final report

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Spray cabinet E.coli intervention project to maintain market access to US and other markets

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Executive Summary

The animal hide (skin) is one of the most significant sources for microbial contamination during animal slaughter and carcass processing. In most cases, the deep tissues of healthy livestock at the time of slaughter are bacteriologically sterile and contamination is introduced onto the meat surfaces during the dressing process.

TwinOxide is a unique sanitizer with advanced delivery system used in food processing, providing approximately 99.9% pure chlorine dioxide solution in a 0.3% concentration without by-products. It is applied as a drinking water disinfectant mainly for washing steps in food industries including the meat industries. TwinOxide reacts to 99.9% pure chlorine dioxide solution with a kinetic halftime as a biocide of 30 days. It is safe to handle and simple to apply, using standard available dosing equipment and without the use of a reactor.

A carcase spray cabinet has been installed to apply TwinOxide to beef bodies post sticking and prior to dressing to reduce TVC, E.coli and coliform prevalence both on the hide and on cutting lines across the carcase.

The spray cabinet consist of two rows of spray nozzles aimed at the hind legs, anus, rump and midline. As bodies enter the cabinet, a sensor triggers a solenoid, activating the application of TwinOxide for a pre-set timeframe.

The most effective configuration of spray volume and time to get suitable coverage without excess runoff was 6.5L/minute nozzles across the top row and 3L/minute nozzles across the bottom row with a spray time of 7 seconds per body.

Validation testing indicated that all beef hides that were treated with TwinOxide in cabinet showed statistically significant reductions in total *E. coli* counts (0.41 $\log_{10}$ reduction), with respect to the control (no washing with TwinOxide). However, the results showed that the current cabinet treatment does not effectively reduce the total bacterial counts on beef hides.

Further review indicates that the installation of a well-designed TwinOxide cabinet has negligible effects on blood recovery or a facilities wastewater treatment system.

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1 Background

The animal hide (skin) is one of the most significant sources for microbial contamination during animal slaughter and carcass processing. In most cases, the deep tissues of healthy livestock at the time of slaughter are bacteriologically sterile and contamination is introduced onto the meat surfaces during the dressing process.

To ensure export market access is safeguarded, processes must ensure all steps are taken to reduce the risk of STEC E.coli contamination.

TwinOxide is a unique sanitizer with advanced delivery system used in food processing, providing approximately 99.9% pure chlorine dioxide solution in a 0.3% concentration without by-products. It is applied as a drinking water disinfectant mainly for washing steps in food industries including meat industries. TwinOxide reacts to 99.9% pure chlorine dioxide solution with a kinetic halftime as a biocide of 30 days. It is safe to handle and simple to apply, using standard available dosing equipment and without the use of a reactor.

A carcase spray cabinet has been installed to apply TwinOxide to beef bodies post sticking and prior to dressing to reduce Total Viable Count (TVC), E.coli and coliform prevalence both on the hide and on cutting lines across the carcase.

2 Project Objectives

The objectives of this project were to:

- Design a spray cabinet, suitable for applying TwinOxide solution to carcasses
- Determine optimal spray pattern and volume configuration
- Investigate the effectiveness of the TwinOxide OFF versus TwinOxide ON treatment on microbial load including total viable count and E. coli on hides.
- Investigate the effect on blood recovery for value adding
- Investigate the effect of the system on the wastewater treatment system

3 Methodology

3.1 Cabinet Design

The cabinet was designed to fit within a park station on the bleed chain stacker. The cabinet is 1.8m wide, 1.8m long and 4.3m high. The cabinet was constructed of 2mm stainless steel panels supported by a 32mm rectangular hollow section frame. The design includes two 200mm diameter exhaust vents attached to an extraction fan mounted on the outside roof to remove spray mist and vapours.

