

1 **Microbiological characterisation and impact of suspended solids on pathogen removal from**
2 **wastewaters in dairy processing factories**

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11 Short title: **Microbiological characterisation and pathogen removal from dairy**
12 **wastewaters**

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19 **Summary**

20 In this Research Communicaiton we investigate the microbiological profile of 12 dairy
21 wastewater streams from three contrasting Irish dairy processing factories to determine
22 whether faecal indicators/pathogens were present and in turn, whether disinfection may be
23 required for potential water reuse within the factory. Subsequently, the impact of suspended
24 solids on the inactivation efficiency of *E.coli* via two means of ultraviolet (UV) disinfection;
25 flow-through pulsed UV (PUV) and continuous low pressure UV (LPUV) disinfection was
26 analysed. Faecal indicators of total coliforms and *E.coli* were detected in 10 out of the 12
27 samples collected at the dairy processing factories while pathogenic bacteria *Listeria*
28 *monocytogenes* was detected in all samples collected at 2 out of the 3 factories. *Salmonella*
29 *spp.* was undetected in all samples. The results also indicated that organic dairy wastewater
30 solids had an impact on the performance efficiency of the PUV system and, to a lesser extent,
31 the LPUV system. The findings indicate that the targeting of key pathogens would be required
32 to enable wastewater reuse (and indeed effluent discharges if regulation continues to become
33 more stringent) and that LPUV may offer a more robust disinfection method as it appears to
34 be less susceptible to the presence of suspended solids.

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39 **Keywords;** dairy wastewater, pathogens, UV

40 Water consumption within the Irish dairy sector is relatively high at 2.5m³/m³ of milk
41 processed and 14.9m³/tonne product (Geraghty, 2011). In comparison, water consumption in
42 the Australian dairy industry has dropped to 1.4m³/m³ of milk processed while the UK dairy
43 industry reported an improved water consumption ratio of 1.1m³/m³ of milk processed in
44 2015 (ADIC, 2013; Dairy UK, 2015). Water is used both internally and externally within
45 factories for manual washing, pasteurization, operational processes and internal pipe washing
46 (i.e. cleaning-in-place: CIP). Research has shown that water reuse practices in Ireland remain
47 low due to the damp climate and low water stress (Deloitte, 2015). Nevertheless, with an
48 increase in sustainability initiatives and stringent legislation within this sector water
49 reclamation and reuse may be a necessary consideration in the near future.

50 Wastewater from dairy processing factories can be divided into three main categories; (i)
51 cooling water, (ii) sanitary wastewater and (iii) industrial wastewater. In terms of the origin of
52 the microbiological contamination within these waste streams there are a multitude of sources
53 including milking machines and bulk tanks on farms and tankers transporting the milk. While
54 the majority of these bacteria are destroyed during the initial pasteurisation process, some
55 pathogenic strains are known to survive post-pasteurisation such as *Listeria monocytogenes*
56 and spore-forming *Bacillus spp.*(Gopal et. al., 2015). Other pathogens associated with the
57 dairy industry include *Salmonella spp.*, *Staphylococcus aureus* and *Campylobacter*
58 *spp.*(Oliver et. al., 2005). Therefore, aside from chemical disinfection of wastewaters for
59 potential reuse there may also be a requirement for enhanced pathogen removal depending on
60 the intended purpose of the reclaimed water. Research studies into the reuse of such treated
61 wastewaters have generally focused on the use of membrane filtration techniques (Riera et.
62 al., 2013). Although filtration techniques are effective, their application in this setting can be
63 hampered by fouling issues (Fitzhenry et. al., 2014). Ultraviolet (UV) technologies for
64 wastewater disinfection are often favoured as they tend to be low maintenance and cost-
65 effective, but they can also be hindered by the presence of suspended solids (SS) (UKWIR,
66 2016).

67 This study aims to investigate (i) the microbiological characterisation of a variety of
68 wastewater streams from three dairy processing factories and (ii) the application of two UV
69 technologies for potential low-level wastewater reuse within dairy processing factories. In
70 addition, the impact of SS on the disinfection efficiency of both a domestic low pressure UV
71 (LPUV) system and a novel pulsed UV (PUV) flow-through system was evaluated.

