EDR001010:

VERIFICATION OF THE CELLAR CONTROL

FINAL REPORT TO HUNTER TECHNOLOGIES

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EcoDiagnostics

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EXECUTIVE SUMMARY

The CellarControl System uses sweeping sound frequencies to reduce the need of beer line cleaning. While the system is not a substitute for cleaning, it slows down the growth of biofilm, which reduces the need to conduct a beer line clean. A normal beer line will require cleaning every 1-2 weeks, in comparison a CellarControl treated beer line only requires a clean every 6 weeks. Hunter Technologies have enlisted EcoDiagnostics to show that the beer line in a CellarControl venue will be of the same quality or better then a non cellarcontrol prior to the beer line clean.

To verify the efficacy of the CellarControl System and the claims made by Hunter Technologies, EcoDiagnostics examined beer samples from five different lines over two six week cycles. Beer samples were tested for the presence of lactic acid bacteria (LAB) and wild yeasts which are known to contaminate beer lines. In addition, a second venue was examined that didn't use CellarControl but was cleaned every two weeks. Results of the CellarControl treat beer lines show that over a six-week period LAB and wild yeast counts increase directly after the clean, but over the subsequent weeks and up to the next clean the counts stay below the previous clean.

An additional two rounds of testing was conducted to examine the CellarControl System after 6 weeks of use and then directly after the line was cleaned. Analysis of the results show that after 6 weeks 67.5% of samples had <10 CFU/ mL of LAB and wild yeasts. Furthermore there was no significant difference (p.value 0.9286) between the samples from the CellarControl System running for 6 weeks and the samples taken directly after a chemical clean.

The testing demonstrates that bacteria and wild yeast levels remained low up until the 6 week clean and verifies the claims made by Hunter Technologies over the use of the CellarControl. Future testing should examine the CellarControl System at nine and 12 week periods as the data here suggests that would continue to inhibit biofilm growth after the 6 weeks.

METHODOLOGY

VENUE LOCATION

The venue chosen was a venue that would give the testing credibility within the marketplace. It was a venue with multiple bars with line lengths varying from short to long compared to average venues in the Australian Marketplace. The system had been installed for 12 months and the beer lines cleaned with Bracton DP1 & DP2 for 3 hours every six weeks. A second venue that didn't use the CellarControl System but cleaned their beer lines with Bracton DP1 & DP2 for 3 hours every two weeks was used as a control group. The two additional sites were the OBH and Vic on the Park.

SAMPLING AND MICROBIOLOGICAL TECHNIQUES

Beer samples were collected from each beer line and stored in a 120mL microbiological container. These samples were stored in ice and transported to ALS (accreditation number 1247) for analysis. Each beer sample was tested on two types of media, RakaRay and MYGP under the following conditions.

Table 1. Microbiological plating condtions for RakaRay and MYGP + Cu.

Plate	Purpose	Incubation	Climate
RakaRay	Lactic Acid species	25°±1C / 120±3 hrs	Microareophilic
MYGP + Cu	Wild Yeast	25°±1C / 120±3 hrs	Microareophilic

T-Test analysis of the results were conducted according to AS/NZS 4659.2.1999.

RESULTS

CELLAR CONTROL SYSTEM

T-TEST

To examine the efficacy of CellarControl system before and after a clean a T-test was performed according to AS/NZS 4659.2.1999. The T-test result was -23.2468 which suggests there is significant difference between the samples taken prior and after the chemical clean. The significant difference was due to the increased LAB and wild yeasts counts after the clean.

WEEK BY WEEK COMPARISON

The average wild yeast and LAB counts from the five beer lines over four sampling events can be observed in Figure 1. The results show that directly after the first clean (Rep1 Week 1) the average LAB and wild yeast count was 38.8 CFU / mL and 56 CFU / mL respectively. At week 4 the wild yeast and LAB had decreased to 7.8 and 1 CFU / mL respectively then slightly increased when sampled again at week 6.