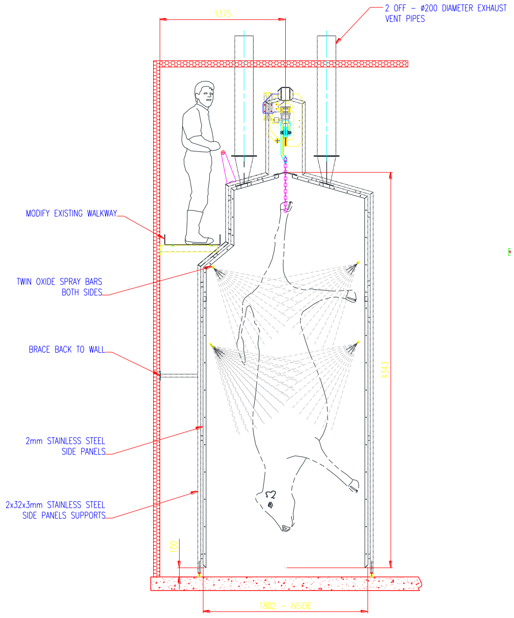
Due to varying cattle sizes, either end of the cabinet was left open to minimise the risk of bodies clashing with the cabinet.

Post installation, an additional tray was installed in the base of the cabinet to capture and segregate overspray and runoff from the blood collection system.

Figure 1: Spray Cabinet nozzle arrangement

Figure 2: Original Spray Cabinet

Figure 3: Modified cabinet with runoff segregation



3.2 Cabinet Configuration & Optimisation

Preliminary trials were undertaken between 14 June 2016 and 23 June 2016. The purposes of the trials were to determine optimal spray nozzle flow rates, spray angle and spray time.

The facility applies a 60ppm TwinOxide solution to the anus and rump area of beef bodies after knocking using a manual handheld spray gun. All spray cabinet trials were undertaken using the same 60ppm TwinOxide solution.

To enable the optimisation of the spray configuration, the effectiveness of the TwinOxide cabinet was assessed by swabbing 100cm^2 of the hide (HO) and along cutting lines (CL) as the hide is removed. Tests were conducted on bodies of cattle within the same run that had TwinOxide applied through the spray cabinet and also bodies that did not have TwinOxide applied. The swabs were analysed for differences in TVC, E.coli prevalence and coliform prevalence.

Figure 4: HO Pre-Spray Cabinet Swab Test

Figure 5: HO Post Spray Cabinet Swap Test

Figure 6: CL without TwinOxide applied to hide

Figure 7: CL after TwinOxide application to hide

3.2.1 Trial 1

The initial configuration of the TwinOxide cabinet utilised 8 spray nozzles with 1L/minute flow rate. Each body would be sprayed for approximately 3.5 seconds. The spray produced a fine mist of TwinOxide solution resulting in light surface coverage across the hide.

Results from the swab tests showed no discernible difference in TVC, E.coli prevalence or coliform prevalence for either HO or CL swabs under Trial 1 conditions.

Observations made during the swabbing indicated that the fine mist was not penetrating into the coarse hairs on the hide, rather only providing a very light coating on the outer surface.

3.2.2 Trial 2

As a result of swab tests and observations from Trial 1, the spray nozzles were changed to provide larger droplet size and greater volume of TwinOxide. The spray time was also increased from 3.5 seconds to 5 seconds. The upper row of 4 nozzles had a flow rate of 3L/minute while the lower 4 nozzles were sized at 2L/minute to ensure minimal runoff from the carcase.

The second set of spray heads produced a much heavier spray and appeared to provide better coverage and wetting of the hide with TwinOxide solution

During the first day of Trial 2, the HO results displayed limited effect between sprayed and non-sprayed carcases. This may be influenced by the level of faecal and other matter on the hides due to the climatic conditions at the time of the trial. The CL swabs demonstrated a reduction in the TVC although this was not significant either statistically or microbiologically, but did demonstrate a reduction in coliform prevalence.

A second set of swabs showed no significant difference in either HO or CL.

3.2.3 Optimisation

Further testing determined the most effective configuration of spray volume and time to get suitable coverage without excess runoff was 6.5L/minute nozzles across the top row and 3L/minute nozzles across the bottom row with a spray time of 7 seconds per body. This spray configuration was utilised for the independent validation trials conducted by the University of Adelaide in September 2016.

3.3 Validation Testing

Independent validation testing was conducted by the University of Adelaide. 60 animals from different cattle types were randomly grouped into either TwinOxide cabinet ON or TwinOxide cabinet OFF.

The right brisket area (10cm x 10cm) was swabbed using 3M Sponge-Sticks before TwinOxide treatment. After treatment, the left brisket and left shank were swabbed using separate sterile sponges. After swabbing, the each sponge was placed in 3 mL bag containing 0.1% peptone water. Collected samples (Table 1) from each group were transport to the lab for microbial enumeration.

