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Reduction of *Listeria monocytogenes* and *Campylobacter* spp. biofilm on conveyor belts using different cleaning and disinfection regimes

Madeleine Moazzami [a,b], Ingrid Hansson [b], Solveig Langsrud [c], Trond Møretrø [c], Sofia Boqvist [b,*]

[a] *Microbiology unit, Swedish Food Agency, 751 26, Uppsala, Sweden*
[b] *Department of Animal Biosciences, Swedish University of Agricultural Sciences, 750 07, Uppsala, Sweden*
[c] *Nofima, Norwegian Institute of Food, Fishery and Aquaculture Research, Ås, N 1430, Norway*

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ABSTRACT

Removal of biofilm is essential in food production environments, such as slaughterhouses and meat processing facilities, to prevent the persistence and spread of spoilage and pathogenic microorganisms that compromise food safety. Thus, efficient cleaning and disinfection (C&D) are crucial. This study aimed to compare the efficacy of 15 commercial C&D treatments, including chemicals diluted at different concentrations of the concentrate, to reduce *Listeria monocytogenes* and *Campylobacter* spp. biofilm. Chlorinated alkaline, acidic, and enzymatic detergents alone or combined with chlorinated alkaline, acidic, electrochemically activated water (ECA), and alcohol disinfection were applied on pre-formed *L. monocytogenes* and *Campylobacter* spp. biofilms on woven conveyor belt material.

The most efficient treatment was high concentration (6%) chlorinated alkaline cleaning combined with 70% alcohol (1.7 and 2.2 log reduction for *L. monocytogenes* and *Campylobacter* spp., respectively), followed by high concentration (6%) chlorinated alkaline cleaning and chlorinated alkaline disinfection (3%) (1.5 and 1.7 log reduction for *L. monocytogenes* and *Campylobacter* spp., respectively). The latter was superior to ECA combined with the same cleaning agent. For certain treatments, an increased concentration resulted in higher bacterial reduction, such as acidic C&D agents which improved reductions with 0.7-1.0 log. Biofilm removal was not enhanced when enzymatic cleaners replaced acidic or chlorinated alkaline cleaning agents.

These findings indicate that, when applied at standard manufacturer concentrations, commonly used C&D agents are unable to remove biofilms of *L. monocytogenes* and *Campylobacter* spp. from conveyor belts. This emphasises the need for science-based guidelines to optimise C&D protocols in meat production facilities.

1. Introduction

Efficient cleaning and disinfection (C&D) procedures are essential in slaughterhouses and other food production establishments to minimise contamination and the transmission of pathogenic and spoilage bacteria. Cleaning primarily removes organic residues, such as remains of proteins, fat, and blood, and inorganic soil (Holah, 2014; Marriott et al., 2018), but further diminishing the levels of microorganisms requires disinfection (Ninios et al., 2014). In addition, periodic and targeted C&D is necessary, particularly to remove biofilms which protect bacteria from environmental stresses, such as heat and desiccation, and increases their resistance to disinfectants (Møretrø et al., 2012; Stoller et al., 2019).

The pathogenic bacterium *Listeria monocytogenes* (*L. monocytogenes*) is known for its ability to form biofilm, which renders it difficult to eliminate using common C&D procedures. *Listeria monocytogenes* can become residential in slaughterhouse environments and has been shown to persist in meat processing facilities for months, or even years, under regular C&D regimes (Fagerlund et al., 2016; Moazzami et al., 2025a; Stoller et al., 2019). This bacterium can cause listeriosis, a severe disease, notably among individuals that belong to risk groups, such as elderly and immunocompromised individuals, and may also cause miscarriages in pregnant women. Moreover, the mortality rate following listeriosis is exceptionally high (up to 22.5%) among individuals above the age of 64 (EFSA Biohazard Panel, 2018; Desai et al., 2019). *Listeria monocytogenes* usually causes sporadic disease, which is consequently challenging to trace back the source of infection (European Food Safety

* Corresponding author.
E-mail address: sofia.boqvist@slu.se (S. Boqvist).

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Authority & European Centre for Disease Prevention and Control, 2024).

Campylobacter spp. is another pathogenic bacterium frequently isolated in slaughterhouse environments (Dzieciolowski et al., 2022; Moazzami et al., 2025a). It is also a biofilm-producing bacteria (Ferrari et al., 2019) known to cross-contaminate poultry carcasses during slaughter and meat processing (García-Sánchez et al., 2017; Kudirkienė et al., 2011). Moreover, *Campylobacter* spp. is the bacteria responsible for most confirmed human cases of food borne disease in Europe since 2005 (European Food Safety Authority & European Centre for Disease Prevention and Control, 2024). Aside from generating gastrointestinal illness, it can also cause severe complications, such as the neurological Guillain-Barré syndrome (Allos, 1997). The impact of insufficient C&D was revealed through an exceptionally large campylobacteriosis outbreak in Sweden during 2016-2017, which originated from improper cleaning of transport crates for chickens (Frosth et al., 2020; Lofstedt, 2019).

