri Cooper
Document No.:	Effective Date: June 2009

KC

1.0 Purpose:

To determine the effect of 7% ozone on Bacillus spores that are embedded in sucrose on glazed tile, stainless steel 316, and Teflon 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* (stern) strain were used

4.0 Equipment and Supplies

- Sterile Conical tubes containing 10 glass beads (diameter 2mm)
- Sterile forceps
- Sucrose
- 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 spores* suspension (BA, BC, BT 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 Dissolve 2g of sucrose in 2ml of dH_20

5.1.2 Obtain 3 coupons for each surface type

5.1.3 Load the coupons with 0.1mL of sucrose solution

5.1.4 Place the coupons in the oven at 180°C (10min for stainless steel, 20min for Teflon and glazed tile)

5.1.5 Remove coupons and allow to cool

5.1.6 Inoculate coupons with 0.05ml of spores (BA, BC, or BT)

5.1.7 Place in the refrigerator to dry for 2hrs

5.2 Analysis of Control Coupons

Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 6/10/09
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MOFFETT CENTER	Page 2 of 3
Title: **Determining the Effect of 7% Ozone on *Bacillus* spores Embedded in Sucrose on Various Coupon Surfaces**	Author: Kerri Cooper
Document No.:	Effective Date: June 2009

5.2.1 Place one coupon per surface type 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
5.2.6 Incubate for 24hrs at 32°C

5.3 Day 1: Analysis for Ozone Treated Coupons
5.3.1 Place 2 coupons per surface type in the Anaerobic Reaction Tank
5.3.2 Set generator parameters to produce 7% ozone and follow SOP: MC SA-105-SOP001-V01
5.3.3 Remove coupons after 120min
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

6.0 References and Supporting Documents

1. Buranov, S.N., V.I. Karelin, V.D. Selemir, O.N. Vorobieva, A.S. Tsareva, V.V. Shkarin. "Sterilizing Effect of Ozone on Live Spores of Anthrax Bacillus." Pulsed Power Conference, 2005 IEEE. June. 1416-1417.

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

3. Oxygen Material Safety Data Sheet (MSDS).

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

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tnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 6/10/09
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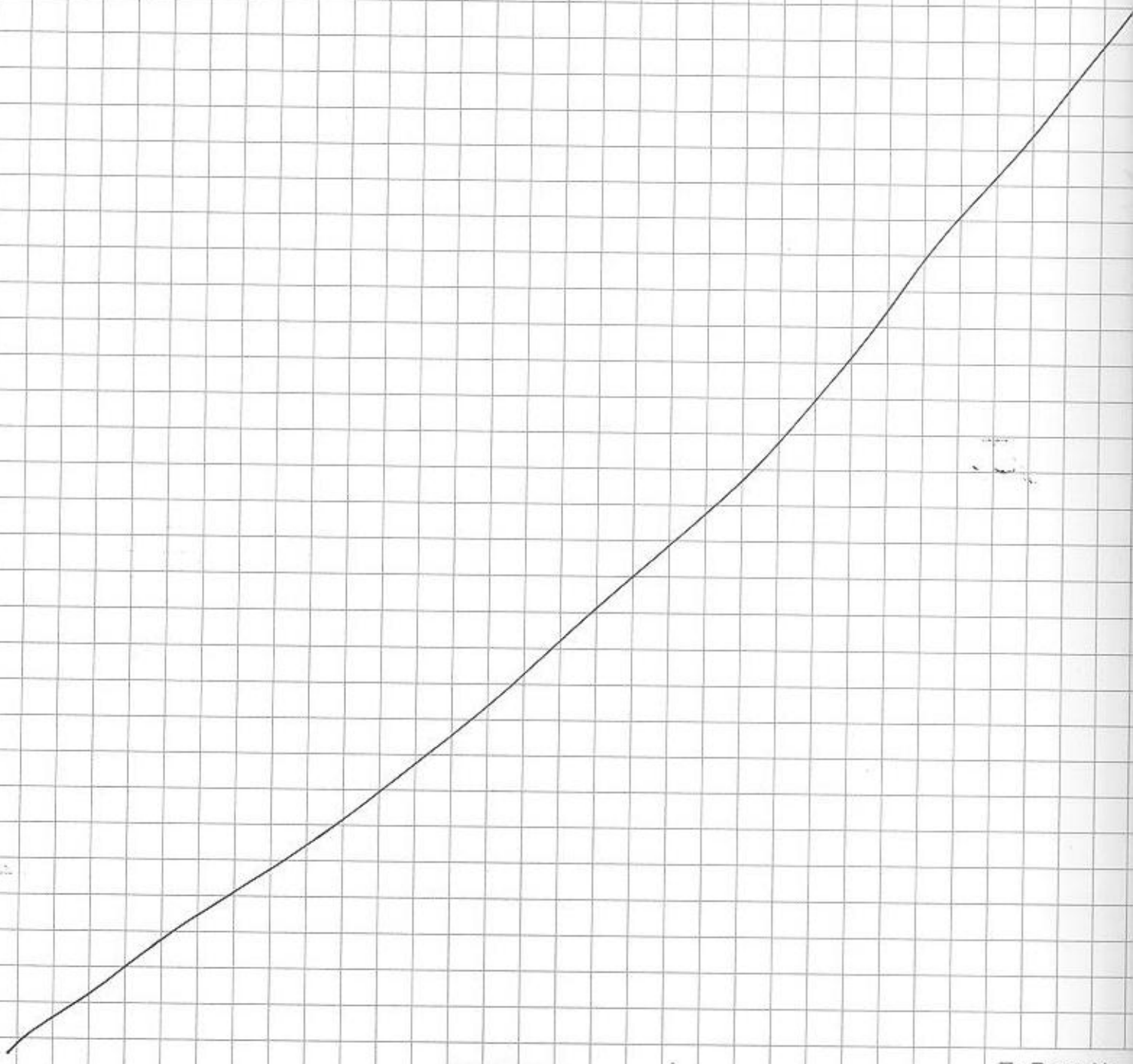
Date: 6/10/09

Effect ~~of~~ of 7% Ozone on Bacillus Anthracis (sterne) Embedded in Sucros

Procedure: Refer to pg. 22



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Raw Data - Run 1 Procedure: Refer to pg. 23

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Date: 6/11/09

Samples	3	4	5	6	7	8	9
Control - SS					76	11	1
					67	11	3
Control - TF					63	5	2
					63	3	0
Control - GT					53	4	0
					47	5	0
SS1	T	T	T	101	14	4	
	T	T	T	114	18	1	
SS2	T	T	T	130	21	0	
	T	T	T	136	17	1	
TF1	T	T	T	163	40	10	
	T	T	T	205	43	7	
TF2	T	T	T	155	27	3	
	T	T	T	132	22	1	
GT 1	T	T	T	166	23	3	
	T	T	T	144	22	1	
GT 2	T	T	T	201	41	1	
				222	42	0	

Calculated Data - Run 1

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	3	4	5	6	7	8	9	avg count
Control - SS					76	11	1	3.82E+09
					67	11	3	
Control - TF					63	5	2	2.03E+09
					63	3	0	
Control - GT					53	4	0	9.50E+08
					47	5	0	
SS1	T	T	T	101	14	4		5.18E+08
	T	T	T	114	18	1		
SS2	T	T	T	130	21	0		3.73E+08
	T	T	T	136	17	1		
TF1	T	T	T	163	40	10		1.45E+09
	T	T	T	205	43	7		
TF2	T	T	T	155	27	3		5.89E+08
	T	T	T	132	22	1		
GT 1	T	T	T					

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Date: 6/11/09

Effect of 7% Ozone on Bacillus Anthracis (sterne) Embedded in Sucrose

Procedure: Refer to pg. 22

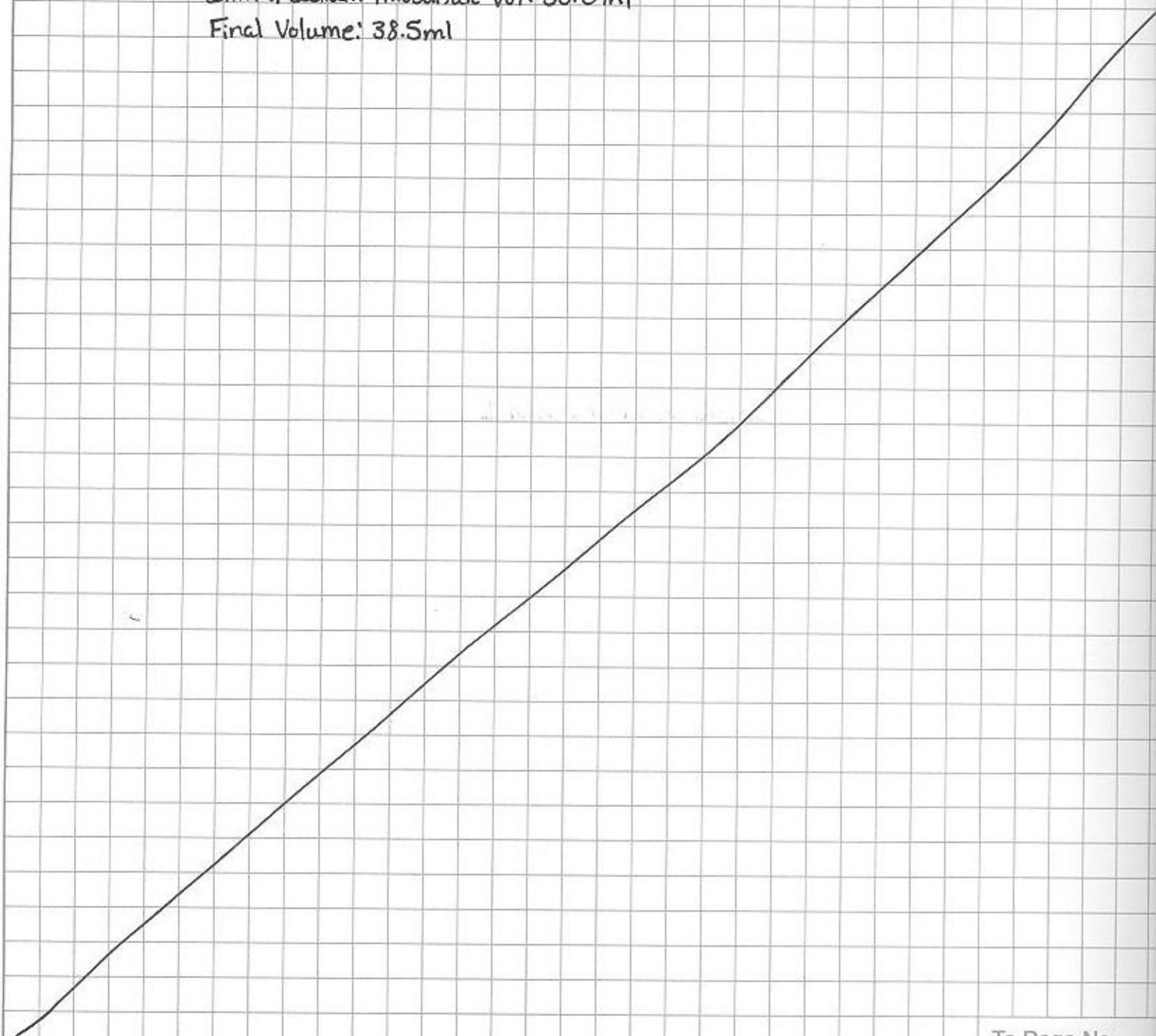
Titration Data

Flow Rate: 2.0 L/min

Time: 0.65 min

Initial Sodium Thiosulfate Vol: 50.0 ml

Final Volume: 38.5 ml



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Calculated Ozone Concentration