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74 **Material & Methods**

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76 *Wastewater Characterisation Analysis*

77 Three dairy processing factories were selected for water/wastewater stream analysis ranging
78 from factories which process milk from 100 million litres per year (Site 1) to those which
79 process up to 1,000 million litres per year (Site 3). Grab samples (1 – 2 L) were collected at
80 various sampling points of the dairy processing factory which included cooling water,
81 condensate water, wastewater treatment plant (WWTP) influent and WWTP effluent. The
82 samples were subjected to a series of standard methods testing (within 8 hours) The following
83 two tests were carried out; (i) heterotrophic plate counts (HPC) at 37°C and 22°C and (ii) total
84 coliform and *E.coli* analysis. These samples (100 mL) were also sent for specific pathogen
85 target analysis at externally accredited laboratory, (Complete Lab Solutions, Rosmuc,
86 Galway) for analysis of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*,
87 *Campylobacter spp.* and *Salmonella spp.* Further details of the sampling points and specific
88 tests are included in the Online Supplementary File. Each dairy wastewater treatment plant
89 was surveyed at least twice.

90 *PUV System Analysis*

91 A bench-scale pulsed power source (PUV-01, Samtech Ltd., Glasgow) was used to power a
92 low pressure (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type; NL4006 series)
93 which produced a high intensity beam of polychromatic pulsed light. The lamp was placed
94 10.75 cm above a sterilised aluminium flow-through vessel (with a plan surface area of 290
95 cm²) which pumped water through the vessel at the desired flow rate corresponding to a
96 hydraulic residence time (HRT). The PUV system allowed for the input voltage and the pulse
97 rate to be varied between 400 and 1000 V and for a pulse frequency of between 0.1 and 10
98 pulses per second (PPS). The UV dose was determined by calculating the output voltage
99 energy, the distance from the lamp, the area of the vessel, the PPS and the HRT. All PUV
100 doses were calculated to only include wavelengths below 300 nm.

101 *LPUV System Analysis*

102 The continuous-flow monochromatic LPUV system (LCD 412 Plus, S.I.T.A., Halpin &
103 Hayward Ltd.) had a fixed power output of 40 W with a maximum flow rate of 45L/min. The
104 UV dose was altered by varying the influent flow rate e.g. influent pumped at a rate of 27
105 L/min gave a retention time of 0.4 seconds and a UV dose output of 11 mJ/cm².

106 *Impact of SS on UV systems*

107 Various concentrations of bentonite, calcium carbonate (CaCO₃) or organic dairy wastewater
108 solids were added to the influent sample of both the PUV (2.5 L distilled water) and LPUV
109 (30 L tap water) to give a range of samples with SS concentrations that varied between 0 and
110 200 mg/L. Subsequently the samples were spiked with *E.coli* to give an initial concentration,
111 prior to UV treatment, of 1×10^6 CFU/mL. Samples were then processed through the LPUV
112 and PUV systems. Influent and effluent samples were analysed using the standard pour plate
113 technique (1 mL) using non-selective nutrient agar. Log inactivation was determined as the
114 difference between log influent concentration (N₀) – log effluent concentration (N).

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116 **Results and Discussion**

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118 *Dairy wastewater characterisation analysis*

119 Table 1 outlines the total abundance of aerobic bacteria in the samples in addition to standard
120 faecal indicator concentrations and results of detection/enumeration tests for five targeted
121 pathogens in the dairy water samples. Faecal indicators of total coliforms and *E.coli* were
122 present in all WWTP influent & effluent samples. *E.coli* was detected in all samples apart
123 from the condensate water samples from Site 2 and Site 3. Thus, if effluent discharge
124 regulations were extended to microbiological monitoring in addition to current regulations, it
125 is likely that tertiary disinfection would be required at all three WWTP sites tested. Separate
126 wastewater streams emerging directly from the dairy processing factories were analysed to
127 determine bacterial contamination levels and suitability for potential low-level water reuse
128 in/around the dairy processing factory. A cooling water waste stream was analysed at Site 2
129 while condensate wastewater was available for collection at both Site 2 and Site 3. Analysis
130 of the cooling water stream yielded the presence of both faecal indicators and four out of the
131 five targeted pathogens (thus disinfection may be required depending on the desired water
132 reuse purpose). Condensate water from Site 2 appeared relatively uncontaminated as aerobic
133 bacterial loads were low and faecal indicators absent. However pathogenic *Listeria*
134 *monocytogenes* was still detected on both sampling days highlighting the importance of
135 rigorous microbiological analysis of dairy wastewater streams if they are to be considered for
136 reuse purposes. Studies have shown this bacteria to survive post-pasteurisation in dairy
137 processing environments, therefore, particular attention may be warranted for this strain in
138 terms of water reclamation in the dairy environment (Oliver et. al., 2005). *Listeria*

139 *monocytogenes* was also detected in all samples at Site 1 and Site 2 and after a further
140 enumeration test the highest levels were detected in Site 1. *Salmonella spp.* went undetected
141 in all 12 samples tested while *Bacillus cereus* was consistently detected in all 12 samples at
142 low concentrations. *Staphylococcus aureus* was found to be most prevalent at Site 1 where
143 process water (pre-treatment) WWTP influent and WWTP effluent streams were tested.