Before new C&D products are marketed, they must undergo laboratory testing to verify sufficient bacterial reduction. These tests are typically limited to four bacterial species (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus hirae*) and are conducted either in suspension or on stainless steel plates within laboratory settings (Swedish Standards Institute, 2012; Swedish Standards Institute, 2019. However, the suspension tests fail to mimic real-life settings in a food production facility as bacterial biofilm attached to surfaces are up to 1000 times more resistant to disinfecting agents than bacteria dispersed in liquids (Marriott et al., 2018). Moreover, testing the bacterial reduction on stainless steel plates, which are smooth, easy to clean, and less conducive to biofilm formation, is not comparable to evaluating reduction on surfaces in real food production facilities, such as conveyor belts (Rashid et al., 2023). Conveyor belts are often present in various food industries such as slaughterhouses and meat processing facilities due to their production properties. However, belts with irregular surfaces, especially those possessing woven material underneath, are difficult to clean and suitable for harbouring niches for biofilm-producing bacteria, such as *L. monocytogenes* and *Campylobacter* spp. (Fagerlund et al., 2020). Thus, additional tests are necessary to determine the C&D efficacy in real-life settings (Wirtanen and Salo, 2003).

For food business operators (FBOs), such as slaughterhouses, it may be difficult to select the most suitable and efficient C&D products and procedures to avoid challenges with a residential microbiota protected in biofilm (Carvalheira et al., 2021; Moazzami et al., 2025b). Frequently used detergents in slaughterhouses and meat processing facilities are alkaline compounds with or without chlorine, acids, and enzyme-based chemicals (Delhalle et al., 2020; García-Sánchez et al., 2017; Hamza, 2017; Nahar et al., 2018). Enzyme-based chemicals are becoming more common in the food industry as they have been shown to be potentially more efficient than other cleaning agents in removing biofilm and as well as less corrosive (Hamza, 2017; Nahar et al., 2018). Regarding disinfection, quaternary ammonium compounds (QACs), chlorine-based compounds, acidic agents, and alcohols (i.e. ethanol and isopropyl alcohol) are often employed (Cherifi et al., 2022; Gantzhorn et al., 2014; Khamisse et al., 2012; Wang et al., 2018). In Swedish slaughterhouses, the most used chemicals are chlorinated alkaline and acidic agents (Moazzami et al., 2025b).

Slaughterhouses face notable challenges regarding C&D and environmental monitoring, including procedural deficiencies, knowledge gaps, and a lack of science-based guidelines (Moazzami et al., 2025b). The selection of proper C&D products and procedures is, therefore, a challenging task for quality assurance managers at both slaughterhouses and other food production establishments. The EU-food legislation, Codex alimentarius, and other guidelines fail to include recommendations on which chemical agents, concentrations, contact time, and application should be used for different purposes (Codex Alimentarius, 2020; EC, 2005). As a result, the responsibility of determining which methods to use lies with the FBO.

The aim of this study was to compare the efficacy of various combinations and concentrations of C&D chemicals commonly used in slaughterhouses and novel agents on the removal of *L. monocytogenes* and *Campylobacter* biofilms from conveyor belts.

2. Materials and methods

2.1. *Listeria monocytogenes strains and growth conditions*

A mixture of four environmental *L. monocytogenes* strains was used as inoculum: PotherB2 (ST7), R5B10 (ST8), R11A6 (ST9), and R5B6 (ST451). The selected strains were originally isolated from surfaces at a cattle and swine slaughterhouse and a poultry slaughterhouse and were of serogroup IIa (serotype 1/2a and 3a), which is known to cause human disease (EFSA Biohazard Panel, 2018). The strain belonging to sequence type 9 has been shown to persist on surfaces over long periods of time (Moazzami et al., 2025a). The strains were cultured on blood agar plates (Swedish Veterinary Agency, Uppsala, Sweden) for 24 ± 3 h at 37 ± 1 °C. A single colony from each strain was inoculated into separate culture tubes containing 5 mL brain heart infusion (BHI) broth (CM1135, Oxoid, Basingstoke, UK). The cultures were incubated with shaking at 150 rpm (Microplate shaker EU plug, Avantor, Pennsylvania, USA) for 24 ± 3 h at 30 ± 1 °C. These overnight cultures were mixed in equal volumes into a single tube and the combined suspension was diluted in BHI to approximately 6.0 log CFU/mL.

2.2. *Campylobacter spp. strains and growth conditions*

A mixture of three *C. jejuni* strains and one *C. coli* strain were used as inoculum: P17_3j (*C. jejuni*, ST257), R6_11j (*C. jejuni*, ST58), P21_2j (*C. jejuni*, ST19), and R4_8c (*C. coli*, ST6585). The selected strains were isolated from surfaces at the same two slaughterhouses that the *L. monocytogenes* strains were isolated from during the same sampling period. These strains have matched with strains found in samples from chickens and cattle and the sequence types of the strains are also known to cause human disease (Frosth et al., 2020; Ghielmetti et al., 2023). The strains were cultured on blood agar plates for 48 ± 4 h at 37 ± 1 °C in microaerobic atmosphere using CampyGen™ (PO5091A, Oxoid) in anaerobic jars. From each strain, 1 μL was transferred to a glass jar with 10 mL BHI and 5 g of glass beads (Glasbeads KS D 2.0 mm, Avantor, Pennsylvania, USA). The jar was shaken at 150 rpm for 3 min and vortexed for approximately 10 s. This combined suspension was diluted in BHI to approximately 7.0 log CFU/mL.