(min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	% O3
.65	50.0	38.5	1.92E-03	5.75E-04	5.75E-04	163	9.37E-02	7.21

Raw Data - Run 2

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	3	4	5	6	7	8	9
Control - SS					50	8	
					53	3	
Control - TF					52	9	
					63	6	
Control - GT					94	8	
					87	8	
SS1			221	101	16	0	
			253	79	12	1	
SS2			215	128	19	1	
			231	114	26	0	
TF1			T	167	50	2	
			T	140	38	1	
TF2			T	123	27	3	
			T	158	39	3	
GT 1			T	144	26	2	
			T	157	80	2	
GT 2			184	67	27	7	
			163	48	18	1	

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Calculated Data - Run 2

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	5	6	7	8	9	avg count
Control - SS			50	8	1	2.07E+09
			53	3	1	
Control - TF			52	9	0	1.33E+09
			63	6	0	
Control - GT			94	8	1	2.71E+09
			87	8	1	
SS1	221	101	16	0		2.91E+08
	253	79	12	1		
SS2	215	128	19	1		4.18E+08
	231	114	26	0		
TF1	T	167	50	2		7.44E+08
	T	140	38	1		
TF2	T	123	27	3		7.71E+08
	T	158	39	3		
GT 1	T	144	26	2		6.31E+08
	T	157	30	2		
GT 2	184	67	27	7		7.00E+08
	163	48	18	1		

Sample	log (avg control)	log (sample)	log reduction			
SS 1	9.31	8.46	0.85	0.77		
SS 2	9.31	8.62	0.69			
TF 1	9.12	8.87	0.25	0.24		
TF 2	9.12	8.89	0.24			
GT 1	9.43	8.80	0.63	0.61		
GT 2	9.43	8.85	0.59			

Summary

- Avg. log reduction for stainless steel is: 0.77
- Avg. $\log_{10}$ reduction for Teflon: 0.24
- Avg. $\log_{10}$ reduction for glazed tile: 0.61
- All $\log_{10}$ reductions for all surfaces are < 1.0
- The glazed tile and teflon surfaces showed a different trend with glazed tile having a higher reduction than in Run 1 (pg 25).
- This experiment will be repeated again for accuracy.

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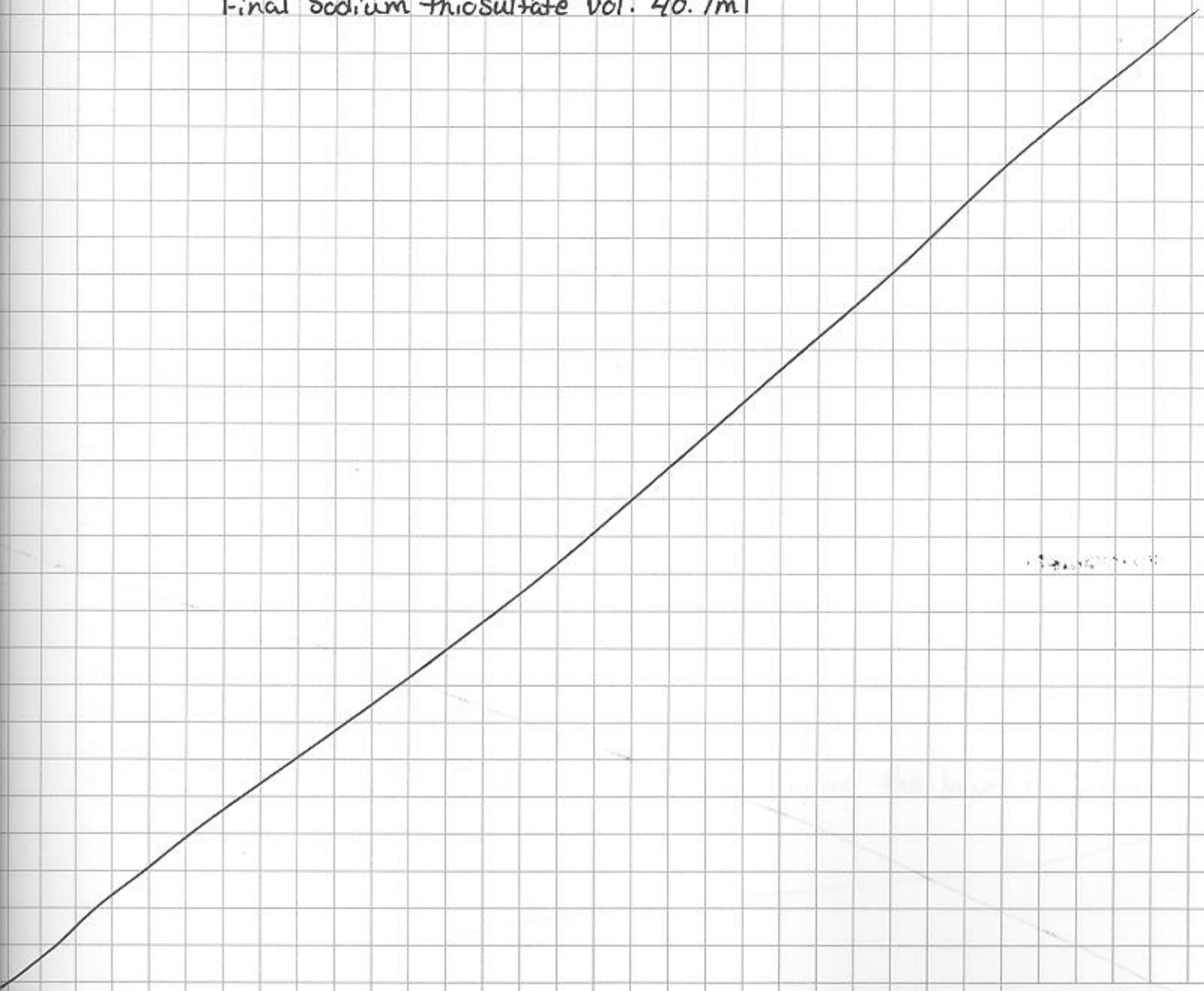
Date: 6/16/09

Effect of 7% Ozone on Bacillus Anthracis (sterne) Embedded in Sucrose

Procedure: Refer to pg. 22

Titration Data
Flow Rate: 2.0 L/min
Time: 0.5 min
Initial Sodium thiosulfate vol: 50 ml
Final Sodium thiosulfate vol: 40.7 ml



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Witnessed & Understood by me,	Date	Invented by: Kuni C. Cooper	Date
		Recorded by:	6/18/09

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Calculated Ozone Concentration

Ozone Flow Rate (L/min)	Time (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol
2	0.50	50.0	40.7	1.55E-03	4.65E

Raw Data - Run 3

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Date: 6/16/09

Samples	KC36	KC49	KC83	6	7	8	9
Control - SS						8	2
						6	1
Control - TF						5	0
						10	2
Control - GT						10	2
						9	1
SS1	118	13	3				
	98	21	2				
SS2	128	23	5				
	119	15	2				
TF1	103	27	2				
	155	33	0				
TF2	189	33	0				
	190	37	4				
GT 1	251	37	6				
	152	48	5				
GT 2	196	41	3				
	145	31	6				

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Witnessed & Understood by me,	Date	Invented by: Thuri C. Cooper	Date 6/17/09
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Calculated Data - Run 3

Effect of 7% Ozone on *Bacillus Anthracis* (sterne) Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	6	7	8	9	avg count
Control - SS			8	2	2.20E+09
			6	1	
Control - TF			5	0	1.75E+09
			10	2	
Control - GT			10	2	2.45E+09
			9	1	
SS1	118	13	3		5.28E+08
	98	21	2		
SS2	128	23	5		6.64E+08
	119	15	2		
TF1	103	27	2		5.29E+08
	155	33	0		
TF2	189	33	0		7.40E+08
	190	37	4		
GT 1	251	37	6		1.18E+09
	152	48	5		
GT 2	196	41	3		9.81E+08
	145	31	6		

Sample	log (avg control)	log (sample)	log reduction		
SS 1	9.34	8.72	0.62	0.57	
SS 2	9.34	8.82	0.52		
TF 1	9.24	8.72	0.52	0.45	
TF 2	9.24	8.87	0.37		
GT 1	9.39	9.07	0.32	0.36	
GT 2	9.39	8.99	0.40		

Summary

- Avg. $\log_{10}$ reduction for stainless steel: 0.57
- Avg $\log_{10}$ reduction for Teflon: 0.45
- Avg $\log_{10}$ reduction for glazed tile: 0.36
- Stainless shows the higher $\log_{10}$ reduction for BA
- Glazed tile + Teflon are close with glazed tile having the lower reduction

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Witnessed & Understood by me, _____ Date _____

Invented by: Kristi C. Coogan

Recorded by: _____

Date 6/17/09

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From Page No. ____ Date: 6/16/09

