Biological Safety Cabinet (BSC) Profiled Decontamination Validation Report

Gaseous Decontamination with Chlorine Dioxide Gas

Report Number: BIO21021

Date of Report: April 7, 2021

Biosafety Pty Ltd – Hygiene & Decontamination Services
Unit 15, 69 Acacia Road, Ferntree Gully Victoria Australia 3156
Phone: 1300 379 996 Fax: 1300 371 199
Email: info@biosafety.com.au Website: www.biosafety.com.au

Title: Biological Safety Cabinet (BSC) Profiled Decontamination Validation Report	
Document Type: Decontamination Validation Report	**Report Number**: BIO20121
	Page: 2 of 15 (Plus Appendices)
	Date: April 7, 2021

Executive Summary

Biosafety Pty Ltd commissioned a validation study of biological safety cabinet decontamination with chlorine dioxide gas (ClorDiSys Solutions Inc) on 16/03/21. The validation study was undertaken to satisfy the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text - Section 16.5 (b)* requirements for a profiled gaseous decontamination process to suit 1.2m biological safety cabinets utilizing the ClorDiSys ChemCD 6.5 decontamination kit.

Realtime chemical monitoring data demonstrated a greater than 720 ppm-hour exposure to Chlorine Dioxide gas. This exposure is normally adequate to provide a 6-log sporicidal reduction (Czarneski, 2010) of the test species and meets the target parameters for Chlorine dioxide gas in *Section 16.4* of the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text.*

Biological indicator validation undertaken as per *Section 16.6* of the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text* returned results of negative for growth. The fumigation cycle showed all the Biological Indicators (BI's) placed in the areas were negative for growth thereby demonstrating greater than a 6-log reduction and that the decontamination cycle performed was successful.

ChemCD 6.5 chlorine dioxide generation kits used to decontaminate 1.2m wide BSCs satisfy the DAWE requirements for a profiled gaseous decontamination when conducted utilizing the methodology contained within this report.

Table of Contents

Introduction

The Australian Department of Agriculture, Water, and Environment (DAWE) recently released updated guidance on Biosecurity containment level 2 (BC2) facility operation, including updated guidance on decontamination requirements for facilities.

Section 16.3 of the new *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text* requires Biological Safety Cabinets (BSC) to be decontaminated in the following circumstances.

- Prior to filter removal
- Prior to performance testing
- When there is a significant change in use or risk of accumulated biological contamination
- Before a cabinet is relocated
- After a serious spill or similar contamination incident within the cabinet, and
- Prior to decommissioning or replacement

Biosafety Pty Ltd conducted a validation study of biological safety cabinet decontamination with chlorine dioxide gas (ClorDiSys Solutions Inc) on 16/03/21. The validation study was undertaken to satisfy the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text - Section 16.5 (b)* requirements for a profiled gaseous decontamination process to suit 1.2m biological safety cabinets utilizing the ClorDiSys ChemCD 6.5 decontamination kit.

The Biological Safety Cabinet used for this validation was located on site at the Biosafety head office. The ClorDiSys (New Jersey, USA) ChemCD 6.5 chlorine dioxide decontamination method was used and approaches every gaseous decontamination project to obtain a 6-log sporicidal reduction equivalent.

The chlorine dioxide (CD) decontamination process consists of the following steps; humidification to soften the spore walls (Relative Humidity, RH > 65%), the introduction of chlorine dioxide (CD) gas into the area to reach the desired concentration, a dwell period, called exposure, where the gas sits for a period of time to obtain the desired kill level and finally aeration to remove the gas.

The humidification range to soften the spore walls, is 60% or higher with an optimal level of 65 – 70%. The exposure level and time that the ChemCD 6.5 kit targets to obtain a 6-log sporicidal reduction is >2 mg/litre (720ppm) for 1 hour. This equates to a >720 ppm-hours of exposure which was completed for the unit used during validation. ClorDiSys has demonstrated 6-log sporicidal reduction as low as 450 ppm-hours. At the end of the exposure period, the gas is aerated until the concentration dropped to or below 0.1 ppm. This is the 8-hour safety level as well as the odour threshold level (Safe Work Australia, 2011).

The Chlorine dioxide (CD) concentration was monitored throughout the process via the EMS, which is located outside of the unit decontaminated. Sample tubing was connected via decontamination ports to interior of the chamber which allowed for continuous samples to be drawn from the unit so that the gas concentration was constantly monitored throughout the cycle.

