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Research Note

Ultrasonic Cleaning of Conveyor Belt Materials Using *Listeria monocytogenes* as a Model Organism

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ABSTRACT

Persistent *Listeria monocytogenes* contamination of food industry equipment is a difficult problem to solve. Ultrasonic cleaning offers new possibilities for cleaning conveyors and other equipment that are not easy to clean. Ultrasonic cleaning was tested on three conveyor belt materials: polypropylene, acetal, and stainless steel (cold-rolled, AISI 304). Cleaning efficiency was tested at two temperatures (30 and 45°C) and two cleaning times (30 and 60 s) with two cleaning detergents (KOH, and NaOH combined with KOH). Conveyor belt materials were soiled with milk-based soil and *L. monocytogenes* strains V1, V3, and B9, and then incubated for 72 h to attach bacteria to surfaces. Ultrasonic cleaning treatments reduced *L. monocytogenes* counts on stainless steel 4.61 to 5.90 log units; on acetal, 3.37 to 5.55 log units; and on polypropylene, 2.31 to 4.40 log units. The logarithmic reduction differences were statistically analyzed by analysis of variance using Statistical Package for the Social Sciences software. The logarithmic reduction was significantly greater in stainless steel than in plastic materials ($P < 0.001$ for polypropylene, $P = 0.023$ for acetal). Higher temperatures enhanced the cleaning efficiency in tested materials. No significant difference occurred between cleaning times. The logarithmic reduction was significantly higher ($P = 0.013$) in cleaning treatments with potassium hydroxide detergent. In this study, ultrasonic cleaning was efficient for cleaning conveyor belt materials.

Several studies show that *L. monocytogenes* survives and persists in equipment and environment in food processing plants *(12, 15, 17, 21).* Miettinen et al. *(15)* found one *L. monocytogenes* strain to persist in the packaging machine of an ice cream plant for 7 years. This was partially due to the problems in cleaning the conveyor belt of the packaging machine. When finished products have been found to be contaminated with *L. monocytogenes,* the contamination studies indicated that the products were contaminated with the same strain as the environment or equipment of the food plant *(1, 6, 15).*

Several studies identified conveyor systems as a favorable environment for *L. monocytogenes (2, 15, 19).* Cotton and White *(2)* collected samples from the environment of milk and ice cream plants. From *L. monocytogenes*–positive samples, 39% were isolated from conveyors. Conveyor belts consist of multiple small parts and joints, which make the effective mechanical cleaning difficult. Usual cleaning methods used in food industry, such as low-pressure foam cleaning, do not always give satisfactory results in cleaning the conveyor belts.

Ultrasound causes pressure changes, cavitation, in liquid media. Bubble collapse during cavitation generates transient hot areas *(3).* The lower frequencies of ultrasound, 20 to 40 kHz, have proved to be more efficient in microscopic particle (i.e., blood cells, bacteria) cleaning than higher frequencies are *(9, 13).*

Ultrasonic cleaning is widely used in electronic and metal industry, but the use in food manufacture is rare, though it is used in bottling lines and in cleaning cheese molds *(5, 7).* Ultrasound used in combination with heat, pressure, or nonfoaming detergents appears to be more effective than any of the treatments alone is in cleaning or decontamination of foods *(4, 10, 18).* Ultrasonication has been used to remove biofilms and microbial soil from various surfaces *(8, 11, 20, 22).* Little information, however, is available about using ultrasonic cleaning of equipment in industrial food processing.

The aim of this study was to determine the effect of ultrasonic cleaning in combination with detergents on detachment of *L. monocytogenes* from different types of conveyor belt materials, at different temperatures and cleaning times.

MATERIALS AND METHODS

Bacterial strains. A mixture of three *L. monocytogenes* strains (B9, V1, and V3) isolated from meat and dairy plants were used. The Department of Food and Environmental Hygiene (B9) and VTT Technical Research Centre of Finland (V1 and V3) provided the strains. Strains were grown in tryptic soy broth at 37°C for 24 h. After incubation, 100 μl of each strain was transferred