2.3. *Conveyor belt biofilm assay*

Four biofilm assays were initially conducted for each bacterial cocktail. Due to bacterial contamination, two *Campylobacter* assays were excluded from further analysis, resulting in two biological replicates for *Campylobacter* spp. Biofilms of *L. monocytogenes* and *Campylobacter* spp. were produced and harvested following the method described in Fagerlund et al. (2020), with certain modifications. In brief: Coupons (1 × 1.5 cm) of food-grade dectyl and ropanol conveyor belt material (Dectyl EM 6/2 00 + 03 dark blue M1 AS FG MD, Ammeraal beltech, Heerhugowaard, Netherlands) were sterilised (90-100 °C for 15 min) and placed vertically in a 24-well plate (VWR tissue culture plates, Avantor) (Fig. 1). The smooth side of the coupon was positioned facing upward and the impregnated fabric facing downward. Each well was inoculated with 1 mL from the suspension of ~6.0 log CFU/mL of *L. monocytogenes* and ~7.0 log CFU/mL of *Campylobacter* spp. The volume was sufficient to half-filling each well with the suspension, resulting in the air/liquid interface crossing the length of the coupon. *Listeria monocytogenes* biofilms were cultured at 12 ± 1 °C for 4 days and *Campylobacter* spp. biofilms at 25 ± 1 °C for 5 days on rockers (10 rpm, 25° angle) (Rocker adjustable double platform 230 V EU, Avantor, Pennsylvania, USA) before the C&D experiments commenced. On day 3,

Fig. 1. Autoclaved coupons (1 × 1.5 cm) of conveyor belt material placed vertically in a 24-well plate prior to the addition of bacterial suspensions.

BHI of the wells were altered to new BHI, which possessed the temperatures of 12 °C and 25 °C for *L. monocytogenes* and *Campylobacter* spp., respectively. The temperature of *L. monocytogenes* was selected to mimic the conditions typically found in the cutting and meat processing areas of a slaughterhouse. The incubation temperature for *Campylobacter* spp. was chosen to reflect the higher temperature of the slaughter area during warmer seasons. This study was conducted at the Department of Animal Biosciences at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

2.4. Selection of cleaning and disinfection agents

The selected C&D agents and their combinations were applied at contact times and concentrations representative of those commonly employed in the largest slaughterhouses and adjacent meat processing facilities in Sweden (Moazzami et al., 2025b). The exceptions were electrochemically activated water (ECA), which was included because it is a relatively new product, and the combination of an enzyme-based cleaning agent with an acidic disinfectant, which had been recommended by the product manufacturer. In this study, the cleaning agents were also tested separately, as slaughterhouses only apply these in certain areas, such as stables, and when there is limited time for complete C&D practices due to a delay in the slaughter process. The enzymatic agents were preheated in a 40 °C water bath right before treatments (according to the manufacturer's instructions, which were listed as 30-40 °C), while the rest of the chemicals were applied at room temperature (approximately 20 °C), which is in accordance with the manufacturer's instructions and is used in slaughterhouses and meat processing facilities (Moazzami et al., 2025b). To reflect the concentrations applied in a large poultry processing facility, which was consulted before the start of this study, all chemicals were used according to the manufacturer's instructions except for agents A and B, which were applied at higher than recommended concentrations (Table 1). Higher concentrations are often applied on significantly contaminated surfaces such as the slaughter areas, whilst lower concentrations are used in processing areas. The 70% alcohol was used undiluted, as recommended by the manufacturer. The ECA was prepared in the laboratory with an ECA water system. The chemicals were diluted with tap water, as per the user instructions. All chemical agents were freshly prepared 1-2 h prior to the experiments. The same treatments (combination of agents, concentrations, and contact time) were used for *L. monocytogenes* and *Campylobacter* spp. biofilms.

2.5. Neutraliser test

To determine if Dey/Engley (D/E) neutralising broth (Sigma-Aldrich, Darmstadt, Germany) adequately neutralised all the different chemicals at the highest concentration in this study, a test was performed for the *L. monocytogenes,* and the *Campylobacter* spp. strains. This was conducted prior to the experiments according to the standard (SS-EN ISO 1276:2019) and demonstrated a satisfactory performance of the neutraliser.

2.6. Standard cleaning and disinfection biofilm treatment

The standard C&D biofilm treatment was carried out as follows: The coupons were rinsed twice in approximately 12 mL of sterile deionised H_2O (in 15-mL Falcon tubes) to remove non-adherent bacteria. This was performed by adding the coupon to the tube and turning it upside down twice. The water was removed and replaced with new water and the coupon was again turned upside down twice. Subsequently, the coupon was placed vertically in wells of a clean 24-well plate. A cleaning agent at either high or low concentration (Table 2) was applied to each well in the form of foam, as intended by the manufacturers, which were

Table 1
The principal agents, main intended uses, recommended and applied concentrations, contact times, and pH values of the cleaning and disinfection agents used in the current study.

	Product	Type of product and principal agents	Main intended use in the food industry	Used conc	Recommended conc	Used contact time	Recommended contact time	Used pH
Cleaning (C)	Alka C	Chlorinated alkaline foam (sodium hypochlorite, potassium hydroxide, sodium hypochlorite)	Regular cleaning to remove fat, protein and starch from open surfaces and machines	4% 6%	2-5%	15 min	5-20 min	12.5 12.6
	Acid C	Acidic foam (phosphoric acid, sulphuric acid)	Periodic cleaning to remove mineral coatings from open surfaces and machines	4% 6%	2-5%		5-20 min	1.4 1.3
	Enzy C	Tri-enzymatic foam (protease, amylase, lipase)	Periodic cleaning and removal of fat from open surfaces and machines	2% 4%	2-4%		15-20 min	7.7 8.4
Disinfection (D)	Alka D	Chlorinated alkaline foam (sodium hydroxide, sodium hypochlorite)	Regular disinfection of open surfaces and machines	1% 3%	1-3%		5-15 min	11.1 11.4
	ECA	Weak acid (hypochlorous acid), electrochemically activated water	Disinfection of surfaces	100%	100%		nd	6.2
	Alco	70% alcohol (ethanol, Propan-2-o-ol)	Occasional disinfection of surfaces	100%	100%		≥1 min	7.9
	Acid D	Acidic foam (hydrogen peroxide, acetic acid, peracetic acid)	Periodic disinfection of surfaces	4% 6%	0.7-13%		5-15 min	3.0 2.8

nd = Not determined, the manufacturer did not provide the information.