Method

Material and Equipment

The following equipment was used for the decontamination:

Qty. 1 EMS Electronic Monitoring System (ClorDiSys).

Qty. 1 6.5 Chem-CD Chlorine Dioxide generation kit

Qty. 1 ATI CD gas low level sensor (Portasense).

Qty. 1 Recirculation Unit

Qty. 1 Activated Carbon Scrubber

Qty. 1 Tape & Plastic

Decontamination Procedure

Biosafety began preparation on the 16/03/21 including assessment of the area. Following the planning and discussion, Biosafety set up of gaseous decontamination equipment. Photos of all works were taken and are located in Appendix I.

One ChemCD 6.5 chlorine dioxide generation kit was used inject gas into the unit. Biological indicators were distributed according to *Section 16.6* of the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text* (*Geobacillus stearothermophilus* – Crosstex Medical - Product Code TCDS-06, Batch No. RU103, expiry 31/03/22). After setup and sealing was complete the CD gassing process began. The area was checked for leaks using an ATI CD Sensor. Active airflow obtained from the recirculation unit was used to ensure gas penetration of both laminar & exhaust HEPA filters.

When exposure was completed (>720 ppm-hours), the external equipment was packed away. Staff waited until confident that effective exposure was achieved then exhausted the gas until, readings were at or below 0.1ppm (TLV SafeWork Australia, 2012) when the device was deemed safe to enter/open.

The plastic sealing was removed and the BI's gathered for processing. Biological indicators were processed by Biosafety and incubated for 7 days at 56°C as per *Section 15.2* of the *DAWE Approved Arrangement 5.2 – Biosecurity containment level 2 (BC2) Informative Text*.

Biological Indicator Incubation

All Biological Indicators were prepared using good aseptic technique as per manufacturer's recommendations (Crosstex Medical, USA). Biological indicators were processed by opening the Tyvek pouch by peeling the flaps apart with gloved hands (disinfected with 70% Ethanol v/v). Spore strip was then removed from the packaging and transfer to individual tubes of modified growth medium, product code GMPCP-100. Each tube was labeled according to the sample identification number. Tubes were incubated at 56°C for 7 days and results checked at completion of time period.

Results

Figures 1 & 2 show the real time monitoring data for chlorine dioxide gas & accumulated exposure throughout the cycle. Table 1 gives the 7-day biological indicator results obtained from the cycle.

