

Full Length Research Paper

A practical evaluation of detergent and disinfectant solutions on cargo container surfaces for bacteria inactivation efficacy and effect on material corrosion

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Cleaning and disinfection agents were evaluated against selected bacteria on three surfaces: aluminium, stainless steel and fibre re-enforced plastic, used as cargo container linings and to assess their effect on the surface integrity. Nine sanitation chemical solutions: benzalkonium chloride, sodium hypochlorite, nitric acid, levulinic acid, peracetic acid sodium hydroxide, sodium dodecyl sulphate, AT special (commercial detergent) and Disinfect Maxi (commercial disinfectant) were tested against seven bacteria strains: *Escherichia coli* K12, *E. coli* DSM 682, *Salmonella* Senftenberg DSM 10062, *Salmonella* Typhimurium P6, *Pseudomonas aeruginosa* DSM 939, *Listeria monocytogenes* Scott A and *Listeria innocua* P577 in dirty condition as described by standard bactericidal test both in suspension and on the three surfaces. With the exception of sodium dodecyl sulphate (SDS) and AT Special (ATS), the others were efficient in reducing the live bacteria counts as required by the standards for six of the bacteria both in suspension and on the three surfaces; *L. monocytogenes* Scott A was the exception. Only peracetic acid was able to disinfect all seven strains on all surfaces (> 4 log CFU reduction) as well as in suspension (> 5 log CFU reduction) as required by the standards. Accelerated corrosion tests also showed that most of the disinfectant will likely compromise the integrity of the surfaces. Only peracetic acid at the concentration used had minimal corrosion effect. A novel index for practical usability was created to take into account disinfection efficacy and low corrosiveness; peracetic acid had the highest usability index from the chemicals tested. Peracetic acid based disinfectants will be appropriate for an environment with the composite materials studied as found in some cargo containers. Combining disinfection studies, corrosion studies and the index of the two can assist the food and allied industries in making cost-effective choices for disinfectants depending on surface materials present.

Key words: Detergents, bacteria, cargo container surfaces, disinfectant.

INTRODUCTION

Foodborne pathogenic microorganisms are ubiquitous and cause havoc, both in making food unwholesome and causing varied levels of mortality and morbidity (Nyachuba, 2010; Anonymous, 2011). Pathogens easily spread to and from various food and food contact surfaces (Abban and Tano-Debra, 2011; Kusumaningrum et al., 2003), with the spread influenced by several

intrinsic and extrinsic factors (Whitehead and Verran, 2006; Montville and Schaffner, 2003; Rusin et al., 2002). Retained pathogens on surfaces may proceed into the biofilm phenotype and thus become more difficult to clean (Sharma and Anand, 2002) while still presenting a bio-transfer potential (Verran, 2002).

Various cleaning and disinfection regimes have been

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adopted in the food and related industries to reduce pathogens to acceptable levels if not eliminate them completely, using various chemical agents (Dvorak, 2008). The effectiveness of these chemicals are affected by the level and type of soil that may be present of these surfaces, and it is also known that they are generally more effective on bacteria in suspension than on sessile (attached and biofilm phenotype) forms of pathogens (Joseph et al., 2001; Møretrø et al., 2009; Deza et al., 2005). However, many disinfectants on the market have been tested in suspension tests only and thus information about their effect on sessile bacteria is limited (Møretrø et al., 2009).

Various food contact surface materials are encountered, with stainless steel grades being the 'golden standard' in the food processing environment. However, other allied food handling operations such as food cargo containers are commonly lined with other composites including aluminium, fibre plastic materials and other polymers; mostly chosen because of their light weight which is desired in such applications (Abban et al., 2012). Choosing appropriate disinfectants for surfaces in this part of the food handling cycle can be challenging as the active agents must be both effective in reducing or eliminating the pathogens present, while also being non-corrosive to the various composite materials which may be present simultaneously. Corrosion or any other deformation in the surface material integrity is important both in terms of the lifespan of the equipment, and also because cracks and other effects of corrosion provide a suitable place for pathogens to lodge and evade later cleaning and disinfection operations (Dvorak, 2008; Guthrie et al., 2002).

Though there are reports in the literature for work done of some pathogens in the sessile form, most of these investigations have been done on stainless steel. Also the links between the concentrations of active agents used in such test and their effect over time on the food contact surface materials are usually ignored. The aim of this study was to evaluate the efficacy of several active agents and commercial formulations used as part of cleaning and disinfection in reducing selected bacteria levels as required by European standards (Anonymous, 2001; Anonymous, 2009). This has been done both in suspension and the sessile form of the bacteria on three cargo container lining surfaces: aluminium, stainless steel and a fibre plastic material (FRP). Accelerated corrosion tests on these materials in the test solutions have also been made to guide comparison and choosing of practical and effective cleaning and disinfection chemicals.