144 *Impact of SS on UV systems*

145 It was observed that inorganic SS (bentonite and calcium carbonate) concentrations of less
146 than 200 mg/L had limited impact on both LPUV and PUV efficiency for *E.coli* inactivation
147 (data available in online Supplementary File). Organic particles (dairy wastewater solids)
148 appeared to have minimal impact on the LPUV system while a decreasing trend of *E.coli* log
149 inactivation with increasing SS concentration can be seen for the PUV system (Figure 1).
150 These results indicate that priority should be given to organic suspended solids removal if
151 wastewater reuse and disinfection is being considered. They further indicate that the PUV
152 appears to be more readily impacted by the presence of suspended solids in comparison to the
153 LPUV system. A significantly higher UV dose was required from the PUV system in
154 comparison to the LPUV system for *E.coli* inactivation. Further analysis into the cost of a
155 higher energy system may be of interest for comparative purposes between the PUV and
156 LPUV.

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158 **Conclusion**

159 In conclusion, results from the wastewater characterisation analysis indicate that the majority
160 of wastewater streams from different dairy processing factories were contaminated with either
161 faecal indicators or foodborne pathogens or a mixture of both. The condensate wastewater
162 streams appeared to be the most suitable to utilise in terms of water reuse as they appeared to
163 be the least contaminated. As some dairy processing factories produce significant quantities of
164 this wastewater as a by-product of dairy processes (e.g. evaporation and drying of milk
165 powder) it may be a suitable choice for wastewater reclamation and reuse within the factory.
166 Comparative analysis of LPUV and PUV disinfection efficiency suggest that the flow-through
167 PUV system appeared to be more sensitive to the presence of organic SS in wastewater
168 samples. Therefore, the LPUV system may offer a more robust disinfection method as it
169 appears to be less susceptible to the presence of suspended solids.

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211 **Figure and table legends:**

212

213 **Figure 1:**

214 Impact of suspended solids on *E.coli* log reduction via low pressure ultraviolet (LPUV) and
215 pulsed ultraviolet (PUV) disinfection, where the ultraviolet (UV) dose is 11 mJ/cm² and
216 1946 mJ/cm², respectively.

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218 **Table 1:**

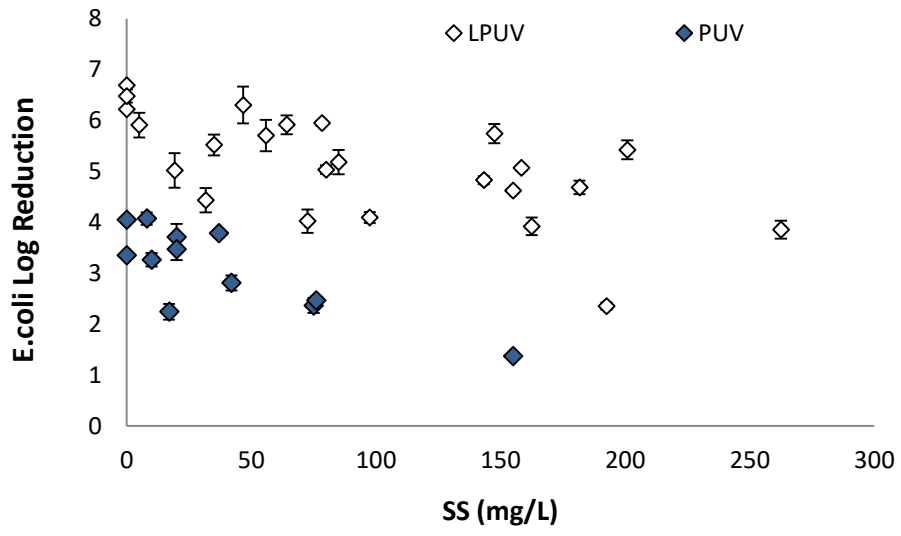
219 Faecal indicator and pathogenic bacteria analysis of various water and wastewater streams at
220 three Irish dairy processing factories.