Chlorine Dioxide EMS Monitoring Data

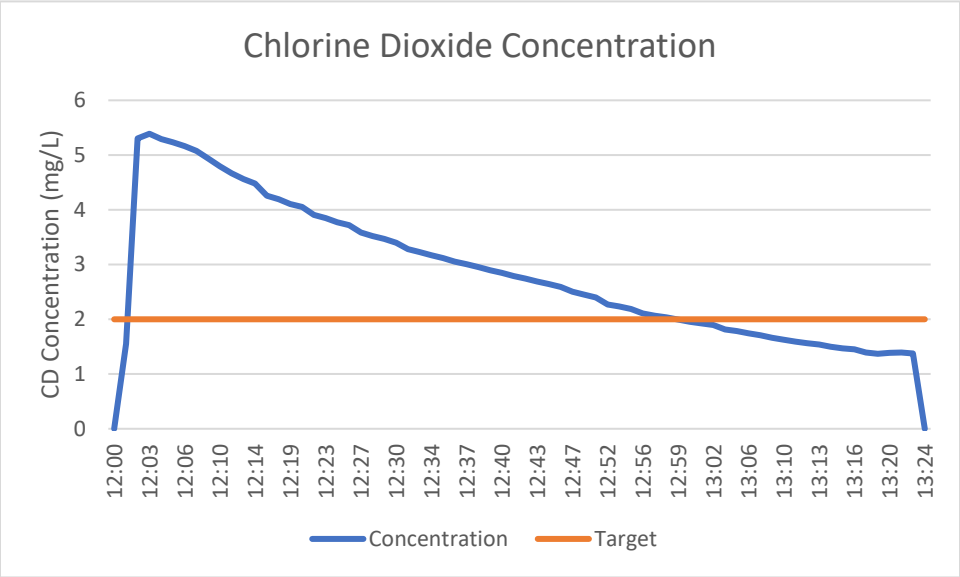


Figure 1: Chlorine Dioxide Concentration Graph

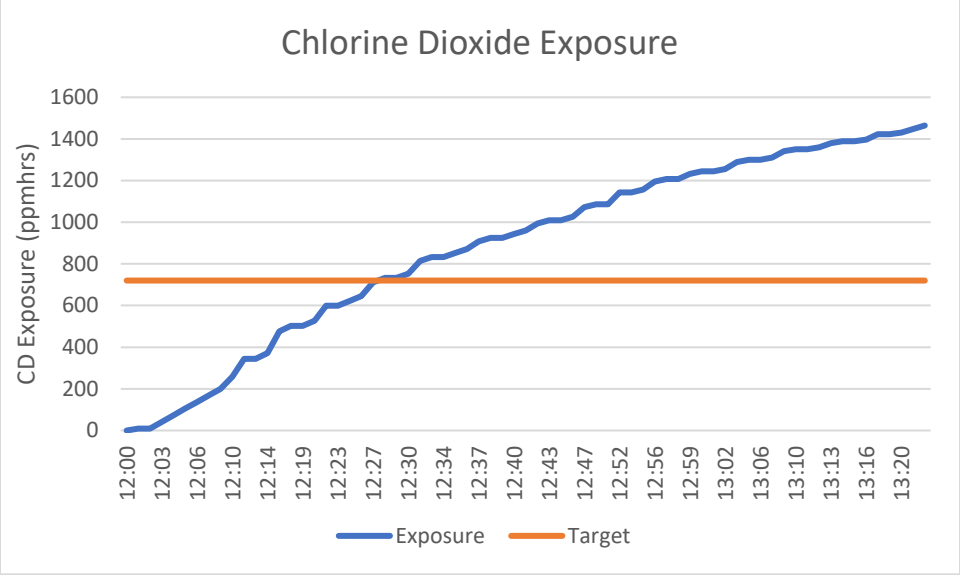


Figure 2: Chlorine Dioxide Exposure Graph

Biological Indicator Results

Table 1: Biological Indicator Results

BSC Decontamination Validation	Growth Status (Pairs) 7 day results	
Sample Pair	1	2
A – Work Floor	Negative	Negative
B - Sump	Negative	Negative
C – Rear Wall	Negative	Negative
D – Downstream Filter Guard – Laminar	Negative	Negative
E – Downstream Filter Guard - Exhaust	Negative	Negative
Controls	Positive	Positive

In all cases, in excess of 720 ppm-hour exposure to Chlorine dioxide gas was acheived which is required for a 6-log sporicidal reduction & to meet DAWE requirements. This was achieved for the validation cycle.

Discussion

The results demonstrate the fumigation cycle achieved the required level of Chlorine dioxide concentration (mg/L) and exposure (ppm-hours) for a standard biological decontamination cycle (720ppm-hours). The Biological Indicators (BI) results from the fumigation on 16/03/21 indicated that all BIs were negative for growth therefore it was assumed that the fumigation cycle performed was successful.

Statistical analysis was completed using the formulas developed by Luftmann et al. (2008) and these are shown below. In all cases a minimum mean statistical log reduction of 6.1 was achieved at a 95% confidence interval. Luftmann's methodology indicates in cycles where all BIs were inactivated, there is a 95% confidence interval that a log-6.1 reduction was achieved and a 50% confidence interval that a log-6.8 reduction was achieved.

Table 2: Biological Indicator Statistical Analysis

BSC Decontamination Validation	Growth Status (Pairs) – 7 days		Confidence Interval	
Sample Pair	1	2	95% Confidence	50% Confidence
A – Work Floor	Negative	Negative	6.1	6.8
B - Sump	Negative	Negative	6.1	6.8
C – Rear Wall	Negative	Negative	6.1	6.8
D – Downstream Filter Guard – Laminar	Negative	Negative	6.1	6.8
E – Downstream Filter Guard - Exhaust	Negative	Negative	6.1	6.8
Mean Log Reduction			**6.1**	**6.8**

Conclusion

For the decontamination validation performed with a ChemCD 6.5 chlorine dioxide gas generation kit (ClorDiSys Solutions Inc) on a 1.2m wide BSC, the Biological Indicator results from the fumigation on 16/03/21 were negative for growth for all locations. The results of the decontamination cycle performed yielded a greater than 720 ppm-hr decontamination exposure to chlorine dioxide gas which is normally adequate to provide a 6-log sporicidal reduction (Czarneski, 2010). The fumigation showed all the Biological Indicators (BI's) placed in the areas were negative for growth thereby demonstrating greater than a 6-log reduction and that decontamination cycle performed was successful. ChemCD 6.5 chlorine dioxide generation kits used to decontaminate 1.2m wide BSCs satisfy the DAWE requirements for a profiled gaseous decontamination when conducted utilizing the methodology contained within this report.