Table 1: Experimental Design for swab samples of hides pre and post Twin Oxide

Cattle Type	**Cabinet OFF TwinOxide**		**Cabinet ON TwinOxide**	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Grass-fed	Brisket (n=10)	Brisket (n=10) Shank (n=10)	Brisket (n=20)	Brisket (n=20) Shank (n=20)
Grain-fed and *Feedlot*	Brisket (n=20)	Brisket (n=20) Shank (n=20)	Brisket (n=10)	Brisket (n=10) Shank (n=10)

4 Results

4.1 Validation Testing

The total viable counts and *E. coli* counts for cabinet OFF and cabinet ON are shown in Figures 8A and 8B respectively. The results revealed that cabinet OFF treatment had no effect on the reduction of total viable and *E. coli* counts (Figure 8 A and 9 A). The use of the cabinet ON showed that TwinOxide® treatment did not reduce the total viable count on the hide of beef carcasses in comparison to cabinet OFF (Figure 8 B and 9 B). However, the results did confirm TwinOxide treatment resulted in a significant reduction in *E. coli* counts (0.41 $\log_{10}$ reduction) post treatment in comparison to control samples (Figure 8 Ba

nd 2 B). Cabinet ON treatment had no effected in the reduction of total viable and *E. coli* counts for the shank region (Figure 2 C).

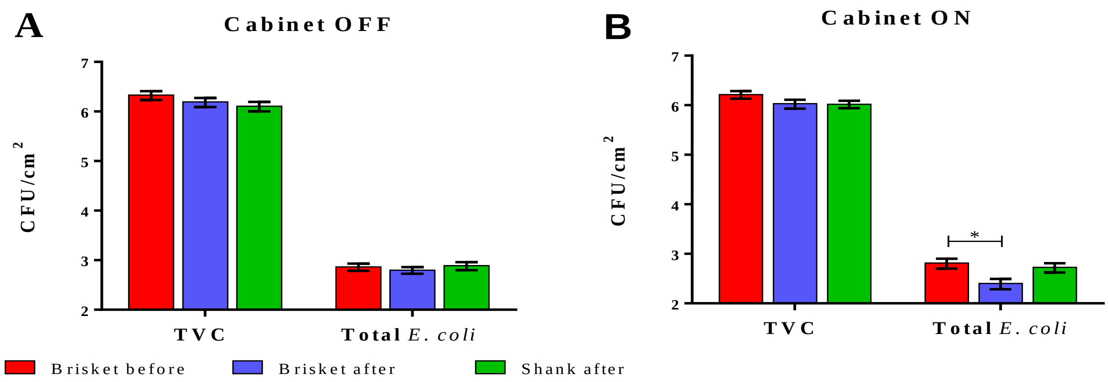


Figure 8: Effect of the cabinet OFF and cabinet ON TwinOxide ($\log_{10}$ CFU/cm^2)

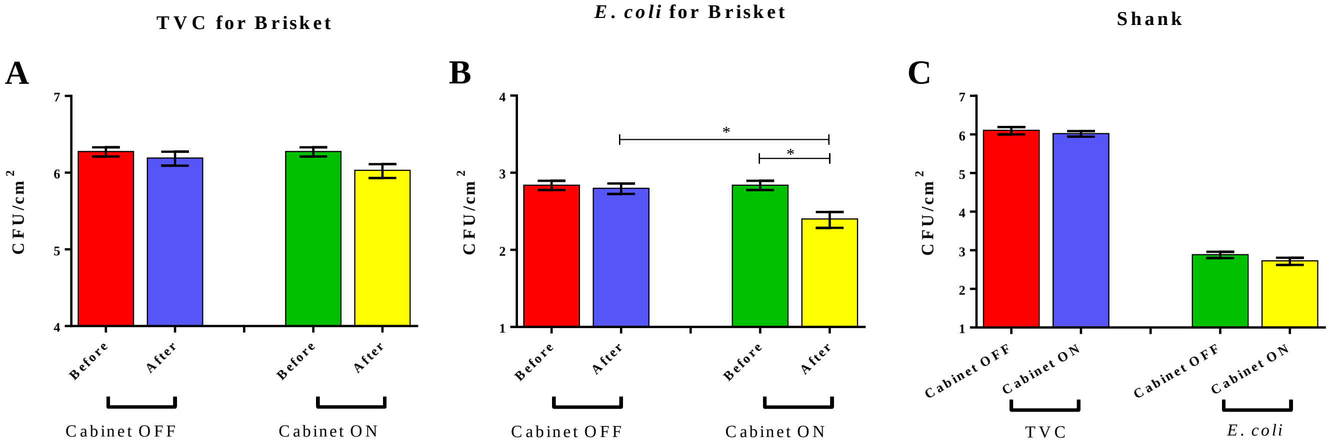


Figure 9: Effect of the cabinet OFF versus cabinet ON TwinOxide ($\log_{10}$ CFU/cm^2)