Table 2
Applied cleaning and disinfection (C&D) treatments used at different concentrations in the current study.

Treatment with only cleaning agents		Combination of treatments with cleaning and disinfecting agents		
Treatment	Cleaning agent	Treatment	Cleaning agent	Disinfection agent
Low alka C	Alka 4 %	Low alka C&D	Alka 4 %	Alka 1 %
High alka C	Alka 6 %	High alka C&D	Alka 6 %	Alka 3 %
Low acid C	Acid 4 %	High alka + ECA	Alka 6 %	ECA 100%
Low acid C	Acid 6 %	High alka + Alco	Alka 6 %	Alcohol 100%
Low enzy C	Enzy 2 %	Low acid C&D	Acid 4 %	Acid 4 %
High enzy C	Enzy 4 %	High acid C&D	Acid 6 %	Acid 6 %
		Low enzy + acid	Enzy 2 %	Acid 4 %
		High enzy + alka	Enzy 4 %	Alka 3 %
		High enzy + acid	Enzy 4 %	Acid 6 %

Acid = Acidic foam; Alcohol = 70% Ethanol, 30% Propanol; Alka = Chlorinated alkaline foam; ECA = Electrochemically activated water; Enzy = Tri-enzymatic foam.

produced in foam pump bottles (Biltema, Uppsala, Sweden). Foam was placed in the wells, and the coupons were dipped in the foam twice and subsequently left in the cleaning agent for 15 min before being rinsed, as described earlier, in H_2O and placed in a second clean 24-well plate. The wells were filled with a disinfecting agent, some in the form of foam, and then dipped in the same way as the cleaning agents. Alcohol and ECA were applied with a pipette to each well (2 mL per well). After 15 min contact time, a final rinse was performed in the same manner as for the cleaning agents. The coupons were placed in 4.5 mL D/E neutralising broth with 2 g glass beads. They were vortexed for 30 s and sonicated with 40 kHZ for 10 min (Bath ultrasonic MT5800-E, Bransonic Ultrasonic Corporation, Soest, Netherlands) to dislodge attached cells and disperse cell aggregates. Following this, dilutions of suspended bacteria were plated on blood agar plates and incubated for 48 ± 4 h at 30 ± 1 °C for *L. monocytogenes* and 48 ± 4 h at 37 ± 1 °C in microaerobic atmosphere for *Campylobacter* spp. The experiment was performed at room temperature (approximately 20 °C).

2.6.1. Control coupons

The bacterial concentrations of three coupons, which had been neither rinsed nor treated with any chemical, were determined on the day of the experiments. Each coupon was placed in 4.5 mL D/E neutralising broth with glass beads and sonicated in the same manner as the treated coupons. Additionally, two control coupons were used to compare the coupons treated with only cleaning agents. These two coupons were treated with 2 mL sterile deionised H_2O rather than a chemical agent for 15 min. Two other coupons were used as controls for coupons treated with both C&D agents. These two coupons were similarly treated with 2 mL sterile deionised H_2O instead of a cleaning and a disinfecting agent for 15 min.

2.6.2. Bacterial concentration of biofilm broth

The bacterial concentration of the biofilm broth was measured at three time points: immediately before incubation of the 24-well plates, on the third day of incubation (prior to replacement of BHI) and immediately before the C&D experiments began (day 4 for *L. monocytogenes* and day 5 for *Campylobacter* spp.). The bacterial suspensions were surface plated on blood agar plates and incubated for 48 ± 4 h at 30 ± 1 °C for *L. monocytogenes* and at 37 ± 1 °C in microaerobic atmosphere for *Campylobacter* spp.

2.7. Statistical analysis

Estimates of the mean and variance (standard error of the mean) for each treatment of four and two biological experiments, for *L. monocytogenes* and *Campylobacter* biofilm assays, respectively, were plotted in the figures (Figs. 2–5). The means were calculated from the $\log_{10}$-transformed values of CFU per coupon (or reduction in CFU per coupon). To evaluate the bacterial reduction, the statistical software R was used (R Core Team, 2024). The values were modelled using analysis of variance, specifically the "lm"-function from the "stats" package and "emmeans"-function from the "emmeans" package (R package version 1.10.6) were used. The treatments were considered as fixed factors in the model and occasion (technical replicate) as a block factor. Residuals were checked to confirm that they fulfilled the assumption of normal distribution and equal variances. Differences between mean values were deemed significant at $P < 0.05$, adjusted for multiple comparisons using the Šidák method. Tukey-test at a 95% significance level was used to determine significant differences between treatments.

3. Results

3.1. Bacterial concentration in the inoculated broth and untreated coupons

The mean concentration of *L. monocytogenes* in the inoculated broth increased during incubation from 6.4 to 9.4 log CFU/mL. The greatest increase (2.7 log) was observed between day 1 and 3. The mean concentration of *Campylobacter* spp. was consistently maintained throughout the 5 days of incubation. After changing the BHI for *L. monocytogenes* (day 4) and *Campylobacter* spp. (day 5) a similar increase of 0.3 log was observed for both bacteria (Table 3).