Attachments:

Appendix I - Images of decontamination fumigation

Report Revisions

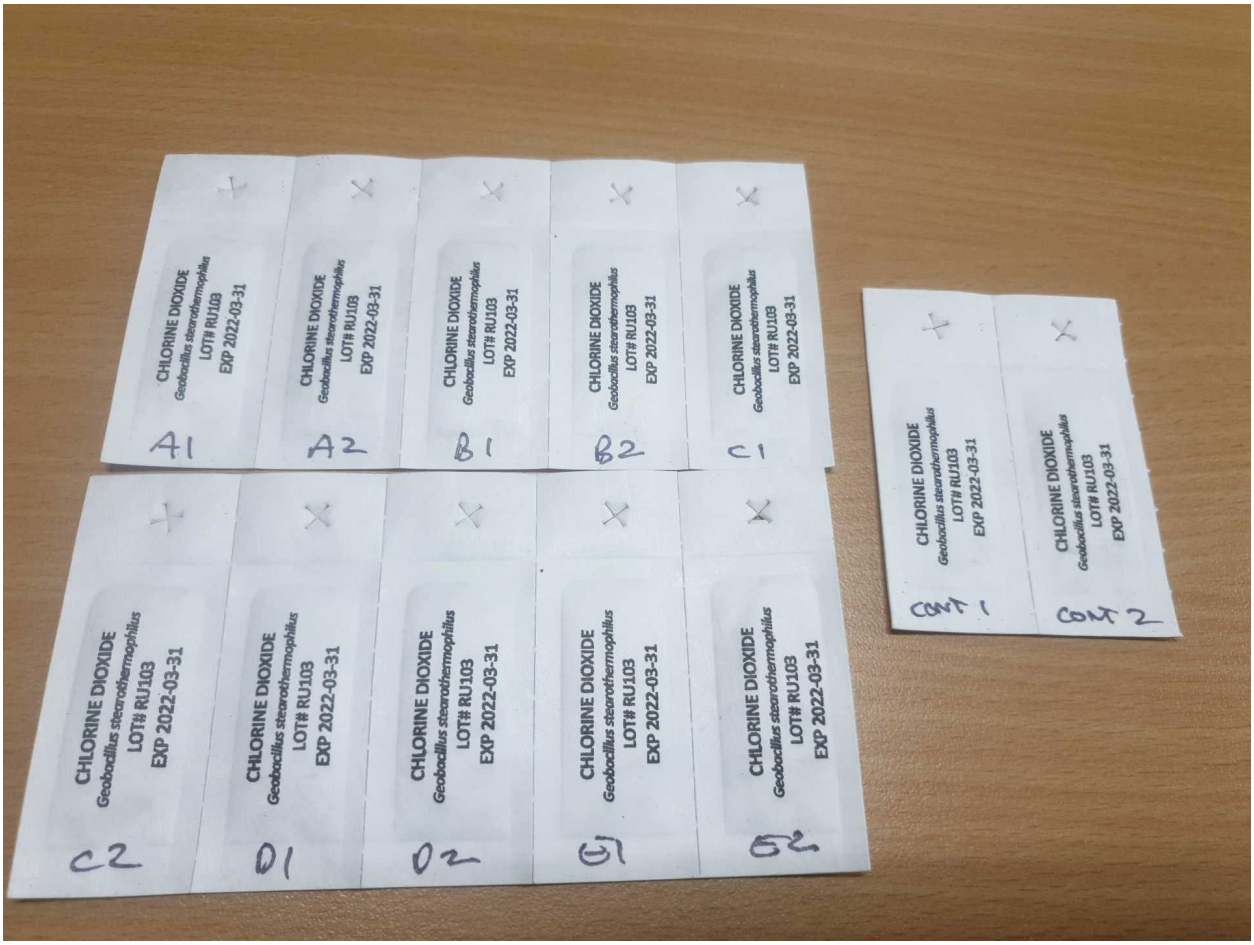
Revision Number	Date	Details
Revision 1	April 7, 2021	

Approvals

Final Report Approvals			
Name	**Title**	**Signature**	**Date**
Brett Cole, B.Sc (Hons) Dip. Bus. Man. M. Occ. Hyg. & Tox. CBP (IFBA) MAIOH MABSANZ MISPE MACPIC	Managing Director – Biosafety		Report – April 7, 2021
Cameron Welch, B.HSc, CBP (IFBA)	Senior Hygiene Technician / Project Manager - Biosafety		Report – April 7, 2021