Effect of 7% Ozone on Bacillus Cereus Embedded in Sucrose - Run 1

Procedure: pg. 22

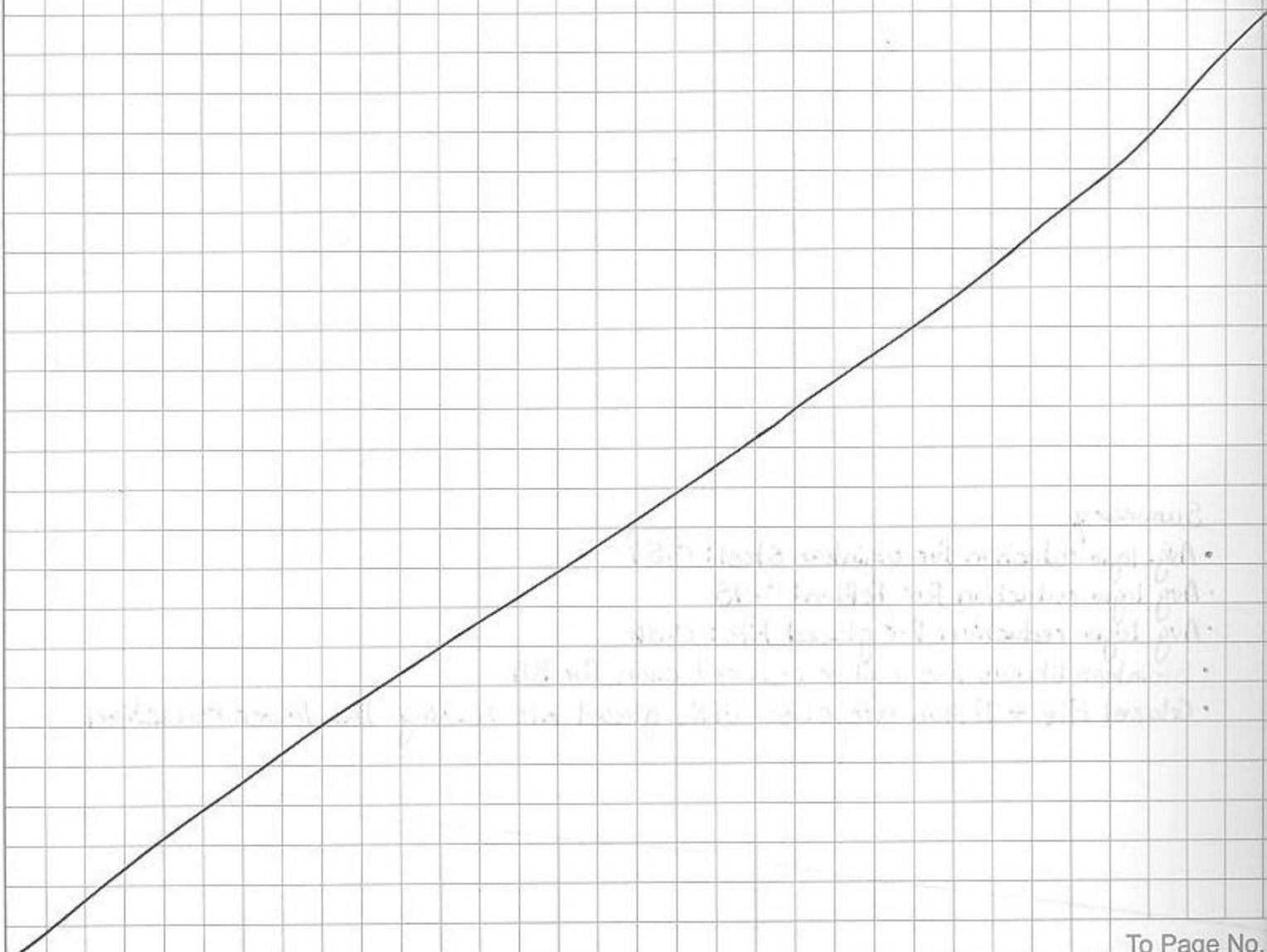
Titration Data

Flow Rate: 2.0 L/min

Time: 0.42 min

Initial Sodium Thiosulfate Vol: 50ml

Final Sodium Thiosulfate Vol: 42.7ml



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Ozone Conc. Calculation

ne (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	% O3
0.42	50.0	42.7	1.22E-03	3.65E-04	3.65E-04	163	5.95E-02	7.14

Raw Data: Run 1

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins
Date: 6/17/09

Samples	3	4	5	6	7	8	9
Control - SS						13	2
						13	2
Control - TF						19	1
						44	2
Control - GT						42	1
						40	2
SS1	T	T	205	138	16	1	
	T	T	210	58	12	1	
SS2	T	T	183	39	11	1	
	T	T	126	46	9	1	
TF1	T	T	T	153	38	5	
	T	T	T	130	39	2	
TF2	T	T	T	172	20	8	
	T	T	T	150	39	9	
GT 1	T	T	T	216	55	8	
	T	T	T	212	36	6	
GT 2	T	T	T	132	55	10	
	T	T	T	109	39	5	

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Calculated Data - Run 1

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	3	4	5	6	7	8	9
Control - SS						13	2
						13	2
Control - TF						19	1
						44	2
Control - GT						42	1
						40	2
SS1	T	T	205	138	16	1	
	T	T	210	58	12	1	
SS2	T	T	183	39	11	1	
	T	T	126	46	9	1	
TF1	T	T	T	153	38	5	
	T	T	T	130	39	2	
TF2	T	T	T	172	20	8	
	T	T	T	150	39	9	
GT 1	T	T	T	216	55	8	
	T	T	T	212	36	6	
GT 2	T	T	T	132	55	10	
	T	T	T	109	39	5	

Sample	log (avg control)	log (sample)	log reduction				
SS 1	9.52	8.55	0.96	1.04			
SS 2	9.52	8.41	1.11				
TF 1	9.67	8.94	0.72	0.64			
TF 2	9.67	9.12	0.55				
GT 1	9.75	9.14	0.61	0.62			
GT 2	9.75	9.13	0.62				

Summary

- Stainless steel log10 avg log10 reduction: 1.04
- Teflon avg log10 reduction: 0.64
- Glazed tile avg log10 reduction: 0.62
- Stainless steel has the highest log10 reduction followed by Teflon then glazed tile
- BC produces a higher log10 reduction than BA for this run

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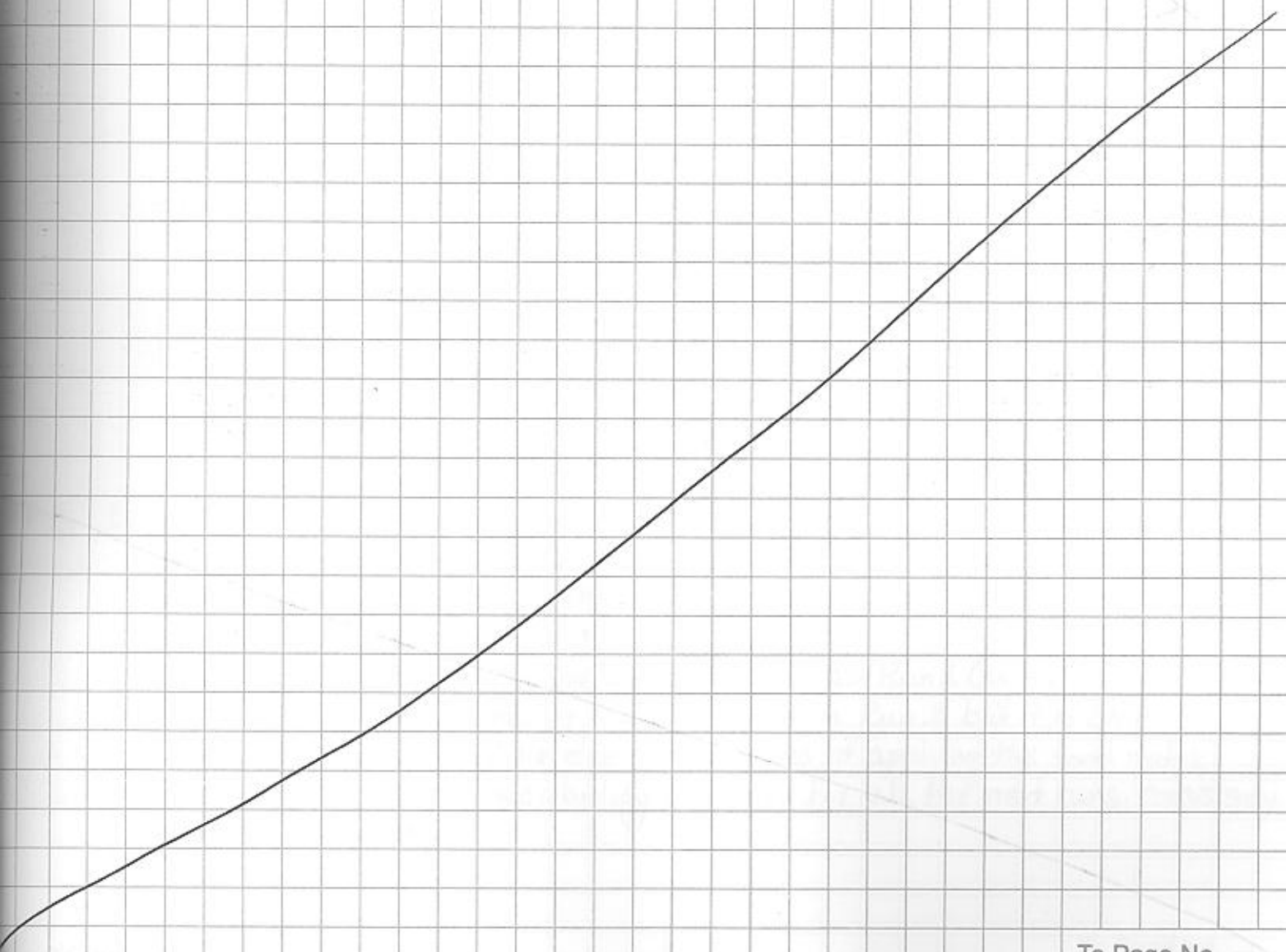
Date: 6/17/09

Effect of ~~Ba~~ Zc 7% Ozone on Bacillus Cereus Embeded in Sucrose - Run 2

Procedure: Refer to pg. 22

Titration Data
Flow Rate: 2.0 L/min
Time: 0.43min
Initial Sodium thiosulfate vol: 50ml
Final Vol: 42.5ml



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Date

Invented by: Kristi C. Cogan

Recorded by:

Date 6/18/09

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KC Calculated Ozone Conc.

Ozone Flow Rate (L/min)	Time (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol
2	0.43	50.0	42.5	1.25E-03	3.75E-04	3.75E

Raw Data - Run 2

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Date: 6/18/09

KC

Samples	3	4	5	6	7	8	9
Control - SS						22	4
						19	6
Control - TF						9	2
						11	0
Control - GT						6	0
						3	2
SS1				148	14	4	
				101	30	7	
SS2				119	21	2	
				117	12	1	
TF1				143	42	5	
				167	50	9	
TF2				12	4	0	
				11	4	1	
GT 1				110	26	5	
				168	16	3	
GT 2				101	26	7	
				96	28	8	

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Calculated Data - Run 2

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	6	7	8	9	avg count
Control - SS			22	4	7.05E+09
			19	6	
Control - TF			9	2	2.00E+09
			11	0	
Control - GT			6	0	1.45E+09
			3	2	
SS1	148	14	4		8.95E+08
	101	30	7		
SS2	119	21	2		4.33E+08
	117	12	1		
TF1	143	42	5		1.32E+09
	167	50	9		
TF2	12	4	0		1.02E+08
	11	4	1		
GT 1	110	26	5		8.69E+08
	158	16	3		
GT 2	101	26	7		1.12E+09
	96	28	8		

Sample	log (avg control)	log (sample)	log reduction	
SS 1	9.85	8.95	0.90	1.05
SS 2	9.85	8.64	1.21	
TF 1	9.30	9.12	0.18	0.74
TF 2	9.30	8.01	1.29	
GT 1	9.16	8.94	0.22	0.17
GT 2	9.16	9.05	0.11	

Summary

- Stainless steel avg. $\log_{10}$ reduction: 1.05
- Teflon avg. $\log_{10}$ reduction: 0.74
- Glazed tile avg. $\log_{10}$ reduction: 0.17
- Same $\log_{10}$ reductions for the surfaces ~~as~~ types as Run 1 (pg 34)
- Glazed tile had a much smaller reduction than in Run 1 but it is still within range. This could be due to techniques of applying the food matrix, ~~In all the~~ or for the microbiology analysis. For all the next runs, consistency will be emphasized.