3.2. General biofilm removal

The mean reductions for the C&D treatments ranged between 0.2 and 1.7 log for *L. monocytogenes* and −0.1-2.2 log for *Campylobacter* spp. The highest mean reduction was observed when chlorinated alkaline cleaning agent at high concentration was combined with alcohol, which resulted in reductions of 1.7 and 2.2 log for *L. monocytogenes* and *Campylobacter* spp., respectively. In contrast, the lowest mean reduction was reported for low concentration of enzymatic cleaning with acidic disinfection (0.2 and −0.1 log for *L. monocytogenes* and *Campylobacter* spp., respectively). In general, the mean reductions for *L. monocytogenes* and *Campylobacter* spp. were similar (±0.2 log) for approximately half of the treatments.

3.3. Biofilm removal by cleaning agents

Increasing the concentration of the chlorinated alkaline agent enhanced the mean removal of *L. monocytogenes* biofilm by 0.7 log. A similar effect was reported for the acidic agent which generated a 0.8 log reduction. In contrast increasing the concentration of the enzymatic agent did not significantly improve the biofilm removal (Fig. 2.).

When comparing the three different cleaning agents at both low and high concentrations, respectively, no significant differences were observed between the treatments. Likewise, for *Campylobacter* spp., no significant differences were observed among the treatments (Fig. 2.).

3.4. Biofilm removal by chlorinated alkaline and acidic cleaning and disinfection agents

Increasing the acidic concentration in C&D resulted in an enhanced mean bacterial reduction of 0.7 log for *L. monocytogenes* and 1.0 log for *Campylobacter* spp. The increased concentration of chlorinated alkaline improved *Campylobacter* spp. biofilm removal, whilst this difference was not significant for *L. monocytogenes*. At low concentration of C&D agents,

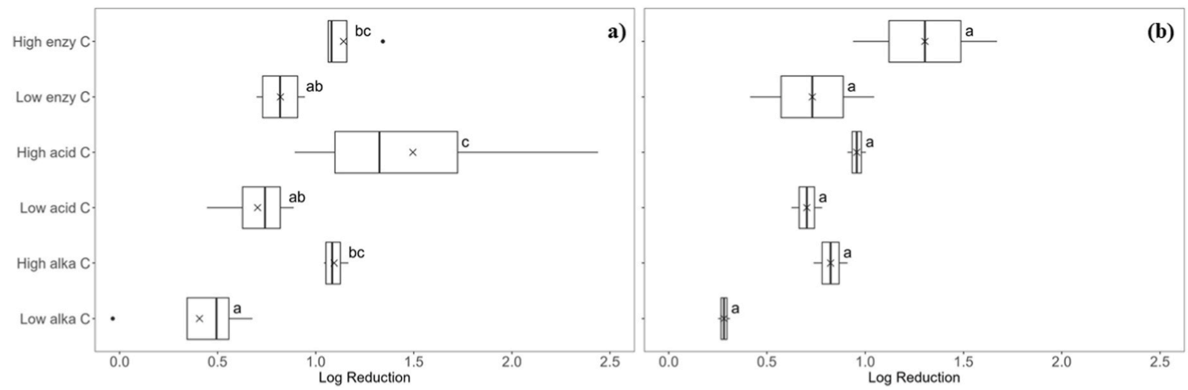

Fig. 2. Reductions of *L. monocytogenes* **(a)** and *Campylobacter* spp. **(b)** biofilm on conveyor belts when treated with three different cleaning agents at high and low concentrations. Enzymes at 4 % (High enzy C) and 2 % (Low enzy C); acids at 6 % (High acid C) and 4 % (Low acid C); chlorinated alkaline at 6 % (High alka C) and 4 % (Low alka C). Treatments not sharing a common letter are significantly different ($p < 0.05$).

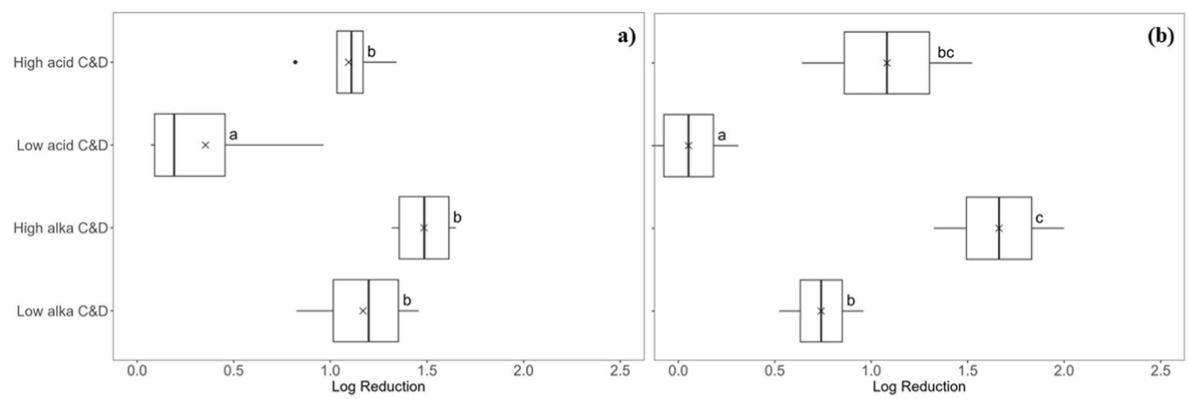

Fig. 3. Reductions of *L. monocytogenes* **(a)** and *Campylobacter* spp. **(b)** biofilm on conveyor belts when treated with chlorinated alkaline and acidic cleaning (C) and disinfecting (D) agents at high and low concentration. Acids at 6 % (High acid C&D) and 4 % (Low acid C&D); chlorinated alkaline at 6 % (High alka C&D) and 4 % (Low alka C&D). Treatments not sharing a common letter are significantly different ($p < 0.05$).