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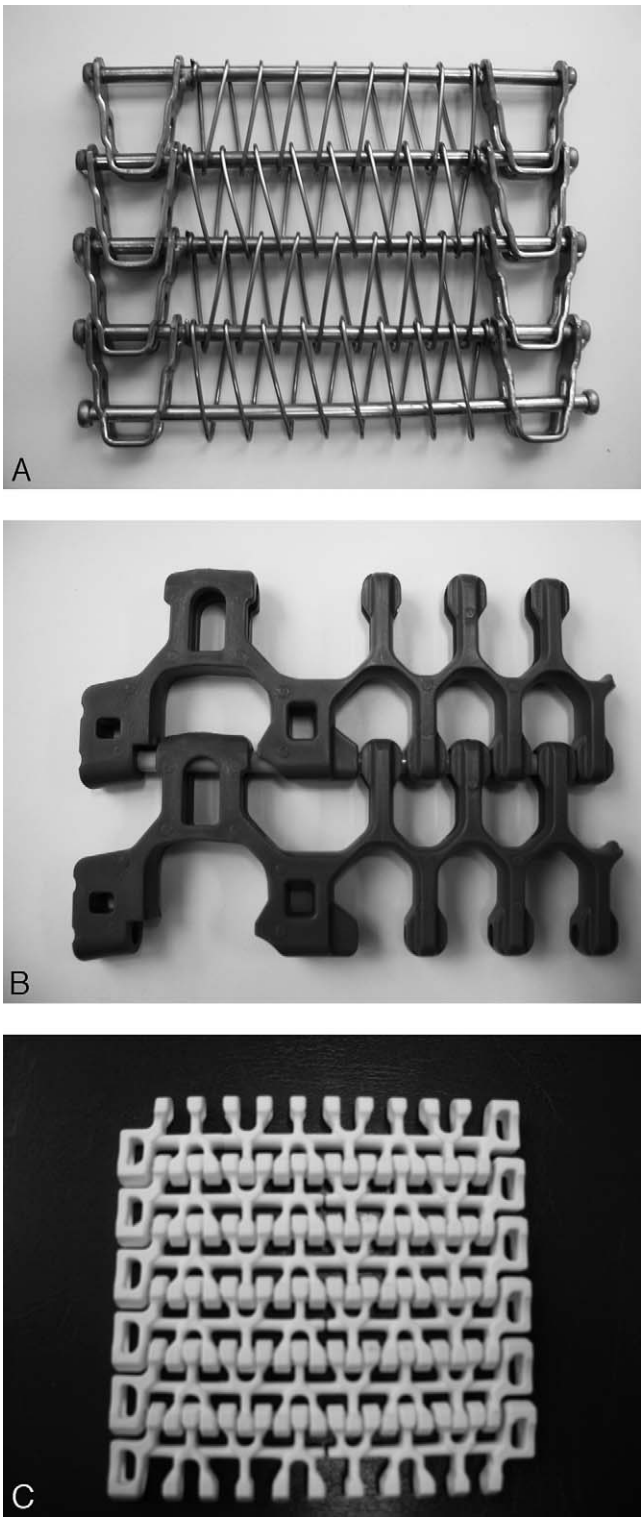

FIGURE 1. *Test pieces of (A) stainless steel (cold-rolled, AISI 304), (B) acetal, and (C) polypropylene.*

to 8 ml of tryptic soy broth, and the *L. monocytogenes* mixture was grown at 37°C for 24 h. This mixture was used for inoculation.

Conveyor belts. Conveyor belt materials of stainless steel mesh (cold-rolled, AISI 304, ESFO BV Transportbanden, Enschede, The Netherlands), polypropylene (Intralox 1100, Amsterdam, The Netherlands), and acetal (Ammeraal Beltec, Heerhugowaard, The Netherlands) were used (Fig. 1). These materials are commonly used in conveyors in the food industry. Belts were cut into pieces measuring 15 by 10 cm.

Organic soil. Organic soil was made according to Wirtanen et al. *(23)* from cream, fat-free milk powder, modified starch, and whey powder. Ingredients were mixed, boiled, and distributed to bottles, which were sterilized (121°C, 15 min) and stored at 4°C.

Inoculation of conveyor belt pieces. Conveyor belt pieces were washed and dried. Dry pieces were sterilized (121°C, 15 min). Pieces were transferred aseptically to individual stomacher bags (Stomacher 400, Seward Medical, London, UK) and 50 ml of organic dirt was added to the bags. Bags were left to stand for 30 min, following which, 4 ml of *L. monocytogenes* suspension (10^8 CFU/ml) and 350 ml of tryptic soy broth were added. Incubation of the bags occurred at 37°C for 72 h. After the incubation, the conveyor belt pieces were rinsed with sterile water.