Appendix I – Images

Decontamination Validation Set Up – Sealed BSC with recirculation blower, EMS real time gas monitoring

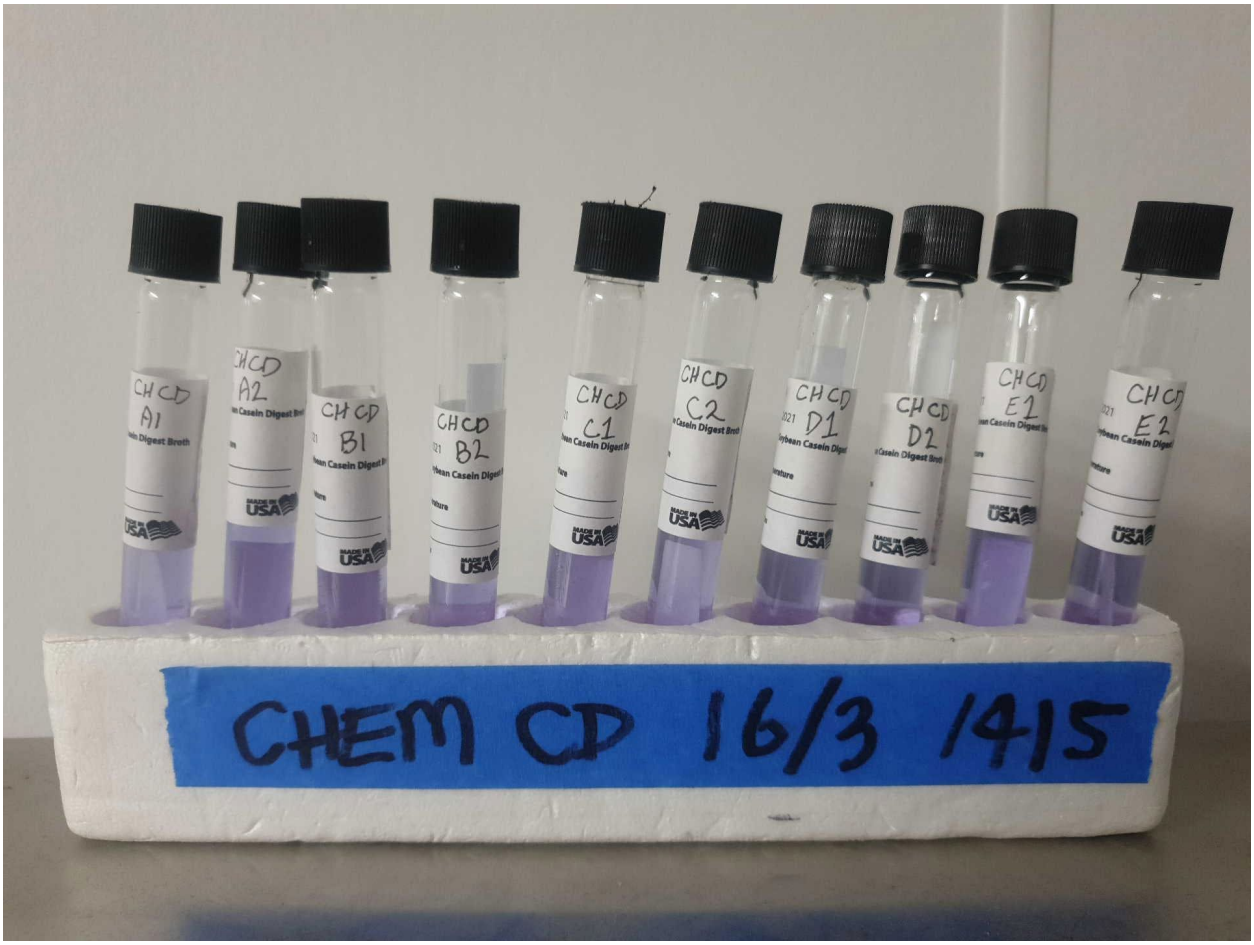


Biological Indicator Pairs Prior to Placement

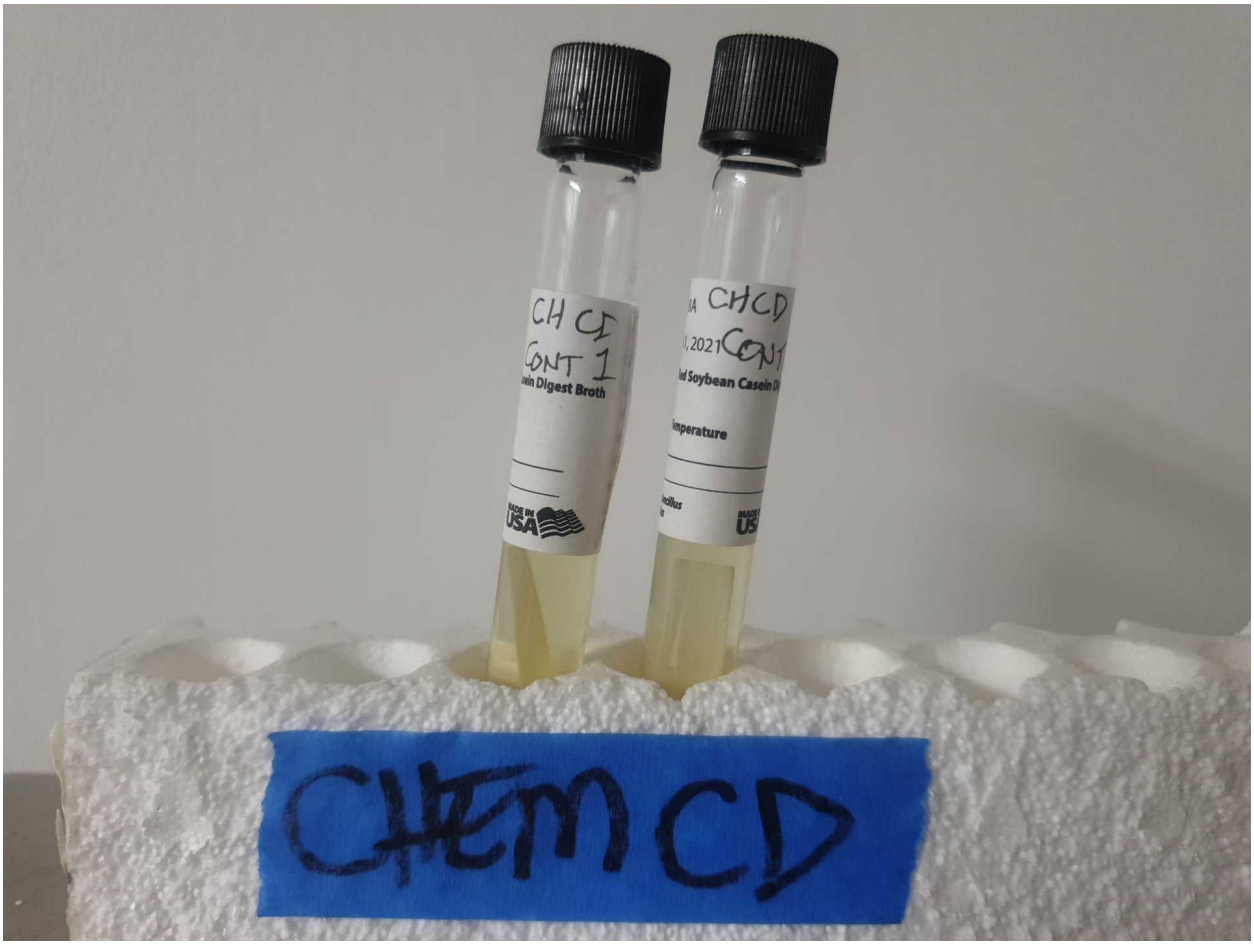
Biosafety Pty Ltd – Hygiene & Decontamination Services
Unit 15, 69 Acacia Road, Ferntree Gully Victoria Australia 3156
Phone: 1300 379 996 Fax: 1300 371 199
Email: info@biosafety.com.au Website: www.biosafety.com.au

ClorDiSys ChemCD 6.5 Chlorine Dioxide Generating Kit

Exemplar EMS monitoring screen. Top left cell demonstrating 1.43 mg/L current concentration, 1405.85 ppm-hrs total accrued exposure



Biological indicator results for treated samples, purple media colour indicates no growth



Biological indicator results for control pair – yellow media colour indicates a pH change as result of growth

END OF REPORT

Biosafety Pty Ltd – Hygiene & Decontamination Services
Unit 15, 69 Acacia Road, Ferntree Gully Victoria Australia 3156
Phone: 1300 379 996 Fax: 1300 371 199
Email: info@biosafety.com.au Website: www.biosafety.com.au