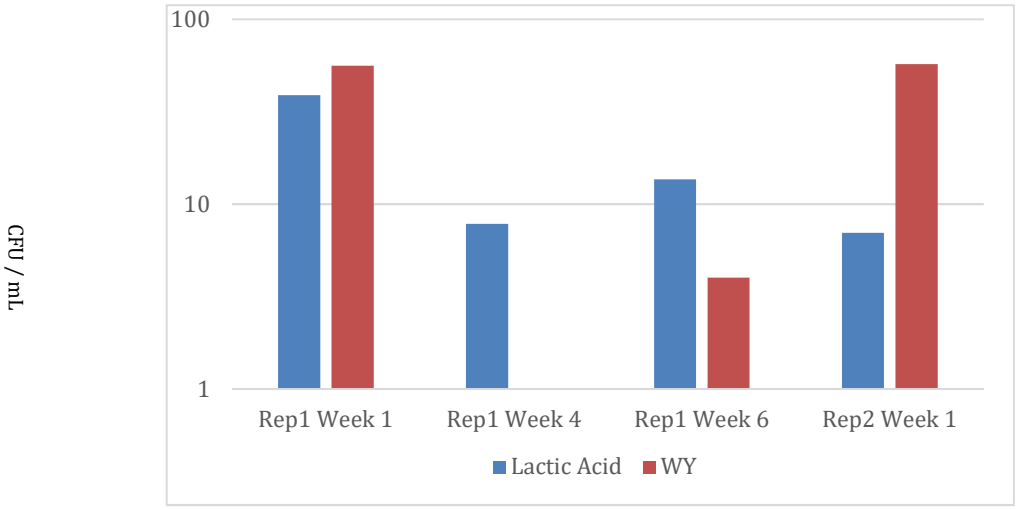

Figure 1. The average wild yeast and lactic acid counts from five beer lines by week.

Sampling after the second clean (Rep2 Week1) showed an increase in wild yeast counts but no significant difference in LAB counts. The likely cause of the increase in counts after a clean is possibly due to the venue performing a "flowback" while cleaning. Flowback is where the beer from lines is returned to the beer kegs, lines are cleaned then the lines refilled. This could contaminate the kegs with bacteria and wild yeast, resulting in a spike in counts levels directly after a chemical line clean. The results show that wild yeast and LAB counts at week 4 and 6 were <14 CFU / mL.

NON-CELLAR CONTROL SYSTEM

WEEK BY WEEK COMPARISON

Compared to the CellarControl system, samples from five beer lines at the non-CellarControl venue had an average wild yeast and LAB count of 526 CFU / mL and <1 CFU /, respectively, prior to the clean. Directly after the two part bracton clean, wild yeast counts significantly decreased to an average of 94.6 CFU / mL, LAB counts remained unchanged. Unfortunately, analysis of the "prior to clean" samples weren't conducted within correct holding times which may have caused an artificially increase the WY counts. Regardless, the after clean results from the non-CellarControl system

were significantly higher than both Rep1 Week 4 and Week 6 samples taken at the CellarControl Venue.

CELLAR CONTROL SYSTEM ADDITIONAL SAMPLING

An additional two sets of samples where taken to increase the sample size of the experiment. In total, 80 samples were taken, 40 after six weeks of Cellar Control usage and 40 directly after the Cellar control systems were cleaned. A T-Test was used to determine if the two datasets where significantly difference. Analysis showed that there was no significant difference (p-value=0.9286) between the two data sets.

CONCLUSION

The testing demonstrates that the following:

1. Bacteria and Wild Yeast levels remained low up until the 6 week clean.
2. Increased sampling showed that there was no significant different (p.value 0.9286) between the samples from the CellarControl System running for 6 weeks and the samples taken directly after a chemical clean.
3. Verifies the claims made by Hunter Technologies over the use of the CellarControl.

APPENDIX 1

Beer Line	Organism	Plate	Method	Rep	Week 1	Week 3	Week 6
Tooheys	WY	MYGP	FM0109	1			10
Coopers	WY	MYGP	FM0109	1			10
Carlton Draught	WY	MYGP	FM0109	1			23
Rechs	WY	MYGP	FM0109	1			10
Hahn Super Dry	WY	MYGP	FM0109	1			46
Tooheys	Lactic Acid	RakaRay	FM0124	1			300
Coopers	Lactic Acid	RakaRay	FM0124	1			23
Carlton Draught	Lactic Acid	RakaRay	FM0124	1			1
Rechs	Lactic Acid	RakaRay	FM0124	1			8
Hahn Super Dry	Lactic Acid	RakaRay	FM0124	1			1
Tooheys	WY	MYGP	FM0109	2	240	1	1
Coopers	WY	MYGP	FM0109	2	10	1	11
Carlton Draught	WY	MYGP	FM0109	2	10	1	1
Rechs	WY	MYGP	FM0109	2	10	1	3
Hahn Super Dry	WY	MYGP	FM0109	2	10	1	4
Tooheys	Lactic Acid	RakaRay	FM0124	2	110	16	43
Coopers	Lactic Acid	RakaRay	FM0124	2	72	20	20
Carlton Draught	Lactic Acid	RakaRay	FM0124	2	3	1	3
Rechs	Lactic Acid	RakaRay	FM0124	2	7	1	1
Hahn Super Dry	Lactic Acid	RakaRay	FM0124	2	2	1	1
Tooheys	WY	MYGP	FM0109	3	2		
Coopers	WY	MYGP	FM0109	3	6		
Carlton Draught	WY	MYGP	FM0109	3	93		
Rechs	WY	MYGP	FM0109	3	65		
Hahn Super Dry	WY	MYGP	FM0109	3	120		
Tooheys	Lactic Acid	RakaRay	FM0124	3	14		
Coopers	Lactic Acid	RakaRay	FM0124	3	10		
Carlton Draught	Lactic Acid	RakaRay	FM0124	3	9		
Rechs	Lactic Acid	RakaRay	FM0124	3	1		
Hahn Super Dry	Lactic Acid	RakaRay	FM0124	3	1		