Ultrasonic cleaning. The stomacher bags were filled with 400 ml of 2% washing detergent, either potassium hydroxide (Solo VC27, Diversey Johnson, Sturtevant, Wis.), or potassium hydroxide–sodium hydroxide (MP3-Mip SP, Ecolab, St. Paul, Minn.). The concentration of potassium hydroxide in the first-mentioned washing detergent according to the manufacturer was 0.3 to 0.6%, and in the latter washing detergent it was 0.1 to 0.3%. The concentration of sodium hydroxide was 0.3 to 0.6%. The stomacher bags were degassed for 5 min in an ultrasound bath (Finnsonic m40, 600 W, 30 kHz, FinnSonic, Lahti, Finland). The tank volume was 38 liters. The conveyor belt pieces were then put into the degassed bags, which were transferred back to the ultrasonic bath and attached to a frame. Cleaning was carried out at 30 and 45°C for 30 s and 60 s with two detergents, using eight different cleaning treatments. After the cleaning treatment, the detergent was rinsed from pieces with sterile water, and the *L. monocytogenes* count was determined. Every treatment was done with two replicate pieces, and each treatment was performed twice.

Determination of *L. monocytogenes*. To determine the amount of *L. monocytogenes* attached to the surfaces before cleaning treatments, one contaminated piece of each material was dismantled with tweezers and transferred to a stomacher bag with 400 ml of degassed sterile water. Degassing was performed in a small ultrasonic bath (Branson 2100-DTH, 70 W, 47 kHz, 2.8 liters, Branson Ultrasonics, Soest, The Netherlands) for 5 min. The pieces in the individual bags were treated with ultrasound at 25°C for 10 min in the same ultrasonic bath to remove *L. monocytogenes* cells from the pieces. *L. monocytogenes* cells detached into the liquid were determined by spread plating serial dilutions in duplicate onto tryptic soy agar plates. The colonies were counted after aerobic incubation at 37°C for 24 h and 48 h. The cleaned pieces were treated similarly after the cleaning treatment. After the cleaning treatment, 1 ml of the liquid was divided on four plates in duplicate. The lowest level of detection was 1 CFU/ml. The difference between cell counts before treatment and after the treatment was calculated, and the effect of the cleaning treatment expressed in logarithmic reduction. When cell counts were below the detection level, a value of 1 was used in calculations. The differences of logarithmic reduction between different cleaning temperatures, times, and detergents were analyzed statistically with the paired *t* test. Analysis of variance was used with multiple comparisons Tukey test with Statistical Package for the Social Sciences software (SPSS Inc., Chicago, Ill.) to analyze differences between materials.

TABLE 1. *Logarithmic cell counts of* Listeria monocytogenes *in ultrasonic cleaning treatments*[a]

Material	Temp (°C)	Time (s)	Detergent solution	Mean log CFU/ml ± SD		Mean log reduction ± SD (n = 4)
				Prior to cleaning (n = 2)	After cleaning (n = 4)	
Stainless steel	30	30	KOH, NaOH	5.25 ± 0.45	ND	5.25 ± 0.18
			KOH	4.66 ± 0.18	ND	4.66 ± 0.14
		60	KOH, NaOH	5.09 ± 0.19	ND	5.09 ± 0.16
			KOH	4.46 ± 0.23	0.12 ± 0.24	4.34 ± 0.20
	45	30	KOH, NaOH	4.98 ± 0.58	ND	4.98 ± 0.48
			KOH	5.54 ± 0.91	ND	5.54 ± 0.74
		60	KOH, NaOH	4.80 ± 0.33	ND	4.80 ± 0.27
			KOH	4.63 ± 0.16	ND	4.63 ± 0.13
Polypropylene	30	30	KOH, NaOH	4.18 ± 0.53	1.93 ± 0.34	2.25 ± 0.73
			KOH	4.93 ± 0.01	1.50 ± 0.49	3.43 ± 0.49
		60	KOH, NaOH	4.73 ± 0.15	2.05 ± 0.28	2.68 ± 0.23
			KOH	4.76 ± 0.44	1.64 ± 0.33	3.12 ± 0.26
	45	30	KOH, NaOH	4.05 ± 1.14	1.12 ± 0.34	2.92 ± 0.62
			KOH	4.56 ± 0.30	0.20 ± 0.41	4.35 ± 0.58
		60	KOH, NaOH	4.11 ± 0.30	0.98 ± 0.87	3.13 ± 0.69
			KOH	3.50 ± 0.00	0.10 ± 0.20	3.40 ± 0.20
Acetal	30	30	KOH, NaOH	4.40 ± 0.42	0.55 ± 0.80	3.85 ± 1.09
			KOH	5.16 ± 0.04	1.58 ± 0.42	3.58 ± 0.44
		60	KOH, NaOH	4.78 ± 0.17	1.29 ± 0.24	3.49 ± 0.35
			KOH	5.73 ± 1.27	0.65 ± 0.39	5.08 ± 1.19
	45	30	KOH, NaOH	4.74 ± 0.04	0.14 ± 0.27	4.60 ± 0.29
			KOH	5.27 ± 0.18	ND	5.27 ± 0.15
		60	KOH, NaOH	4.76 ± 0.69	0.15 ± 0.19	4.62 ± 0.65
			KOH	5.17 ± 0.77	0.29 ± 0.22	4.88 ± 0.50

[a] KOH, potassium hydroxide; NaOH, sodium hydroxide; ND, not detected.

