CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2020
DateRun:	11/11/2020
Experimenters:	Alicia McCarthy, Zoe Lawson
ClientType:	
ProjectNumber:	Project #2
Substrates:	Stainless Steel
PartType:	Coupon
Contaminants:	
Cleaning Methods:	Pour Plate
Analytical Methods:	Organism count
Purpose:	To evaluate the Skrubbr ECA device on MS2 Bacteriophage.

Experimental Procedure:

The Skrubbr ECA device was prepared using one scoop of salt, 400ml of tap water, and one full cycle. Total chlorine of the tap water, pre-cycle, and post cycle using a Lovibond MD100 instrument set to the CLHR mode. The pH and total dissolved solids (TDS) of the tap water, pre-cycle, and post cycle were measured using an APERA Instrument. Each run consisted of one contact time on one surface and had 26 plates that included two of each of the following dilutions: Negative 1:1; Positive 1:1, 1:10, 1:100, 1:1000; Test 1 1:1, 1:10, 1:100, 1:1000; and Test 2 1:1, 1:10, 1:100, 1:1000.

Pour Plate Method - MS2 Bacteriophage

Six hours prior to one run (26 plates), E.coli 15597 was sub cultured into three milliliters of tryptic soy broth (TSB) screw-cap tubes and incubated at 37°C (98.6°F). Four glass petri dishes, each containing one stainless steel coupon, along with 27 screw-cap tubes filled with 10ml of 0.5X tryptic soy agar (TSA) were autoclaved. The biosafety cabinet (BSC) was sprayed with 70% v/v isopropyl alcohol using a paper towel before spraying any items going into the BSC. Once autoclaving was complete, the TSA tubes were placed into a 45°C (113°F) D.I. water bath inside biosafety cabinet (BSC). The four glass petri dishes were marked using a black sharpie to designate the positive (P+), negative (N-), Test 1 (T1), and Test 2 (T2). Ten microliters of the organism was pipetted onto the P+, T1, and T2 stainless steel coupons and air dried for 15 minutes. A motorized pipette with 10ml tips was used to pipet 15 ml of Dey-Engley (D/E) neutralizing broth into four separate 50ml conical tubes labeled P+, N-, T1, and T2. Once the MS2 bacteriophage dried on the coupons, the P+ coupon was placed into the conical tube. The N-, T1, and T2 were pipetted with 1000µl of the Skrubbr generated solution onto each coupon for 30 seconds before immediately placing them in the conical tube with an autoclaved forceps. The conical tubes were then placed on the shaker for 10 minutes. During this time, using the 1000ml pipette, 900ml of 1x phosphate-buffered saline (PBS) was pipetted into nine autoclaved dilution tubes, and serial dilutions were made for P+, T1, and T2 up to 10^{-4} using 100µl of the shaken D/E broth.

When the six hour sub time was complete, the E. coli 15597 subculture was removed from the incubator for use. For each variable (N-, P+, T1, and T2), 100µl of the stock and serial dilutions of MS2 bacteriophage, and 100µl of the E.coli 15597 subculture were combined into an empty dilution tube. A screwcap tube of 0.5X TSA was removed from the water bath, wiped with a paper towel to remove moisture, and poured into the dilution tube. The mixture was immediately poured into a sterile polystyrene petri dish; swirled to cover the entire plate surface; and then air dried before covering. Dried petri dishes were placed into a clean labelled zip lock bag that was partially closed and incubated at 37°C overnight. Plates were counted the following day based on the clear lysis zones in the bacterial lawn of growth (1 plate forming unit) to calculate log reduction and percent removal.

Results:

Type	TDS (ppm)	pH	Total Cl (ppm)
Tap Water	1.58	7.54	11
Pre Cycle	1.98	7.47	30
Post Cycle	2.1	8.43	134

The following table shows the results from the disinfection run.

Plate	Dilution	Count #1	Count #2
Negative Control	1:1	0	0
Positive Control	1:1	TNTC	TNTC
	1:10	140	95
	1:100	12	13
	1:1000	8	3
Test 1	1:1	1	0
	1:10	0	0
	1:100	0	0

CLEANING LABORATORY EVALUATION SUMMARY

	1:1000	0	0
Test 2	1:1	1	0
	1:10	0	0
	1:100	0	0
	1:1000	0	0

TNTC = Too Numerous to Count

Calculated Log Reduction: 3.3630

Calculated Percent (%) Reduction: 99.9574

Summary:

Substrates:	Stainless Steel				
Contaminants:					
Company Name:	**Product Name:**	**Conc.:**	**Efficiency:**	**Effective:**	**Observations:**
KOHI Plus	Skrubbr Multipurpose Cleaner	134 ppm	99.95	☑	

Conclusion: Skrubbr device was 99.96% effective at a 3.36 Log Reduction with a 30 second contact time on stainless steel coupons.