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224 **Figure 1:**



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Site	Day	Sample Type	HPC - abundance (CFU/100mL)		Total coliforms (MPN/100mL)	E.coli (MPN/100mL)	Salmonella detection (100mLs)	Listeria monocytogenes detection & enumeration (cfu/100mL)	Campylobacter spp detection (100mL)	S.aureus (cfu/100mL)	B.cereus (cfu/100mL)
			37°C	22°C							
1	1	Process water pre-treatment	Inconclusive	8.35E+05	1.87E+02	3.10E+00	*ND	Detected	ND	4.40E+03	4.48E+03
		WWTP influent	Inconclusive	7.30E+09	4.61E+06	1.85E+04	ND	Detected	ND	4.32E+03	5.04E+03
		WWTP effluent	Inconclusive	2.65E+08	4.28E+05	8.66E+02	ND	Detected	ND	4.08E+03	5.26E+03
	2	Process water	2.85E+05	6.20E+04	3.26E+02	3.00E+00	*N/A	<1 cfu/mL	N/A	1.63E+03	1.04E+03
		WWTP influent	3.75E+09	4.80E+09	1.50E+06	1.15E+04	N/A	<1 cfu/mL	N/A	1.63E+03	9.60E+02
		WWTP effluent	1.41E+09	4.20E+08	2.42E+05	1.73E+03	N/A	<1 cfu/mL	N/A	1.85E+03	1.07E+03
	3	Process water	5.00E+03	4.00E+03	6.49E+02	4.22E+01	ND	8.40E+03	ND	<1	9.80E+02
		WWTP influent	5.70E+09	4.60E+09	3.89E+05	4.48E+03	ND	7.90E+03	ND	<1	9.40E+02
		WWTP effluent	7.00E+07	9.10E+07	3.45E+04	1.07E+03	ND	6.20E+03	ND	<1	9.23E+02
2	1	WWTP influent	8.10E+07	7.80E+07	8.66E+04	1.46E+01	ND	Detected	ND	1.46E+03	1.99E+03
		WWTP effluent	2.02E+07	3.20E+07	5.17E+06	2.75E+01	ND	Detected	Detected	1.25E+03	1.67E+03
		Condensate	0.00E+00	1.40E+04	0.00E+00	0.00E+00	ND	Detected	ND	1.10E+03	1.84E+03
		Cooling water	5.30E+06	4.20E+06	1.02E+04	5.48E+02	ND	Detected	Detected	1.16E+03	1.96E+03
	2	WWTP influent	6.30E+08	6.80E+08	4.11E+06	1.11E+04	ND	3.60E+02	ND	<1	1.05E+03
		WWTP effluent	5.50E+05	2.50E+05	5.56E+03	1.83E+01	ND	6.40E+02	ND	<1	1.06E+03
		Condensate	0.00E+00	0.00E+00	0.00E+00	0.00E+00	ND	<1	ND	<1	1.05E+03
		Cooling water	7.60E+06	8.40E+06	1.31E+04	2.42E+03	ND	1.10E+02	ND	<1	9.60E+02
3	1	Cheese process effluent	2.03E+09	4.20E+09	2.42E+08	5.83E+01	ND	ND	ND	<1	1.08E+03
		Mixed process effluent excl. whey	2.00E+08	1.40E+08	1.55E+05	2.42E+03	ND	ND	ND	<1	1.06E+03
		Whey process effluent	3.32E+08	2.85E+08	7.80E+03	5.37E+03	ND	ND	ND	<1	1.05E+03
		Condensate	3.40E+06	3.30E+05	0.00E+00	0.00E+00	ND	ND	ND	<1	9.67E+02
		WWTP effluent	7.00E+05	2.80E+06	6.30E+04	2.28E+02	ND	ND	ND	<1	1.02E+03
	2	Cheese process effluent	2.41E+09	3.00E+09	4.48E+07	3.10E+04	ND	ND	ND	<1	1.04E+03
		Mixed process effluent excl. whey	2.00E+08	4.80E+08	9.32E+05	1.78E+02	ND	Detected	ND	<1	1.01E+03
		Whey process effluent	1.07E+07	9.10E+07	4.10E+02	3.10E+02	ND	ND	ND	<1	1.06E+03
		Condensate	3.36E+07	2.92E+07	1.05E+03	0.00E+00	ND	ND	ND	<1	9.84E+02
		WWTP effluent	7.40E+06	9.70E+06	6.13E+04	2.61E+02	ND	ND	ND	<1	1.06E+03