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Invented by: Kimi C-Coagm Date 6/18/09

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Date: 6/18/09

Effect of 7% Ozone on Bacillus Cereus Embedded in Sucrose

Procedure: Refer to pg. 22

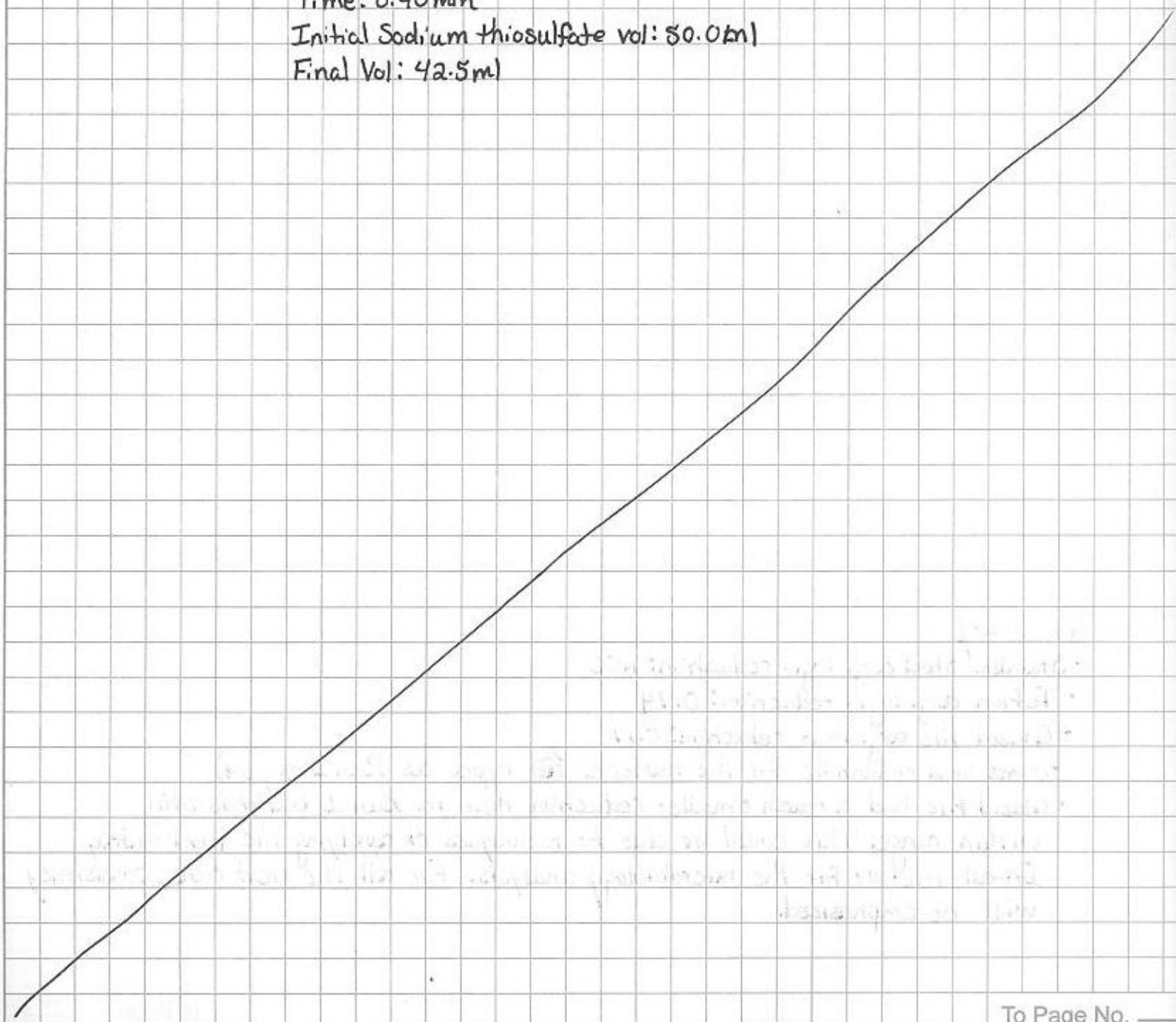
Titration Data

Flow Rate: 2.0 L/min

Time: 0.40 min

Initial Sodium thiosulfate vol: 50.0ml

Final Vol: 42.5ml



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Ozone Concentration

	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	% O3
	50.0	42.5	1.25E-03	3.75E-04	3.75E-04	163	6.11E-02	7.64

Raw Data - Run 1

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	3	4	5	6	7	8	9
Control - SS						9	2
						8	3
Control - TF						12	0
						13	4
Control - GT						7	1
						6	3
SS1				59	12	0	
				44	13	0	
SS2				87	34	2	
				88	12	3	
TF1				191	64	9	
				173	59	7	
TF2				153	56	10	
				122	50	6	
GT 1				193	58	10	
				202	53	6	
GT 2				115	63	14	
				101	45	19	

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Calculated Data - Run 3

Effect of 7% Ozone on *Bacillus Cereus* (sterne) Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	6	7	8	9	avg count
Control - SS			9	2	3.35E+09
			8	3	
Control - TF			12	0	3.25E+09
			13	4	
Control - GT			7	1	2.65E+09
			6	3	
SS1	59	12	0		1.77E+08
	44	13	0		
SS2	87	34	2		5.68E+08
	88	12	3		
TF1	191	64	9		1.60E+09
	173	59	7		
TF2	153	56	10		1.29E+09
	122	50	6		
GT 1	193	58	10		1.47E+09
	202	53	6		
GT 2	115	63	14		2.30E+09
	101	45	19		

Sample	log (avg control)	log (sample)	log reduction	
SS 1	9.53	8.25	1.28	
SS 2	9.53	8.75	0.77	1.02
TF 1	9.51	9.20	0.31	
TF 2	9.51	9.11	0.40	0.35
GT 1	9.42	9.17	0.25	
GT 2	9.42	9.36	0.06	0.16

Summary

- Stainless steel avg. log10 reduction: 1.02
- Teflon avg log10 reduction: 0.35
- Glazed tile avg log10 reduction: 0.16
- The log reduction for Glazed tile is similar to Run 2's results (pg 37).
- For all runs stainless steel produced an avg. >1.0 but the other surfaces still had avgs. <1.0

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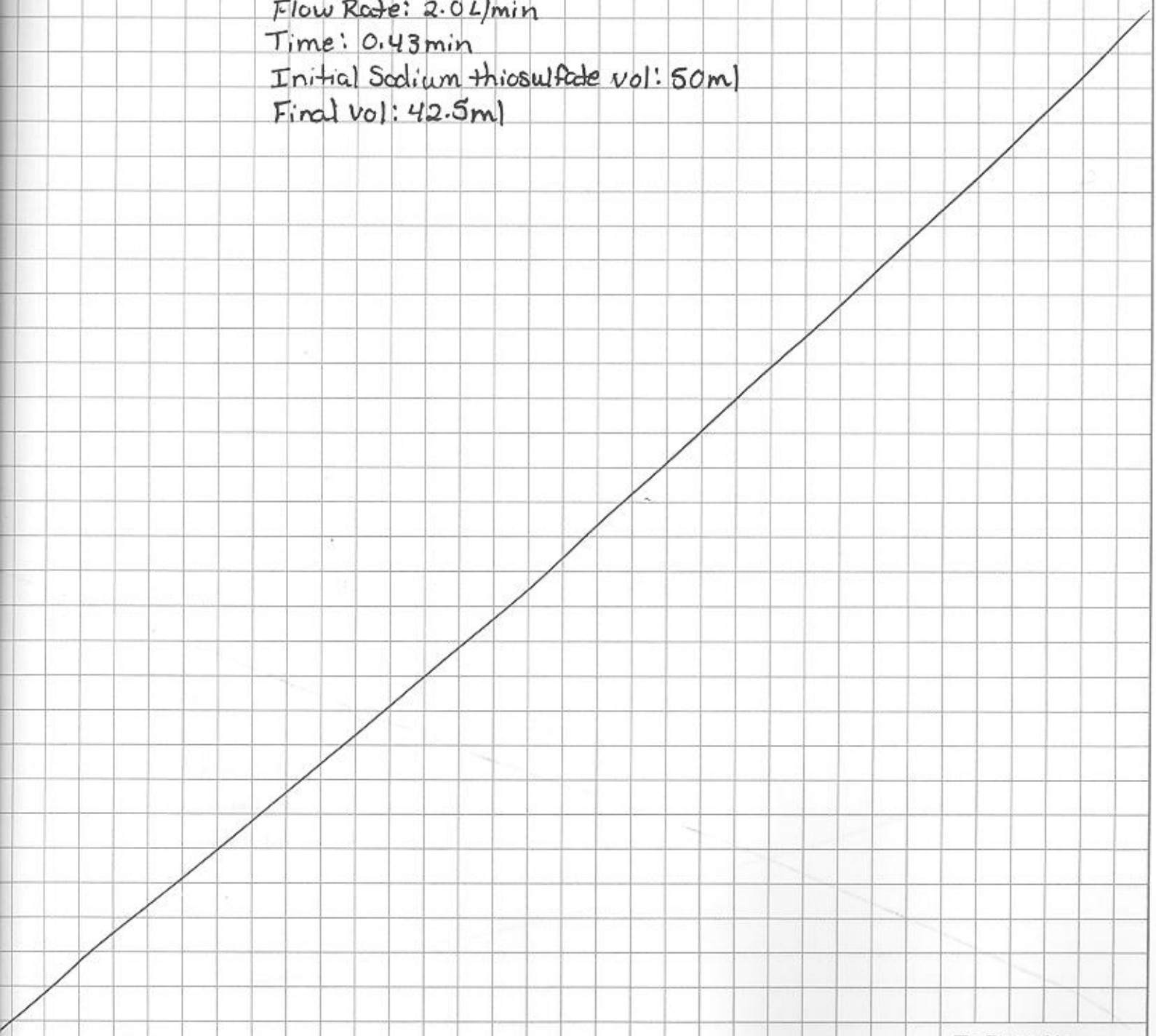
Date: 6/22/09

Effect of 7% Ozone on Bacillus Thuringiencis Embedded in Sucrose

Procedure: Refer to pg. 22

Titration Data
Flow Rate: 2.0 L/min
Time: 0.43 min
Initial Sodium thiosulfate vol: 50ml
Final vol: 42.5ml



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Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	%
50.0	42.5	1.25E-03	3.75E-04	3.75E-04	163	6.11E-02	7.