Results are expressed as CFU / mL

APPENDIX 2

	Beer Line	Organism	Plate	Method	Rep	Before	After
1	VB	WY	MYGP	FM0109	1	160	340
2	New	WY	MYGP	FM0109	1	170	1
3	Draught	WY	MYGP	FM0109	1	600	2
4	Gold 150	WY	MYGP	FM0109	1	880	37
5	Lashes	WY	MYGP	FM0109	1	820	93
1	VB	Lactic Acid	RakaRay	FM0124	1	1	1
2	New	Lactic Acid	RakaRay	FM0124	1	1	1
3	Draught	Lactic Acid	RakaRay	FM0124	1	1	1
4	Gold 150	Lactic Acid	RakaRay	FM0124	1	1	1
5	Lashes	Lactic Acid	RakaRay	FM0124	1	1	1

Results are expressed as CFU / mL

APPENDIX 3.

Beer Line	Organism	Before	After
Tooheys	WY	10	240
Coopers	WY	10	10
Carlton Draught	WY	23	10
Rechs	WY	10	10
Hahn Super Dry	WY	46	10
Tooheys	Lactic Acid	300	110
Coopers	Lactic Acid	23	72
Carlton Draught	Lactic Acid	1	3
Rechs	Lactic Acid	8	7
Hahn Super Dry	Lactic Acid	1	2
Tooheys	WY	1	2
Coopers	WY	11	6
Carlton Draught	WY	1	93
Rechs	WY	3	65
Hahn Super Dry	WY	4	120
Tooheys	Lactic Acid	43	14
Coopers	Lactic Acid	20	10
Carlton Draught	Lactic Acid	3	9
Rechs	Lactic Acid	1	1
Hahn Super Dry	Lactic Acid	1	1
XXXX Gold	WY	84	1

Tooheys	WY	6	9
Carlton Draught	WY	2	4
Hahn Super Dry	WY	140	17
V.B	WY	5	2
XXXX Gold	Lactic Acid	1	1
Tooheys	Lactic Acid	1	1
Carlton Draught	Lactic Acid	1	1
Hahn Super Dry	Lactic Acid	1	1
V.B	Lactic Acid	1	1
V.B	WY	2	1
Gritter	WY	220	1
Carlton Draught	WY	1	1
Batch	WY	3	10
Young Henry	WY	8	1
V.B	Lactic Acid	1	1
Gritter	Lactic Acid	1	1
Carlton Draught	Lactic Acid	1	1
Batch	Lactic Acid	1	1
Young Henry	Lactic Acid	2	1

Results are expressed as CFU / mL

APPENDIX 4.

EcoDiagnostics Pty Ltd is a West Australian company established in 2013 to provide molecular diagnostic capability for clients requiring analysis of environmental, veterinary, and food samples. The three directors have, in combination, over 40 years of experience applying molecular diagnostic techniques to the agriculture, fisheries, biosecurity, environmental, and water testing sectors. Further, the directors have extensive academic research experience with over 40 peer reviewed publications and are actively engaged, both directly and collaboratively, in various research projects. EcoDiagnostics, by way of minority private equity investment, become part of the ARL group of companies in March 2016.

EcoDiagnostics is NATA accredited to ISO/IEC 17025 with specific accreditations for qPCR and conventional microbiological analysis of environmental, food and water samples. Notably, EcoDiagnostics is the only company in Australia to hold both NATA accreditation for qPCR analysis of water samples for identification and enumeration of *Legionella pneumophila* and NATA accreditation for species identification using nucleotide sequence analysis. EcoDiagnostics is undergoing assessment (July 2016) for NATA accreditation to provide immunological assays (ELISA).

EcoDiagnostics is operated out of state-of-art laboratory facilities at the University of Western Australia, Floreat campus; these facilities provide a convenient central location with significant capacity to expand the operation. EcoDiagnostics laboratory is fully equipped with the very latest in nano-bead based DNA extraction and PCR setup robotics, and qPCR diagnostic capability. Furthermore, EcoDiagnostics is a Qiagen reference laboratory in Australia, providing priority access to new techniques and services from one of the world's most highly regarded providers of sample and assay technologies for molecular diagnostics.