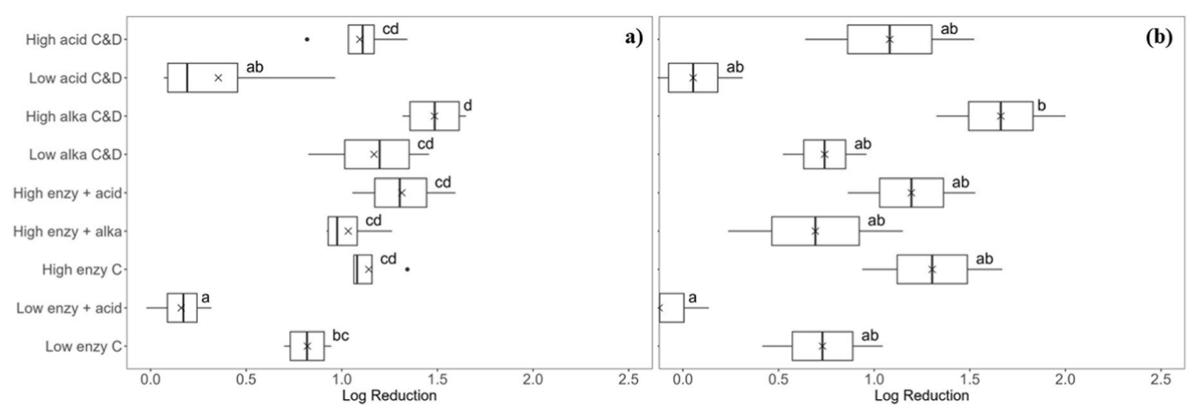

Fig. 4. Reductions of *L. monocytogenes* **(a)** and *Campylobacter* spp. **(b)** biofilm on conveyor belts when comparing cleaning (C)/cleaning and disinfection (C&D) using enzymatic agents alone or in combination with other agents at different concentrations. Acids at 6 % (High acid C&D) and 4 % (Low acid C&D); chlorinated alkaline at 6 % (C) and 3 % (D) (High alka C&D) and 4 % (C) and 1 % (D) (Low alka C&D); enzymes at 4 % with acids at 6 % (High enzy + acid) or with chlorinated alkaline 3 % (High enzy + alka); enzymes at 4 % (High enzy C); enzymes at 2 % with acids at 4 % (Low enzy + acid); enzymes at 4 % (Low enzy C). Treatments not sharing a common letter are significantly different ($p < 0.05$).

a greater mean bacterial reduction (0.8 and 0.7 log for *L. monocytogenes* and *Campylobacter* spp., respectively) was achieved when chlorinated alkaline treatment was applied compared to acidic treatment (Fig. 3).

3.5. Biofilm removal by enzymatic cleaning agents

Overall, replacing acidic or chlorinated alkaline cleaning agents with enzymatic cleaners did not enhance biofilm removal. The use of chlorinated alkaline C&D agents at high concentration was superior to that of enzymes at low concentration. The lowest mean reduction was

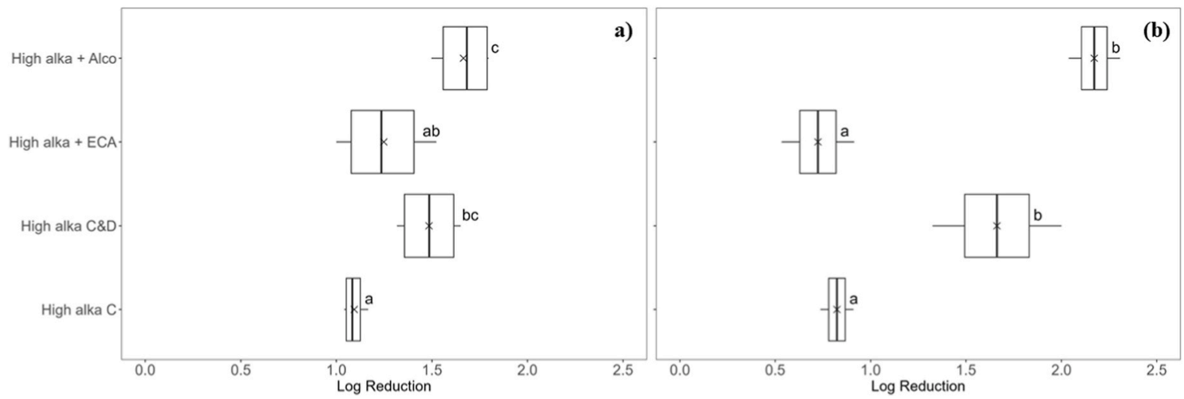

Fig. 5. Reductions of *L. monocytogenes* **(a)** and *Campylobacter* spp. **(b)** biofilm on conveyor belts when electrochemically activated water (ECA) and alcohol are used as disinfectants compared with chlorinated alkaline agents. Chlorinated alkaline cleaner at 6 % with alcohol at 100% (High alka + Alco) or with ECA at 100 % (High alka + ECA); chlorinated alkaline cleaner at 6% and disinfection at 3 % (High alka C&D); chlorinated alkaline cleaning agent at 6% (High alka C). Treatments not sharing a common letter are significantly different ($p < 0.05$).