Overall, the results indicate that with the TwinOxide cabinet ON, there is a significant reduction (0.41 $\log_{10}$ reduction) in *E.coli* counts in comparison to the cabinet OFF treatment.

4.2 Effects on Blood Recovery

The installation of the Twin Oxide cabinet had an immediate effect on blood recovery. Feedback received from the by-products department indicated that blood yield had dropped at the same time as the commissioning of the twin-oxide cabinet.

An experiment was set up to determine the effect of twin oxide on blood. A 400ml sample of blood was mixed with 1ml, 2ml, 5ml and 50ml of twin oxide solution and compared to a control with no twin oxide added after 30 minutes and 60 minutes. The samples were observed for coagulation and congealing effects. There was no noticeable increase in coagulation or congealing of blood between the samples compared to the control.

	30 Minutes	**60 Minutes**
Control	No Change	No Change
1ml Twin Oxide	No Change	No Change
2ml Twin Oxide	No Change	No Change
5ml Twin Oxide	No Change	No Change
50ml Twin Oxide	No Change	No Change

Figure 10: Control Sample

Figure 11: 50ml Twin Oxide

Following the experiment, a review of the spray system was undertaken and a conclusion drawn that the reduction in blood recovery was due to the volume of additional water (through overspray and runoff) entering the blood capture system rather than the twin oxide chemical itself.

The selected spray configuration results in approximately 4.5L of solution applied to each body. It was estimated that between 30-50% of this ended up in the blood recovery system as a result of overspray and runoff. The additional water load caused reduced performance of the blood coagulation, decanting and drying process resulting in loss of blood solids to the effluent stream and a reduction in blood yield.

To reduce the impact of the twin-oxide spray cabinet, a tray was fabricated and installed below the cabinet to capture any initial overspray and excess runoff of the solution. The tray was connected to a separate drainage system which was direct to the effluent stream as opposed to the blood recovery stream.

The capture system was effective in reducing the volume of excess solution entering the blood capture system and as a result, blood yields returned to expected levels.

Figure 12: Spray cabinet before capture tray

Figure 13: New capture tray and drain

4.3 Effects on Covered Anaerobic Lagoons

Post installation of the twin oxide cabinet, TFI's wastewater treatment system was closely monitored. Fortnightly sampling of the covered anaerobic lagoons (CALs) showed no discernible change in key parameters being COD, BOD, Volatile Fatty Acids and Total Alkalinity..

An estimated maximum of 2.25L of twin oxide solution per body is added to the effluent stream, equating to approximately 2,025L per day. This volume is negligible compared to the daily effluent volume of the site of approximately 3.5ML at the time of trials. The volume of twin oxide solution added to the effluent steam is approximately 0.05% of the daily flow. As a result, no adverse impacts to the CALs are expected from the use of Twin Oxide.

5 Conclusions/Recommendations

All beef hides that were treated with TwinOxide in cabinet showed statistically significant reductions in total *E. coli* counts (0.41 $\log_{10}$ reduction), with respect to the control (no washing with TwinOxide®). The results showed that the current cabinet treatment does not effectively reduce the total bacterial counts on beef hides.

Excess runoff and overspray from a spray cabinet can have impacts on blood recovery by increasing moisture content. A well designed runoff capture system can avert such issues.

There is negligible impact on wastewater from the use of the TwinOxide cabinet in this facility due to the small volume of runoff in relation to the volume of wastewater treated.

The installation of a TwinOxide cabinet must be accompanied by other good hygiene, sanitary and dressing practices to reduce potential for *e.coli* transfer from the hide to carcase during dressing, as there are minimal effect on TVCs.

6 Bibliography

Khazandi, Manouchehr et al. *Effect Of TwinOxide Treatment On Microbial Load Hide During Cattle Slaughter*. 2016. Print.