Raw Data - Run 1

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Date: 6/23/09

Samples	3	4	5	6	7	8	9
Control - SS				8	1		
Control - TF				11	0		
				10	3		
Control - GT				12	0		
				19	0		
SS1	100	39	5	16	1		
	110	21	1	0	0		
SS2	172	38	9	0	0		
	132	36	2	0	0		
TF1	T	116	51	5	0		
	T	99	50	4	0		
TF2	203	52	13	5	0		
	180	41	17	2	0		
GT 1	162	31	6	3	0		
	137	47	16	1	0		
GT 2	142	64	6	0	0		
	131	61	11	5			

Witnessed & Understood by me, _____ Date _____

Invented by: Thui C. Cooper

Recorded by: _____

Date 6/23/09

Calculated Data - Run 1

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	3	4	5	6	7	avg count
Control - SS				8	1	1.45E+07
				11	0	
Control - TF				10	3	2.60E+07
				12	0	
Control - GT				19	0	2.25E+07
				16	1	
SS1	100	39	5	0	0	4.45E+05
	110	21	1	0	0	
SS2	172	38	9	0	0	1.07E+06
	132	36	2	0	0	
TF1	T	116	51	5	0	1.06E+07
	T	99	50	4	0	
TF2	203	52	13	5	0	5.66E+06
	180	41	17	2	0	
GT 1	162	31	6	3	0	3.34E+06
	137	47	10	1	0	
GT 2	142	64	6	0	0	4.06E+06
	131	51	11	5	0	

Sample	log (avg control)	log (sample)	log reduction	
SS 1	7.16	5.65	1.51	1.32
SS 2	7.16	6.03	1.13	
TF 1	7.41	7.03	0.39	0.53
TF 2	7.41	6.75	0.66	
GT 1	7.35	6.52	0.83	0.79
GT 2	7.35	6.61	0.74	

Summary
- Stainless steel 1.E avg log10 reduction: 1.32
- Teflon avg log10 reduction: 0.53
- Glazed tile avg log10 reduction: 0.79
- BT has a lower concentration of 10^{-7} than BC + BA
- This experiment will be run again

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Effect of 7% Ozone on Bacillus Thuringiencis Embedded in Sucrose

Procedure: Refer to pg. 22

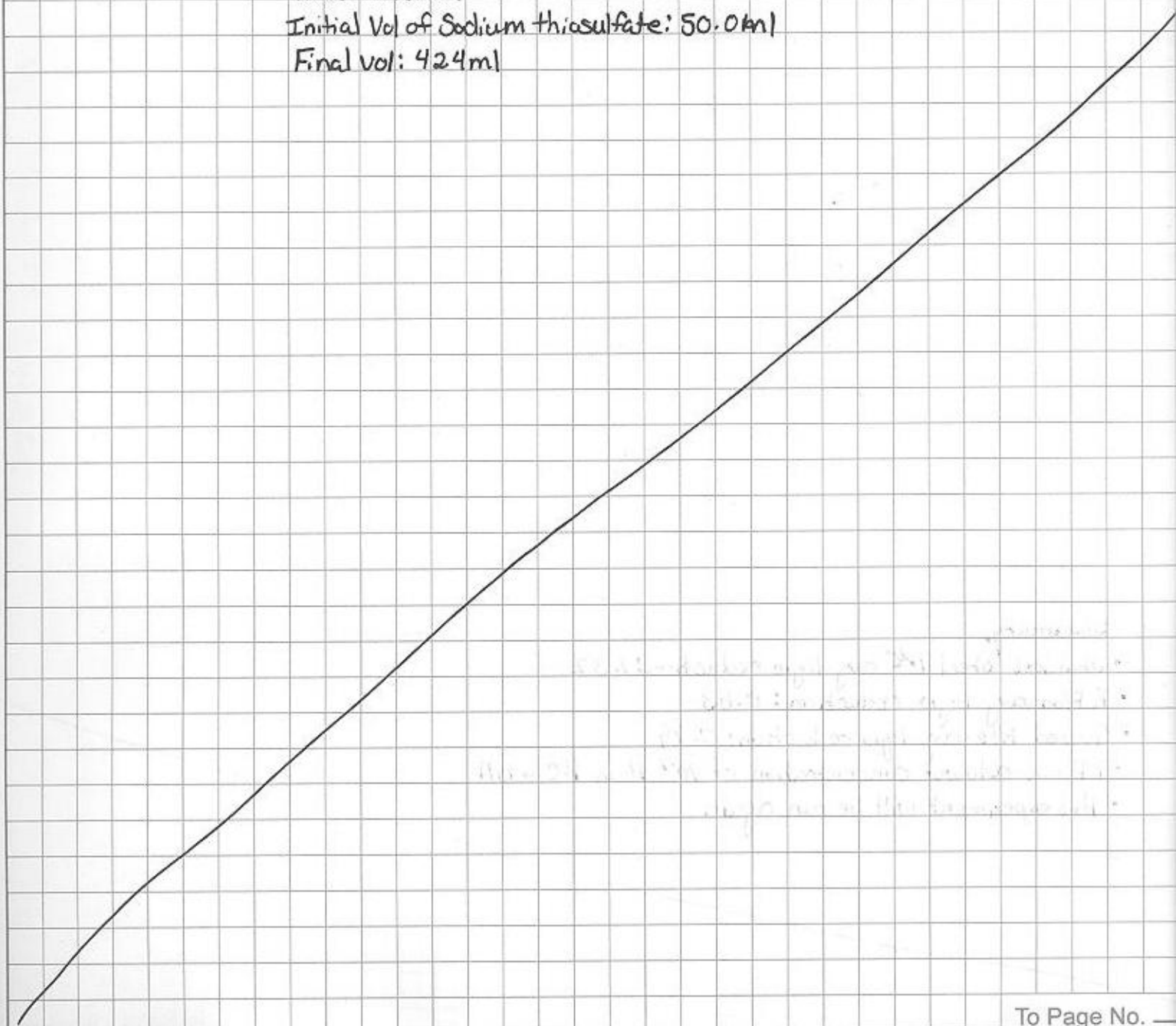
Titration Data

Flow Rate: 2.0L/min

Time: 0.43min

Initial Vol of Sodium thiosulfate: 50.0ml

Final vol: 424ml



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Witnessed & Understood by me,	Date	Invented by: Kim C. Coyne Recorded by:	Date 6/24/09

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Calculated Ozone Concentration

Ozone Flow Rate (L/min)	Time (min)	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3
2	0.43	50.0	42.4	1.27E-03	3.80E-04	3.80E-04

Raw Data - Run 2

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	3	4	5	6	7	8	9
Control - SS				11	0		
				3	5		
Control - TF				9	1		
				8	0		
Control - GT				14	1		
				11	0		
SS1		26	14	0			
		24	8	5			
SS2		37	11	0			
		39	8	0			
TF1		36	16	2			
		24	8	0			
TF2		28	6	0			
		29	6	2			
GT 1		32	3	1			
		27	10	1			
GT 2		57	12	2			
		52	4	0			

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nessed & understood by me, | Date | Invented by: Kristi C. Coggin | Date 6/24/09

Recorded by:

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Calculated Data - Run 2

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose					
Coupons: Stainless Steel 316, Glazed Tile, Teflon					
Contact Time: 120mins					
Samples	**4**	**5**	**6**	**7**	**avg count**
Control - SS			11	0	3.70E+07
			3	5	
Control - TF			9	1	1.35E+07
			8	0	
Control - GT			14	1	1.75E+07
			11	0	
SS1	26	14	0		3.85E+06
	24	8	5		
SS2	37	11	0		1.33E+06
	39	8	0		
TF1	36	16	2		2.50E+06
	24	8	0		
TF2	28	6	0		1.89E+06
	29	6	2		
GT 1	32	3	1		1.95E+06
	27	10	1		
GT 2	57	12	2		2.35E+06
	52	4	0		
Sample	**log (avg control)**	**log (sample)**	**log reduction**		
SS 1	7.57	6.59	0.98	1.21	
SS 2	7.57	6.12	1.44		
TF 1	7.13	6.40	0.73	0.79	
TF 2	7.13	6.28	0.86		
GT 1	7.24	6.29	0.95	0.91	
GT 2	7.24	6.37	0.87		

Summary

- Stainless ~~steel~~ Steel avg $\log_{10}$ reduction: 1.21
- Teflon avg $\log_{10}$ reduction: 0.79
- Glazed tile avg $\log_{10}$ reduction: 0.91
- For this run + Run 1 the glazed tile had a higher log reduction than Teflon for BT
- This experiment will be repeated again for accuracy

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		Recorded by:	6/24/09

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Date: 5/24/09

Effects of 7% Ozone on Bacillus Thuringiencis Embedded in Sucrose - Run 3

Procedure: Refer to pg. 22

Titration Data
Flow Rate: 2.0 L/min
Time: 0.43 min
Initial Sodium thiosulfate vol: 50 ml
Final vol: 42.5 ml

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Witnessed & Understood by me,	Date	Invented by: Kui C. Coopm Recorded by:	Date 5/25/09

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Calculated Ozone Conc.

	Initial Sodium Thiosulfate Volume (ml)	Final Sodium Thiosulfate Volume (ml)	mol/L I2	mol I2	mol O3	Molar Volume O3	Volume O3	% O3
KC	50.0	42.5	1.25E-03	3.75E-04	3.75E-04	163	6.11E-02	7.06

Raw Data - Run 3

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	4 KC	5 KC	5	6	7	8	9
Control - SS				13	1		
				5	0		
Control - TF				15	1		
				8	1		
Control - GT				12	3		
	5 KC			11	0		
SS1	56	6		1			
	37	7		0			
SS2	54	14		0			
	51	14		0			
TF1	43	10		1			
	39	5		0			
TF2	55	16		2			
	52	5		1			
GT 1	43	10		0			
	45	9		1			
GT 2	21	3		2			
	17	4		1			

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Witnessed & Understood by me, _____ Date _____

Invented by: Kurt C. Coogan

Recorded by: _____

Date 6/25/09

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Calculated Data - Run 3

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	4	5	6	7	avg count
Control - SS			13	1	1.40E+07
			5	0	
Control - TF			15	1	2.45E+07
			8	1	
Control - GT			12	3	2.65E+07
			11	0	
SS1	56	6	1		1.62E+06
	37	7	0		
SS2	54	14	0		1.93E+06
	51	14	0		
TF1	43	10	1		1.66E+06
	39	5	0		
TF2	55	16	2		2.89E+06
	52	5	1		
GT 1	43	10	0		1.89E+06
	45	9	1		
GT 2	21	3	2		2.04E+06
	17	4	1		
Sample	**log (avg control)**	**log (sample)**	**log reduction**		
SS 1	7.15	6.21	0.94	0.90	
SS 2	7.15	6.28	0.86		
TF 1	7.39	6.22	1.17	1.05	
TF 2	7.39	6.46	0.93		
GT 1	7.42	6.28	1.15	1.13	
GT 2	7.42	6.31	1.11		

Summary

- Stainless steel avg $\log_{10}$ reduction: 0.90
- Glazed tile: 1.13
- Teflon $\log_{10}$ reduction: 1.05
- The log reductions for this experiment was higher than Runs 1 + 2 but they are still within a 1 log range for GT + TF
- Stainless steel had a < 1 $\log_{10}$ reduction

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Witnessed & Understood by me, _____ Date _____

Invented by: Kimi C. Cooper Date 6/25/09

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Effect of Ozone on *Bacillus* Spores Status Update

Submitted By: Kerri C. Cooper

Date: July 6, 2009

Determination of Optimal Ozone Conditions

Table 1: Log Reduction Comparison for 7 & 8% Ozone Concentration for Various Contact Times

Concentration	60min			90min			120min		
6.97-7.21	1.41	1.43	1.58	3.01	3.36	3.06	5.19	4.90	4.64
7.80-8.55	3.19	2.41	2.87	4.36	4.66	4.58	$>10^{-2}$	$>10^{-2}$	$>10^{-2}$

The table above compares the log reductions for the effect of 7 & 8% ozone on *Bacillus anthracis* (sterne) on stainless steel coupons. Fro the information above the concentration log reduction for a higher ozone concentration requires less contact time. Although the contact time may be shorter, the 7% ozone concentration requires less power and oxygen for production as well as decreases the safety hazard in the case of potential exposure. Therefore, the concentration of 7% at 120min will be used as the optimal conditions for the *Bacillus* spores embedded in sucrose.