Table 3
Mean log CFU/mL of *Listeria monocytogenes* and *Campylobacter* spp. in the broth and biofilm on untreated coupons (per coupon) before C&D treatment on day 4 and 5 for *L. monocytogenes* and *Campylobacter* spp., respectively. Four and two biological experiments, for *L. monocytogenes* and *Campylobacter* spp. biofilm assays were conducted, respectively.

	L. monocytogenes	*Campylobacter* spp.
Inoculated broth:	Mean (SD)	Mean (SD)
Day 1	6.4 ± 0.1	7.1 ± 0.3
Day 3	9.1 ± 0.7	7.0 ± 2.0
Day 4/5	9.4 ± 0.2	7.3 ± 2.1
Coupons	8.0 ± 0.1	6.9 ± 0.1

SD = Standard deviation.

observed for low concentration enzymatic cleaning combined with acidic disinfection. No additional reduction was observed when acidic or chlorinated alkaline disinfectants were applied together with enzymatic cleaning compared to enzymatic cleaning alone. Interestingly, a higher biofilm reduction (1.0 log) was achieved with low concentration chlorinated alkaline C&D compared to low concentration of enzymatic cleaning and acidic disinfection. However, these findings were only observed for *L. monocytogenes* biofilm (Fig. 4).

3.6. Biofilm removal by chlorinated alkaline cleaner and electrochemically activated water or alcohol

The combination that provided the most efficient C&D was chlorinated alkaline at high concentration together with alcohol. The additional effect of alcohol was 0.6 and 1.3 log for *L. monocytogenes* and *Campylobacter* spp., respectively. The least efficient combination was found to be chlorinated alkaline cleaning agent together with ECA (Fig. 5).

4. Discussion

The reduction of *L. monocytogenes* and *Campylobacter* spp. biofilms following different treatment protocols were similar across this study. However, it is important to note that only two biological experiments were performed for *Campylobacter* spp. biofilm, compared to four for *L. monocytogenes*. Nevertheless, previous studies have also used two biological replicates to evaluate the efficacy of different C&D regimes (Fagerlund et al., 2020), which supports the validity of the results obtained in the present study. The highest reductions of bacteria were achieved when chlorinated alkaline cleaner at high concentration was combined with alcohol disinfection. The promising effect of alcohol disinfection in the removal of *L. monocytogenes* biofilm from conveyor belt material has been previously described (Fagerlund et al., 2020). Despite this, it should be mentioned that alcohol is generally unsuitable for the daily C&D procedures that occur in slaughterhouses for reasons such as flammability, volatility, and high cost. Thus, its use is more appropriate for disinfecting hands, surfaces, and equipment, which must be decontaminated regularly during working hours (Fagerlund et al., 2017; Moazzami et al., 2025b). Moreover, it is noteworthy that no significant difference between the use of alcohol disinfection compared to chlorinated alkaline disinfection was found, which is reassuring considering that the latter is commonly used in C&D regimens in slaughterhouses (Moazzami et al., 2025b; Naim et al., 2025). However, in the current study, the reduction of bacterial counts was moderate (1.7-2.2 log), and substantially lower than the 5.0 log reduction required by the European standards for chemicals (SS-EN 1276:2019). Further, high bacterial loads were still present on the conveyor belts after C&D. This could be partly explained by the stability and persistence of *L. monocytogenes* and *Campylobacter* spp. biofilms on surfaces (Fagerlund et al., 2025; Monteith et al., 2023; Techaruvichit et al., 2016). In earlier investigations, chemical concentrations as high as 40% of chlorinated alkaline/alkaline chemicals had to be applied to achieve complete biofilm removal (Fagerlund et al., 2020). However, the use of such high concentrations is not recommended by the chemical producers, nor should it be used due to the safety risks for the cleaners, corrosion, tear of the equipment and surfaces, and economic reasons. A potential explanation for the relatively low reductions observed in this study is the absence of mechanical force during the cleaning process. Mechanical action is typically part of industrial C&D procedures and is recommended when applying the types of chemical agents evaluated here. Nevertheless, challenge studies performed by chemical suppliers generally do not include mechanical force, as reflected in standardized test protocols (ISO 14349:2012; ISO 1276:2019). Incorporating mechanical action in our experiments was considered, but ultimately not implemented for several reasons. First, applying mechanical force to the small test coupons in a controlled and reproducible manner was deemed technically impractical. Second, the contribution of mechanical action to bacterial removal on partially porous materials, such as woven conveyor belts, remains uncertain. Bacteria embedded within the belt structure may be largely inaccessible to mechanical abrasion, limiting the potential impact of such an approach (Møretrø et al., 2026).

Ideally, in industrial settings manual mechanical cleaning such as scrubbing should be included in C&D regimens, as it enables the use of a lower concentration of chemicals (Dallagi et al., 2023). This is a time-consuming practice and is therefore often not feasible in industrial practice in slaughterhouses and meat processing facilities. Instead, pressure systems for rinsing and applying chemicals are widely used