7 Appendix – Raw Data

Cabinet ON					**Cabinet OFF**				
			TVC	**TEC**				**TVC**	**TEC**
Cow 1-10	1	A	1070000	34	**Cow 11-20**	31	A	520000	1050
Grass-fed		B	1400000	29	Grass-fed		B	100000	480
A Brisket before wash		C	990000	13			C	450000	660
Brisket after wash	2	A	1800000	2900		32	A	210000	1020
Shank afte rwash		B	1600000	680			B	100000	240
		C	1700000	1380			C	200000	330
	3	A	3670000	1580		33	A	740000	330
		B	3700000	860			B	200000	450
		C	230000	280			C	690000	390
	4	A	1730000	400		34	A	1350000	960
		B	450000	170			B	190000	1080
		C	850000	180			C	1150000	390
	5	A	3480000	300		35	A	2880000	750
		B	2280000	740			B	710000	1110
		C	1600000	120			C	1580000	570
	6	A	650000	1450		36	A	1700000	780
		B	750000	2220			B	2100000	270
		C	130000	390			C	1200000	360
	7	A	3310000	2670		37	A	7000000	330
		B	2250000	1960			B	760000	660
		C	1200000	750			C	3850000	1740
	8	A	3450000	770		38	A	5800000	1170
		B	1100000	1700			B	670000	2850
		C	2800000	340			C	3700000	900
	9	A	3800000	1990		39	A	450000	1110
		B	1600000	1160			B	510000	1080
		C	3600000	820			C	400000	270
	10	A	4600000	280		40	A	5800000	1410
		B	2100000	990			B	2400000	690
		C	4600000	810			C	2100000	1980
Cow 21-40	11	A	3500000	170	**Cow 41-60**	41	A	500000	110
Grain-fed and Feedlot		B	1600000	84	Grain-fed and Feedlot		B	550000	320
		C	1650000	13			C	340000	230
	12	A	3000000	207		42	A	60000	360
		B	2900000	142			B	180000	350
		C	2800000	89			C	90000	310
	13	A	1350000	35		43	A	470000	510
		B	1400000	41			B	440000	680
		C	700000	60			C	530000	490
	14	A	1900000	250		44	A	160000	90
		B	1500000	480			B	240000	400
		C	1750000	30			C	150000	280
	15	A	260000	104		45	A	120000	870
		B	240000	108			B	110000	300
		C	110000	32			C	120000	130
	16	A	380000	78		46	A	470000	220
		B	200000	88			B	340000	420
		C	380000	31			C	140000	160
	17	A	440000	71		47	A	220000	190
		B	500000	125			B	250000	160
		C	220000	71			C	210000	310
	18	A	440000	53		48	A	600000	500
		B	190000	73			B	480000	500
		C	180000	21			C	550000	460
	19	A	2400000	24		49	A	330000	570
		B	2200000	61			B	110000	480
		C	2300000	53			C	300000	440
	20	A	560000	161		50	A	650000	110
		B	100000	149			B	160000	160
		C	400000	100			C	580000	690
	21	A	100000	770		51	A	33000	500
		B	130000	540			B	32000	660
		C	60000	13			C	34000	880
	22	A	620000	45		52	A	13000	320
		B	860000	32			B	18000	110
		C	400000	29			C	10000	880
	23	A	250000	390		53	A	115000	250
		B	80000	500			B	10000	290
		C	50000	250			C	15000	520
	24	A	5000000	260		54	A	21000	410
		B	3900000	64			B	19000	230
		C	2400000	71			C	10000	3000
	25	A	480000	700		55	A	180000	2490
		B	300000	175			B	140000	800
		C	170000	290			C	270000	3030
	26	A	33000	400		56	A	260000	620
		B	32000	210			B	280000	770
		C	34000	610			C	310000	170
	27	A	36000	340		57	A	180000	2390
		B	38000	340			B	230000	1300
		C	21000	240			C	150000	540
	28	A	18000	2000		58	A	180000	2320
		B	15000	2200			B	500000	930
		C	16000	105			C	200000	680
	29	A	440000	150		59	A	250000	370
		B	410000	168			B	190000	60
		C	500000	162			C	60000	1760
	30	A	400000	1140		60	A	250000	300
		B	450000	1200			B	150000	530
		C	340000	210			C	80000	140