KC

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Witnessed & Understood by me,	Date	Invented by: Kerri C. Cooper	Date 7/29/09
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Effect of 7% Ozone on Bacillus Spores Embedded in Sucrose on Various Food Contact Surfaces for a 120min Exposure Time

Table 2: BA Embedded in Sucrose

Bacillus Anthracis (sterne)			
Date	Coupon Type	Concentration (%)	Average Log Reduction (cfu/ml)
6/11/2009	Stainless Steel 316	7.05	0.94
6/112/2009		7.21	0.77
6/16/2009		7.58	0.57
6/11/2009	Teflon	7.05	0.34
6/112/2009		7.21	0.24
6/16/2009		7.58	0.45
6/11/2009	Glazed Tile	7.05	0.18
6/112/2009		7.21	0.61
6/16/2009		7.58	0.36

The average log reduction of BA embedded in sucrose is < 1.0. The breakdown for each coupon is as follows:
Stainless Steel 316: 0.76 cfu/ml
Teflon: 0.34 cfu/ml
Glazed Tile: 0.38 cfu/ml

Table 3: BC Embedded in Sucrose

Bacillus Cereus			
Date	Coupon Type	Concentration (%)	Average Log Reduction (cfu/ml)
6/17/2009	Stainless Steel 316	7.14	1.04
6/18/2009		7.05	1.05
6/20/2009		7.64	1.02
6/17/2009	Teflon	7.14	0.64
6/18/2009		7.05	0.74
6/20/2009		7.64	0.35
6/17/2009	Glazed Tile	7.14	0.62
6/18/2009		7.05	0.17
6/20/2009		7.64	0.16

The average log reduction for the samples on Teflon and Glazed Tile are 0.58 and 0.32 cfu/ml respectively. The Stainless Steel samples show an average log reduction of 1.04 cfu/ml.

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Witnessed & Understood by me,	Date	Invented by: Kevin C. Cooper	Date 7/29/09
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Effect of 7% Ozone on Bacillus Spores Embedded in Sucrose on Various Food Contact Surfaces for a 120min Exposure Time

Table 4: BT Embedded in Sucrose

Bacillus Thuringiencis			
Date	Coupon Type	Concentration (%)	Average Log Reduction (cfu/ml)
6/23/2009	Stainless Steel 316	7.1	1.32
6/24/2009		7.15	1.21
6/25/2009		7.06	0.9
6/23/2009	Teflon	7.1	0.53
6/24/2009		7.15	0.79
6/25/2009		7.06	1.05
6/23/2009	Glazed Tile	7.1	0.79
6/24/2009		7.15	0.91
6/25/2009		7.06	1.13

The average log reduction for stainless steel 316: 1.14 cfu/ml
The average log reduction for Teflon: 0.79 cfu/ml
The average log reduction for Glazed Tile: 0.94 cfu/ml

Summary

The log reductions for BA, BC and BT show the same trend of BA being more resistant to sanitizers than the other two strains. Also, the average log reductions for all the spores on the coupons also show that stainless steel 316 has a higher log reduction and glazed tile is the harder surface to sterilize due to its texture, except for the BT spores. Since the effect of ozone directly on sucrose embedded with the spores does not produce a high log reduction, a wash step will be incorporated before the sanitizing step to help increase ozone's effect.

Witnessed & Understood by me, _____ Date _____

Invented by: Kuni C. Cooper

Recorded by: _____

Date 7/29/09

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Run 1 - B.C with wash step Date: 8/24/09

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose After a Previous W

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8
Control - TF							7
							6
Conrol - SS							6
							6
Control - GT							14
							11
R - GT1		82	29	10	1		
		92	37	8	1		
R-SS1		41	10	1			
		35	6	0			
R-GT1		92	21	5			
		111	52	4			
D-TF1		36	19	3			
		50	17	1			
D-SS1		18	8	1			
		25	3	0			
D-GT1		105	23	4			
		93	33	3			
O-TF1	69	22	1				
	51	10	2				
O-TF2	118	28	1				
	145	26	3				
O-SS1	17	0	0	0			
	22	0	0	0			
O-SS2	52	0	0	0			
	31	0	0	0			
O-GT1	T	122	31	4			
	T	116	25	5			
O-GT2	T	152	56	8			
	T	136	24	9			

Samples	log(avg control)	log (avg count)	log reduction
R-Tf1	9.50	6.36	3.13

Summary

- No major change b/t rinse + dry
- TF: 4.25 log reduction w/wash + ozone
- SS: 5.87 log reduction w/wash + ozone
- GT: 3.21 log reduction w/wash

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Witnessed & Understood by me, | Date | Invented by: Yuri C Cooper | Date 8/24/09

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From Page No. ____ Date: 8/25/09

Run 1: BT w/ wash step

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg reduction
Control - TF					8	0			3.10E+08
					10	1			
Conrol - SS					11	4			3.55E+07
					10	1			
Control - GT					5	0			5.50E+06
					6	0			
R - GT1	10	1							1450
	9	0							
R-SS1	5	1							9.50E+02
	4	0							
R-GT1	15	2							3.40E+03
	13	2							
D-TF1	8	1							1.25E+03
	7	0							
D-SS1	2	0							
	6	0							4.00E+02
D-GT1	21	3							4.00E+03
	19	1							
O-TF1	0	0	0						5.00E+01
	1	0	0						
O-TF2	0	0	0						6.02E+04
	0	0	0						
O-SS1	0	0	0	0					0.00E+00
	0	0	0	0					
O-SS2	0	0	0	0					0.00E+00
	0	0	0	0					
O-GT1	15	1	0	0					2.95E+03
	14	2	0	0					
O-GT2	3	0	0	0	0				9.50E+02
	6	1	0	0	0				

Samples	log(avg control)	log (avg count)	log reduction
R-Tf1	8.49	3.16	5.33
R-SS1	7.55	2.98	4.57
R-GT1	6.74	3.53	3.21
D-TF1	8.49	3.10	5.39
D-SS1	7.55	2.60	4.95
D-GT1	6.74	3.60	3.14
O-TF1	8.49	1.70	6.79
O-TF2	8.49	3.47	5.02
O-SS1	7.55	#NUM!	#NUM!
O-SS2	7.55	#NUM!	#NUM!
O-GT1	6.74	3.47	3.27
O-GT2	6.74	2.98	3.76

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Witnessed & understood by me, Date

Invented by: Yun C. Cho

Recorded by:

Date 8/25/09

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Run 1: BA w/wash step Date: 8/26/09

Effect of 7% Ozone on *Bacillus Anthracis (sterne)* Embedded in Sucrose After a Previou

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8
Control - TF							6
							5
Conrol - SS							6
							4
Control - GT							22
							41
D-TF1		63	7				
		63	9				
D-TF2		53	7				
		59	6				
D-SS1		44	4				
		48	0				
D-SS2		18	2				
		22	5				
D-GT1		244	32				
		215	29				
D-GT2		156	31				
		149	35				
O-TF1	42	8					
	25	7					
O-TF2	34	1					
	20	4					
O-SS1	1	0					
	0	0					
O-SS2	0	0					
	0	0					
O-GT1	222	48					
	155	44					
O-GT2	178	37					
	156	33					

Samples	log(avg control)	log (avg count)	log reduction
D-TF1	9.02	5.16	3.87
D-TF2	9.02	5.09	3.93
D-SS1	8.70	4.82	3.88
D-SS2	8.70	4.64	4.05
D-GT1	10.38	5.73	4.65
D-GT2	10.38	5.68	4.70
O-TF1	9.02	4.04	4.99
O-TF2	9.02	3.72	5.31
O-SS1	8.70	1.70	7.00
O-SS2	8.70	#NUM!	#NUM!
O-GT1	10.38	4.81	5.57
O-GT2	10.38	4.71	5.67

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Witnessed & Understood by me, _____ Date _____

Invented by: Kristi C Cooper Date 8/26/09

Recorded by: _____

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From Page No. ____ Date: 8/27/09

Run 2: BA w/wash step + ozone

Effect of 7% Ozone on *Bacillus Anthracis (sterne)* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF							4	1	9.50E+08
							5	0	
Conrol - SS							7	0	1.05E+09
							4	1	
Control - GT							7	0	1.25E+09
							8	1	
D-TF1		86	6						1.40E+05
		64	7						
D-TF2		64	7						1.37E+05
		60	8						
D-SS1		61	6						1.10E+05
		58	4						
D-SS2		28	1						3.90E+04
		20	2						
D-GT1		253	95						1.28E+06
		273	109						
D-GT2		212	97						1.01E+06
		206	63						
O-TF1	1	0							1.50E+02
	2	0							
O-TF2	12	2							3.15E+03
	11	2							
O-SS1	1	0							5.00E+01
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	156	30							4.29E+04
	121	28							
O-GT2	297	103							1.96E+05
	316	228							

Samples	log(avg control)	log (avg count)	log reduction	
D-TF1	8.98	5.15	3.83	3.84
D-TF2	8.98	5.14	3.84	
D-SS1	9.02	5.04	3.98	4.21
D-SS2	9.02	4.59	4.43	
D-GT1	9.10	6.11	2.99	3.04
D-GT2	9.10	6.00	3.09	
O-TF1	8.98	2.18	6.80	6.14
O-TF2	8.98	3.50	5.48	
O-SS1	9.02	1.70	7.32	#NUM!
O-SS2	9.02	#NUM!	#NUM!	
O-GT1	9.10	4.63	4.46	4.13
O-GT2	9.10	5.29	3.80	

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Witnessed & Understood by me, _____ Date _____

Invented by: Ruth C. Cooper Date 8/27/09

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From Page No. ____ Run 2: BC w/wash step + ozone

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose After a Previous Wash Step
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF							13	0	7.35E+09
							10	1	
Conrol - SS							17	5	5.35E+09
							20	2	
Control - GT							19	2	3.70E+09
							15	2	
D-TF1		43	7						1.08E+05
		43	6						
D-TF2		22	1						3.00E+04
		18	1						
D-SS1		30	4						5.40E+04
		23	3						
D-SS2		29	6						8.10E+04
		23	5						
D-GT1		282	83						1.12E+06
		214	92						
D-GT2		222	83						8.57E+05
		212	45						
O-TF1	0	0							0.00E+00
	0	0							
O-TF2	0	0							0.00E+00
	0	0							
O-SS1	0	0							0.00E+00
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	10	1							1.35E+03
	7	0							
O-GT2	11	2							2.55E+03
	10	1							