RESULTS

L. monocytogenes attached to tested materials in different amounts. In most cases, the highest *L. monocytogenes* counts after the inoculation were attained from stainless steel and acetal, and the lowest on polypropylene. On the other hand, *L. monocytogenes* was easily detached from stainless steel, and in most of the treatments there was no *L. monocytogenes* detected after the cleaning treatment (Table 1). The logarithmic reduction was lower than 3 log units in only 3 of the 24 treatments, and they were all obtained from polypropylene (Table 1). The analysis of variance test showed difference ($P < 0.001$) between material groups. In all treatments the logarithmic reductions on stainless steel (mean, 4.91) were greater than on polypropylene (mean, 3.61), and the difference between these materials was statistically significant (Tukey, $P < 0.001$). The logarithmic reductions in polypropylene were also smaller than in acetal (mean, 4.42), and the difference between these materials was statistically significant (Tukey, $P < 0.001$). The logarithmic reductions in stainless steel were statistically greater than in acetal (Tukey, $P = 0.023$).

A higher temperature enhanced the efficiency of the cleaning treatments. The difference between the 45°C (mean, 4.43) and 30°C (mean, 3.90) treatments was statistically significant (paired t test, $P < 0.001$). The difference in the logarithmic reductions between 30-s (mean, 4.22) and 60-s (mean, 4.10) treatments was not statistically significant (paired t test, $P > 0.05$). The logarithmic reduction was higher in cleaning treatments with potassium hydroxide detergent (mean, 4.36) than with potassium and sodium hydroxide detergent (mean, 3.97), and this difference was statistically significant (paired t test, $P = 0.013$).

DISCUSSION

Despite the high amount of *L. monocytogenes* in stainless steel before the washing treatment, the amount was either small or undetectable after the washing treatment. All eight of the tested ultrasonic cleaning parameter combinations were efficient in cleaning the conveyor made of stainless steel. The cleaning treatment was considered effective if *L. monocytogenes* reduction after treatment was at least 3 log units *(16).* In three of the tested ultrasonic cleaning combinations, the logarithmic reduction of *L. monocytogenes* was less than 3 log units on polypropylene, and *L. monocytogenes* contamination was present on polypropylene after all cleaning treatments.

The logarithmic reduction of *L. monocytogenes* was significantly greater in stainless steel than it was in plastic materials. The difference in cleaning efficacy for the various materials tested can be partly explained by the hardness of the material. Ultrasonic cleaning is more efficient on hard surfaces than it is on soft materials. The surface hardness of stainless steel is greater than is the surface hardness of plastic materials, and also the attachment of *L. monocytogenes* to stainless steel is weaker than it is to polymeric materials *(14).* Acetal has a greater surface hardness in comparison with polypropylene, which can explain the greater logarithmic reduction obtained in acetal than in

polypropylene. In addition, the amount of joints was greater in the pieces of polypropylene than it was in the pieces of acetal or stainless steel, which might have resulted in weaker washing results.

With the plastic conveyor materials, the higher cleaning temperature (45°C) resulted in 0.5 to 1.7 log units greater reductions of cell counts than did the lower cleaning temperature (30°C). The difference was statistically significant in all of the treatments. At low temperatures it is difficult to remove fat from surfaces, even when combined with ultrasound. The milk-fat residue on material surfaces provides protection to *L. monocytogenes* cells and impairs the cleaning result. Therefore it is advisable to use a higher cleaning temperature than 30°C.

The cleaning results were similar with both tested cleaning times in this study. This means that the cleaning time could be as short as 30 s without impairing the cleaning result, and perhaps even shorter cleaning times could be used. This should be, however, ascertained with further studies. Ultrasonic treatment has been found to dislodge 83% of a biofilm on stainless steel in 10 s *(18).* It is significant that ultrasonic cleaning is effective even with short treatment times. This facilitates the application of ultrasonic cleaning of conveyors in the actual food processing environment.

Even though a statistically significant difference in logarithmic reductions between detergents was found, the actual difference between the detergents was small, 0.38 log units. This means that the correlation between the statistically calculated parameter and the actual detergent performance in industrial use should be interpreted with caution.

In conclusion, ultrasonic cleaning was an effective method of detaching *L. monocytogenes* from conveyor materials. Short ultrasonic washing treatment may provide a new possibility in cleaning conveyor belts that are difficult to clean with conventional methods.

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