(Moazzami et al., 2025b; Wang et al., 2018). Difficulty to sufficiently remove bacteria from conveyor belts by C&D have previously been observed in slaughterhouses and meat processing facilities, in which mechanical force was applied in the C&D procedure. For instance, when comparing the highest reductions in the current study (1.7 and 2.2 log for *L. monocytogenes* and *Campylobacter* spp., respectively) with reductions of the total aerobic count (1.6, 2.5, 2.8 log/100 cm^2) reported for woven conveyor belts in a comparable study, similar levels of reductions were observed (Moazzami et al., 2023). In another study, reductions of aerobic bacteria after C&D were only 0.3 log/1000 cm^2, which is comparable to the lowest reductions achieved in the current study (0.2 and 0.1 log for *L. monocytogenes* and *Campylobacter* spp., respectively) (Wang et al., 2018).The lowest reduction was reported when low concentration of enzymatic cleaning followed by acidic disinfection was used. Further, biofilm reduction did not improve when enzymatic cleaner was followed by acid or chlorinated alkaline disinfection agent. According to the manufacturer, the efficacy should improve when enzyme-based cleaning is followed by alkaline and acidic disinfection, and this has also been observed in previous research (Delhalle et al., 2020; Nahar et al., 2018). Enzymes have been used without disinfectant on biofilm composed of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which resulted in a slightly higher reduction (2.1 log) than that in the current study (1.1 log for *L. monocytogenes*) (Stiefel et al., 2016). The discrepancy of bacterial reductions between different studies testing enzymatic cleaners could be due to the use of products with different enzymatic composition, different disinfectants applied after enzymatic cleaning, composition of the biofilm, materials studied and the C&D procedures applied (Waldhans et al., 2023).

In slaughterhouses, higher concentrations of chemicals (typically 2-3% more) can be used in heavily soiled areas such as the slaughter area/stable. When different concentrations were compared in this study, increased efficacy was observed for chlorinated alkaline and acidic cleaners. However, this was not the case for the enzymatic cleaners. The failure of improved efficacy when increasing the enzymatic concentration may be due to substrate saturation (Bisswanger, 2014). Moreover, as discussed previously, the concentrations of C&D chemicals should be kept as low as possible (Marriott et al., 2018).

When comparing different cleaning products, such as acidic or alkaline agents, no significant difference was observed regarding their efficacy on biofilm removal. This applied both when cleaners were used alone or in combination with their corresponding disinfectant. In contrast, when chlorine and peracetic acid were applied on conveyor belts, marginally higher reductions (2.2 log) of *L. monocytogenes* were achieved compared to the present study (Ohman et al., 2024). It should be highlighted that the selection of C&D products also must be based on their ability to remove different types of soil. For instance, chlorinated alkaline products are used to remove protein and fat, whilst acidic products are more efficient in removing mineral salts (Holah, 2014; Marriott et al., 2018). Enzymatic agents can be used to remove biofilm (Hamza, 2017; Nahar et al., 2018), however this was not confirmed in the current study.

Furthermore, ECA is a relatively novel product and thus not widely used in slaughterhouses and other food premises. According to the manufacturer's instructions, ECA could be used in the food industry and advertised as being more environmentally friendly and affordable than other commercial agents, as only salt and water is required to produce the solution. Despite this, it was shown that the biofilm removal efficacy of ECA was moderate (0.7 and 1.2 log reduction for *Campylobacter* spp. and *L. monocytogenes*, respectively). Similar results were observed in an earlier study, in which ECA together with acidic or alkaline agents was unable to remove *E. coli* from stainless steel coupons when meat residues were present (Khalid et al., 2024).

In challenge studies, such as this one, different bacterial loads applied on the conveyor belts can be used to evaluate the C&D efficacy. The bacterial loads applied in this study are consistent with those used in comparable research (Fagerlund et al., 2020; Khalid et al., 2024; Mazaheri et al., 2022; Merino et al., 2024). Additionally, high bacterial loads have been reported on slaughterhouse surfaces (Fagerlund et al., 2025; Moazzami et al., 2023), thereby supporting the bacterial loads that were applied in the present study.

Few studies have evaluated the efficacy of different C&D regimes, conducted in laboratory or industrial settings. Even fewer have been adapted to slaughterhouses and meat production facilities. There is also a lack of studies which have evaluated different C&D regimes to remove *Campylobacter* spp. biofilm from surfaces and which have included products considered environmentally friendly such as ECA. Sufficient C&D in these settings is crucial, as the surfaces become heavily soiled (especially in the slaughter area). Ready-to-eat (RTE) foods are often manufactured in these facilities and the legal requirements for monitoring bacterial contamination of meat products and surfaces must be complied (EC, 2005). In meat production environments, a variety of conveyor belt materials are used, as their characteristics are suitable for different production purposes (Moazzami et al., 2023; Rashid et al., 2023). Woven conveyor belts were selected in this challenge study, as they are commonly used in the type of facilities targeted in the present study. They are known to be difficult to clean and may therefore constitute as harbourage sites for biofilm-producing bacteria (Moazzami et al., 2025b; Waldhans et al., 2023).

In conclusion, this study demonstrated that commonly used C&D agents in slaughterhouses and meat processing facilities do not sufficiently reduce biofilms of *L. monocytogenes* and *Campylobacter* spp. on woven conveyor belts commonly found in slaughterhouses and cutting plants. Differences in efficacy between chemical agents and their applied concentrations were relatively small, rendering it difficult to advise the FBO on which C&D procedure to implement. In particular, *L. monocytogenes* constitutes a serious threat to human health and it is therefore crucial to develop specific C&D guidelines for FBOs to ensure safer operations. Testing C&D protocols under conditions that are similar to those in real-life industrial settings is key to develop science-based guidelines that can improve C&D regimens at slaughterhouses and other meat production facilities. As this study showed that biofilm is difficult to remove from woven conveyor belts, food companies should consider using belts with a more hygienic design.

CRediT authorship contribution statement

Madeleine Moazzami: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ingrid Hansson:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Solveig Langsrud:** Writing – review & editing, Methodology, Conceptualization. **Trond Møretrø:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sofia Boqvist:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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