Samples	log(avg control)	log (avg count)	log reduction
D-TF1	9.87	5.03	4.83
D-TF2	9.87	4.48	5.39
D-SS1	9.73	4.73	5.00
D-SS2	9.73	4.91	4.82
D-GT1	9.57	6.05	3.52
D-GT2	9.57	5.93	3.64
O-TF1	9.87	#NUM!	#NUM!
O-TF2	9.87	#NUM!	#NUM!
O-SS1	9.73	#NUM!	#NUM!
O-SS2	9.73	#NUM!	#NUM!
O-GT1	9.57	3.13	6.44
O-GT2	9.57	3.41	6.16

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Witnessed & Understood by me, _____ Date

Invented by: Kurt C Cooper

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Date 8/27/09

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From Page No. ____ Run 3: BC w/wash step + ozone

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF							6	0	1.40E+09
							12	1	
Conrol - SS							10	4	3.90E+09
							8	2	
Control - GT							11	2	2.50E+09
							9	1	
D-TF1		40	5						6.70E+04
		34	8						
D-TF2		63	16						2.27E+05
		51	18						
D-SS1		24	5						5.95E+04
		25	2						
D-SS2		28	2						4.65E+04
		25	2						
D-GT1		283	99						1.34E+06
		293	112						
D-GT2		233	92						1.09E+06
		212	81						
O-TF1	0	0							0.00E+00
	0	0							
O-TF2	0	0							0.00E+00
	0	0							
O-SS1	0	0							0.00E+00
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	127	52							5.53E+04
	139	32							
O-GT2	145	25							4.31E+04
	157	31							

Samples	log(avg control)	log (avg count)	log reduction
D-TF1	9.15	4.83	4.32
D-TF2	9.15	5.36	3.79
D-SS1	9.59	4.77	4.82
D-SS2	9.59	4.67	4.92
D-GT1	9.40	6.13	3.27
D-GT2	9.40	6.04	3.36
O-TF1	9.15	#NUM!	#NUM!
O-TF2	9.15	#NUM!	#NUM!
O-SS1	9.59	#NUM!	#NUM!
O-SS2	9.59	#NUM!	#NUM!
O-GT1	9.40	4.74	4.66
O-GT2	9.40	4.63	4.76

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Witnessed & Understood by me, _____ Date _____

Invented by: Kim C. Cooper

Recorded by: _____ Date 8/28/09

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From Page No. ____ Run 4: BC w/wash step + ozone Date: 9/1/09

Effect of 7% Ozone on *Bacillus Cereus* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF							10	0	1.00E+09
							10	0	
Conrol - SS							8	0	2.10E+09
							14	2	
Control - GT							10	3	3.85E+09
							7	3	
D-TF1		23	3						4.40E+04
		25	1						
D-TF2		9	3						1.65E+04
		4	1						
D-SS1		44	3						8.10E+04
		38	5						
D-SS2		29	3						4.75E+04
		26	1						
D-GT1		39	3						5.25E+04
		50	8						
D-GT2		73	15						1.92E+05
		80	8						
O-TF1	0	0							0.00E+00
	0	0							
O-TF2	0	0							0.00E+00
	0	0							
O-SS1	0	0							0.00E+00
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	4	1							9.50E+02
	5	0							
O-GT2	9	1							2.45E+03
	10	2							

Samples	log(avg control)	log (avg count)	log reduction
D-TF1	9.00	4.64	4.36
D-TF2	9.00	4.22	4.78
D-SS1	9.32	4.91	4.41
D-SS2	9.32	4.68	4.65
D-GT1	9.59	4.72	4.87
D-GT2	9.59	5.28	4.30
O-TF1	9.00	#NUM!	#NUM!
O-TF2	9.00	#NUM!	#NUM!
O-SS1	9.32	#NUM!	#NUM!
O-SS2	9.32	#NUM!	#NUM!
O-GT1	9.59	2.98	6.61
O-GT2	9.59	3.39	6.20

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Witnessed & Understood by me, Date Invented by: Teri C Coop Date

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From Page No. ___ Run2! BT w/wash step + ozone Date: 9/2/0

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF				60	8				1.71E+07
				62	14				
Conrol - SS				51	8				1.32E+07
				63	7				
Control - GT				63	14				1.47E+07
				50	4				
D-TF1	0	0							5.00E+01
	1	0							
D-TF2	1	0							1.00E+02
	1	0							
D-SS1	2	0							2.00E+02
	2	0							
D-SS2	1	0							1.00E+02
	1	0							
D-GT1	4	1							8.00E+02
	2	0							
D-GT2	1	1							9.00E+02
	7	1							
O-TF1	0	0							0.00E+00
	0	0							
O-TF2	0	0							0.00E+00
	0	0							
O-SS1	0	0							0.00E+00
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	1	0							5.00E+01
	0	0							
O-GT2	2	0							1.00E+02
	0	0							

Samples	log(avg control)	log (avg count)	log reduction
D-TF1	7.23	1.70	5.53
D-TF2	7.23	2.00	5.23
D-SS1	7.12	2.30	4.82
D-SS2	7.12	2.00	5.12
D-GT1	7.17	2.90	4.26
D-GT2	7.17	2.95	4.21
O-TF1	7.23	#NUM!	#NUM!
O-TF2	7.23	#NUM!	#NUM!
O-SS1	7.12	#NUM!	#NUM!
O-SS2	7.12	#NUM!	#NUM!
O-GT1	7.17	1.70	5.47
O-GT2	7.17	2.00	5.17

Witnessed & Understood by me, _____ Date _____

Invented by: Kui C. Cooper

Recorded by: _____ Date _____

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From Page No. ____ Run 3: BT w/wash step + ozone Date: 9/3/09

Effect of 7% Ozone on *Bacillus Thuringiencis* Embedded in Sucrose After a Previous Wash Step

Coupons: Stainless Steel 316, Glazed Tile, Teflon

Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - TF				47	8				1.13E+07
				49	5				
Conrol - SS				83	15				1.90E+07
				56	9				
Control - GT				50	9				1.20E+07
				39	6				
D-TF1	1	0							5.00E+01
	0	0							
D-TF2	1	0							5.00E+01
	0	0							
D-SS1	1	0							5.00E+01
	0	0							
D-SS2	2	0							1.05E+02
	0	0							
D-GT1	18	0							2.30E+03
	18	1							
D-GT2	13	1							4.70E+03
	1	7							
O-TF1	0	0							0.00E+00
	0	0							
O-TF2	0	0							0.00E+00
	0	0							
O-SS1	0	0							0.00E+00
	0	0							
O-SS2	0	0							0.00E+00
	0	0							
O-GT1	1	0							5.00E+01
	0	0							
O-GT2	0	0							0.00E+00
	0	0							

Samples	log (avg control)	log (avg count)	log reduction
D-TF1	7.05	1.70	5.35
D-TF2	7.05	1.70	5.35
D-SS1	7.28	1.70	5.58
D-SS2	7.28	2.02	5.26
D-GT1	7.08	3.36	3.72
D-GT2	7.08	3.67	3.41
O-TF1	7.05	#NUM!	#NUM!
O-TF2	7.05	#NUM!	#NUM!
O-SS1	7.28	#NUM!	#NUM!
O-SS2	7.28	#NUM!	#NUM!
O-GT1	7.08	1.70	5.38
O-GT2	7.08	#NUM!	#NUM!

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Witnessed & Understood by me, | Date | Invented by: Kim C. Ong | Date

Recorded by:

Project No. _____

Book No. _____ TITLE _____

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Run 4: BT w/ wash step + ozone (GT only)

Effect of 7% Ozone on *Bacillus Thuringiensis* Embedded in Sucrose After a Previous Wash Step
Coupons: Stainless Steel 316, Glazed Tile, Teflon
Contact Time: 120mins

Samples	2	3	4	5	6	7	8	9	avg count
Control - GT				50	14				1.58E+07
				46	8				
D-GT1	14	1							2.95E+03
	25	1							
D-GT2	16	1							2.90E+03
	22	1							
O-GT1	1	0							5.00E+01
	0	0							
O-GT2	2	0							2.00E+02
	2	0							

Samples	log(avg control)	log (avg count)	log reduction
D-GT1	7.20	3.47	3.73
D-GT2	7.20	3.46	3.74
O-GT1	7.20	1.70	5.50
O-GT2	7.20	2.30	4.90

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Summary of Ozone Effect after Wash Step

Bacillus anthracis (sterne)

Coupon Type	Ozone Effect after Rinse Step (cfu/ml)	Overall Ozone Effect (cfu/ml)
Teflon	1.92	5.31
	1.25	5.15
	2.30	6.14
Stainless Steel	-	>7.0
	-	>7.0
	-	>7.0
Glazed Tile	1.4	4.81
	0.94	5.62
	1.09	4.13

Bacillus thuringiensis

Coupon Type	Ozone Effect after Rinse Step (cfu/ml)	Overall Ozone Effect (cfu/ml)
Teflon	-	> 5.0
	-	> 5.0
	-	> 5.0
Stainless	-	> 5.0
	-	> 5.0
	-	> 5.0
Glazed	1.82	6.4
	1.08	5.32
	1.47	5.2

Bacillus cereus

Coupon Type	Ozone Effect after Rinse Step (cfu/ml)	Overall Ozone Effect (cfu/ml)
Teflon	-	> 7.0
	-	> 7.0
	-	> 7.0
Stainless Steel	-	> 7.0
	-	> 7.0
	-	> 7.0
Glazed Tile	2.72	6.3
	1.39	4.71
	1.86	5.4

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essed & Understood by me,

Date

Invented by: Hui C. Cao

Recorded by:

Date

Comparison Tables of Ozone Gas' Effect on Various Food Contact Surfaces Embedded Containing Sucrose Embedded with Bacillus Spores

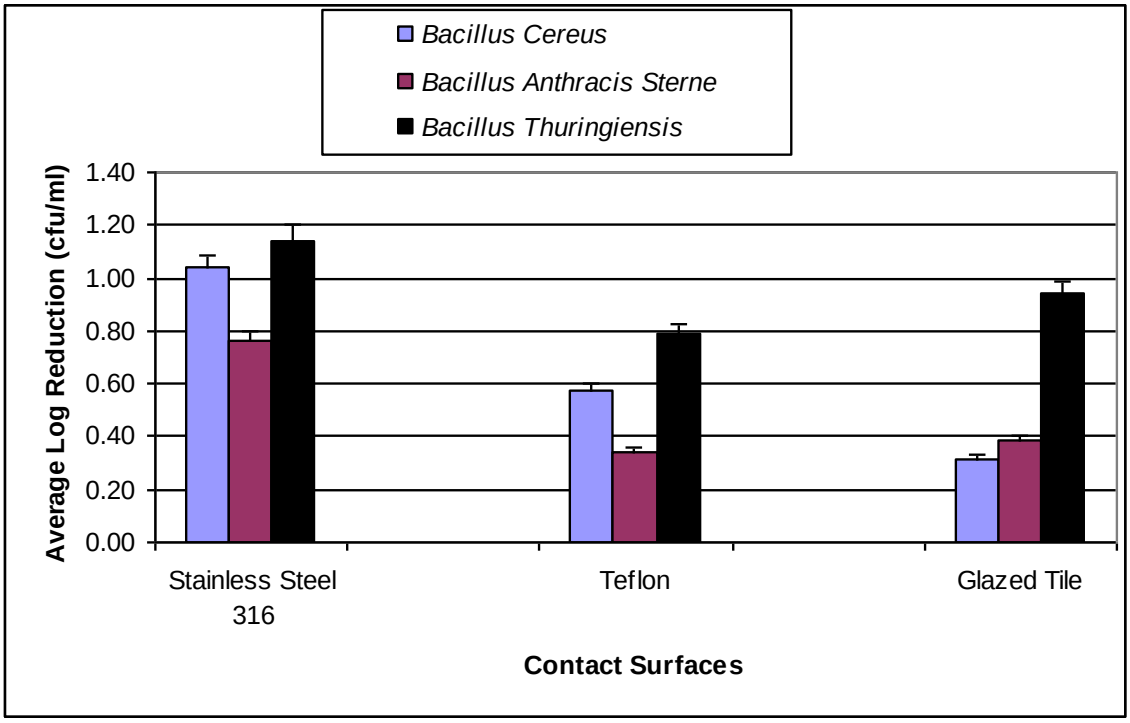

Figure 4.5. Log_{10} Reduction of $7\%_{wt}$ Ozone on *B. anthracis sterne*, *B. cereus*, and *B. thuringiensis* Embedded in Sucrose on Stainless Steel 316, Glazed Tile, and Teflon

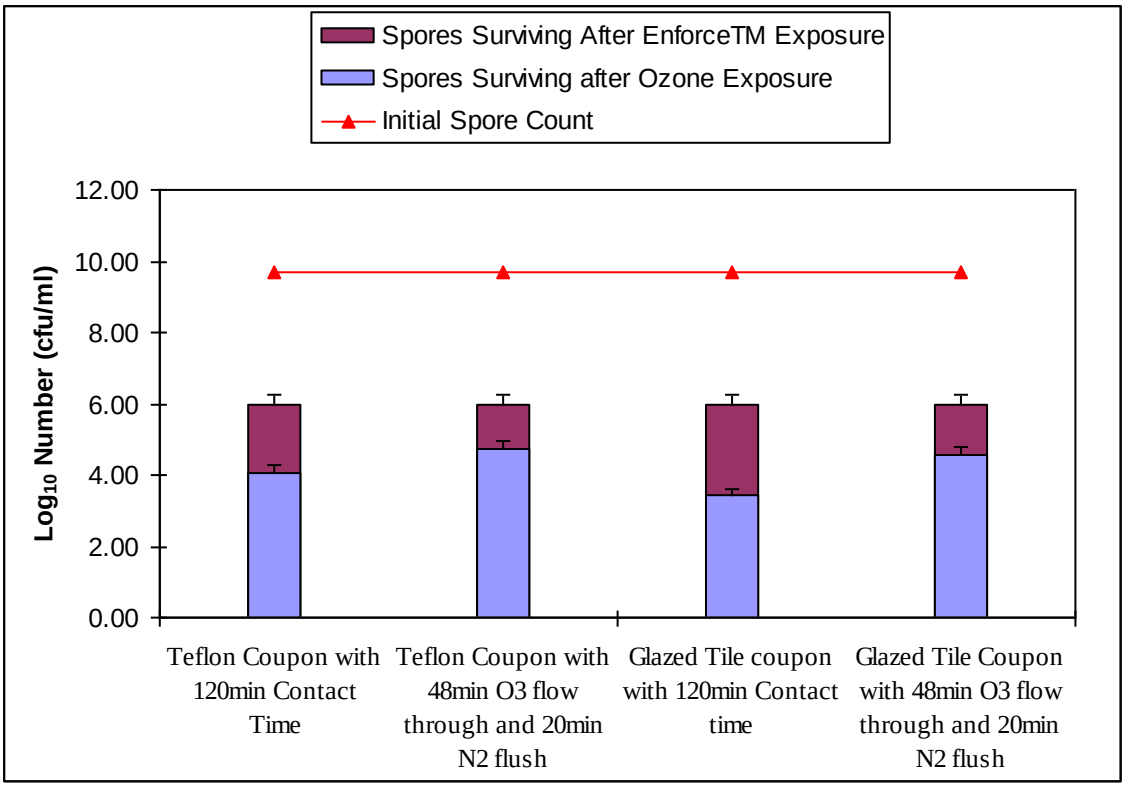

Figure 4.6. Log_{10} number of Surviving *B. anthracis* sterne 34F2 Embedded In Sucrose on Teflon and Glazed Tile Coupons after Enforce™ and 120min O_3 Exposure or 48min Ozone Flow Time and 20min Nitrogen Flush

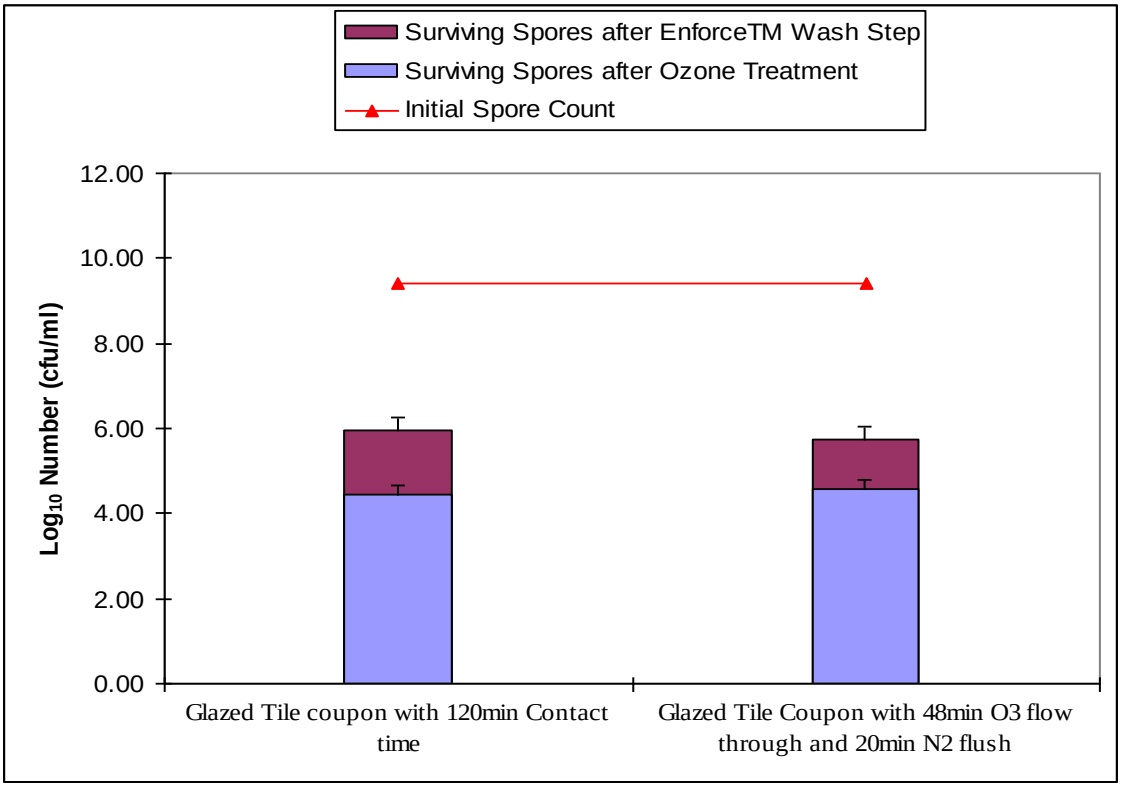

Figure 4.7. Log_{10} number of Surviving *B. cereus* ATCC 21281 Spores Embedded in Sucrose on Glazed Tile Coupons after Enforce^{TM} and 120min O_3 Exposure or 48min Ozone Flow Time and 20min Nitrogen Flush

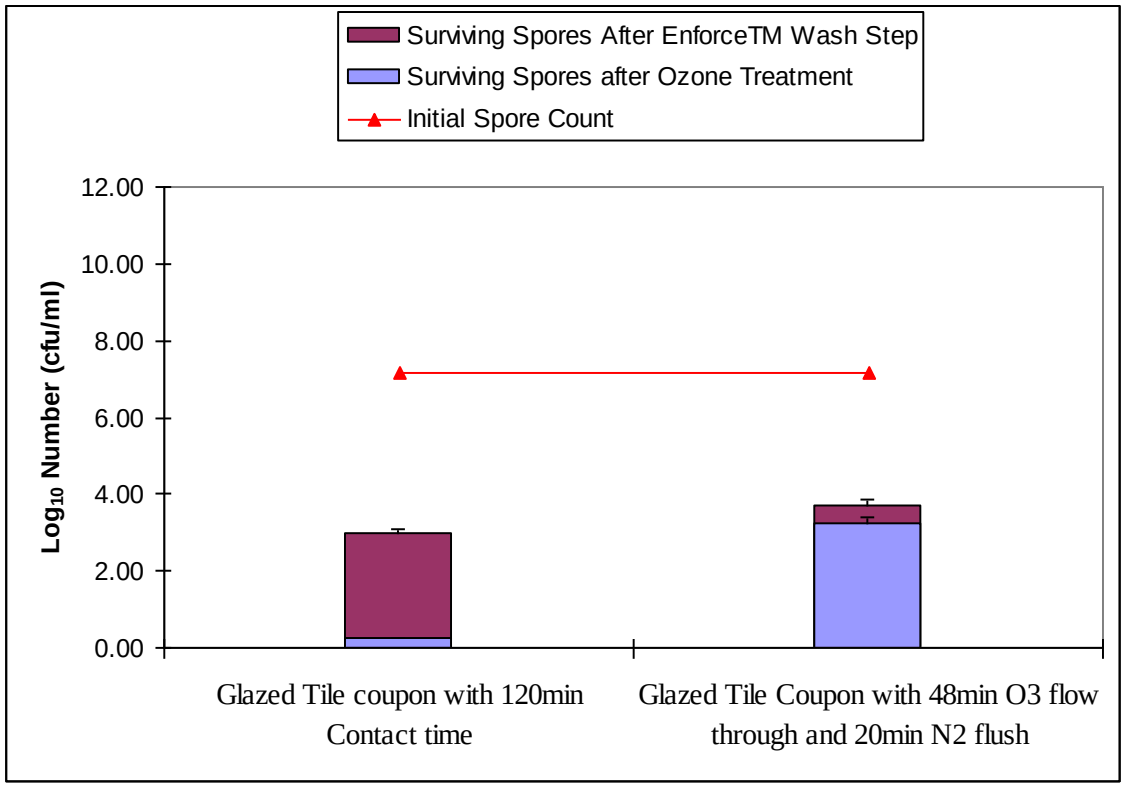

Figure 4.8. Log_{10} number of Surviving *B. thuringiensis* ATCC 33680 Spores Embedded in Sucrose on Glazed Tile Coupons after Enforce™ and 120min O_3 Exposure or 48min Ozone Flow Time and 20min Nitrogen Flush