MATERIALS AND METHODS

Bacterial strains

The following strains were obtained and used for the study: *Escherichia coli* K12 (Staten Serum Institute, Denmark), *E. coli* DSM 682, *Salmonella* Senftenberg DSM 10062, *Pseudomonas*

aeruginosa DSM 939 (German Culture Collection, DSZM, Braunschweig), *S. Typhimurium* P6 (Klingberg et al., 2005), *Listeria monocytogenes* Scott A and *L. innocua* P577 (Department of Food Science bacteria collection, University of Copenhagen). All strains were cultivated in tryptic soy broth (TSB) and tryptic soy agar (TSA) (Oxoid, Hampshire, UK) at 37°C for 24 h and maintained at -80°C with TSB and 20% (v/v) glycerol.

Bactericidal tests

The bactericidal test solutions and their concentrations as used in the assays are shown in Table 1, together with the measured pH of the solutions (PHM 250, Radiometer A/S, Denmark). Concentrations used were the lowest recommended user levels from manufacturers for the commercial products (AT Special and Disinfect Maxi) or concentrations found to be effective in the literature for the other test solutions made from analytical grade laboratory chemicals (Møretrø et al., 2009; Zhao et al., 2010). Assays were conducted in the presence of 3% (w/v) bovine serum albumin (BSA, Sigma-Aldrich, Steinheim, Germany) for a contact time of 5 min at 20°C. Dey-Engley (DE) neutralizing broth (Fluka/ Sigma-Aldrich, Steinheim, Germany) was used in neutralizing the disinfectants after exposure, except for the quaternary ammonium salt and Disinfect Maxi, where the DE broth was supplemented with 30 g/L saponin (Sigma-Aldrich). It was confirmed as part of the standard tests that the neutralizing solutions used neutralized the test solutions and did not have any bactericidal or inhibitory effect on the growth of surviving bacteria cells. All tests were performed in duplicates; two independent repeats experiments of each assay type were performed.

Suspension test

The effectiveness of the test solutions against the test bacteria were investigated according to the European Committee for Standardization (CEN) suspension test DS/EN 1276 (Anonymous, 2001) as also described by Møretrø et al. (2009). Briefly 1 ml of microbial suspension [approximately 10^8 to 10^9 CFU/ml; made by suspending single colonies from 24 h cultures of each test bacteria on TSA into saline-tryptone buffer (8 g NaCl and 1 g tryptone per litre; Oxoid, Hampshire, UK)] was mixed with 1 ml the BSA solution in a test tube. After 2 min of incubation at 20°C, 8 ml of a test solution was added and the components mixed by vortexing. After 5 min contact time at 20°C, 1 ml of this test mixture was transferred into a test tube containing 8 ml of neutralizing solution as defined above and 1 ml of sterilized distilled water. The components were again mixed by vortexing and neutralizer allowed to take effect for 5 min at 20°C. Triplicate 1 ml aliquots of this resulting mixture were serially diluted and plated on TSA plates and incubated at 37°C for 24 h, followed by enumeration of viable bacteria. The efficacy of the chemical test solution using this method was indicated by a > 5 log reduction in the CFU of the test bacteria according to the standard (Anonymous, 2001).

Surface test

The susceptibility of sessile cells of the test bacteria to the test solutions was determined for three surfaces: aluminium (alloy 5754), stainless steel (AISI-304) and fibre plastic material (FRP) (Maersk Container Industry, Tinglev, Denmark); coupons of the surface materials (10 × 10 mm) were cleaned and sterilized as previously described (Abban et al., 2012). Surface disinfection assays were according to the European surface test DS/EN 13697 (Anonymous, 2009), as also described by Møretrø et al. (2009) with few modifications. Briefly, 50 µl of microbial suspension [approximately 10^8 to 10^9 CFU/ml; prepared by as above] was mixed

Table 1. Concentration and pH of disinfectant preparations used in the study.

| Test chemical | Test solution concentration ² | pH ³ |
|---|---|-----------------|
| Benzalkonium chloride (QAS) | 0.2% w/v | 5.47 |
| Disinfect Maxi (DSM) ¹ ; (<5% isopropyl alcohol, 5-15% dodecyldimethyl ammonium chloride) | 3% v/v (user concentration recommended by manufacturer) | 5.69 |
| Sodium hypochlorite (NaOCl) | 5% w/v | 11.44 |
| Nitric acid (HNO ₃) | 0.2% w/v | 1.46 |
| Sodium hydroxide (NaOH) | 2% w/v | 12.39 |
| Peracetic acid (PAA) | 0.2% w/v | 2.91 |
| Levulinic acid (LEV) | 3% w/v | 2.75 |
| AT Special (ATS) ¹ ; (<1% disodium metasilicate, 1-5% anionic surfactant, 1-5% alcohol ethoxylate polymer) | 4% v/v (user concentration recommended by manufacturer) | 11.10 |
| Sodium dodecyl sulphate (SDS) | 2% w/v | 6.42 |

¹ Commercial product from ITW Novadan ApS, Kolding, Denmark. Other agents were of analytical grade.

² Concentrations of commercial product based on minimal use concentration recommended by the manufacturer; concentration of analytical grade chemical based on concentration levels found to have bactericidal effect in the literature (Møretrø et al., 2009; Zhao et al., 2010).

³ pH of freshly prepared test solution as measured with pH meter (PHM 250, Radiometer A/S, Denmark).

with 50 µl of the BSA solution and applied per coupon. The suspension was air-dried onto the coupon at 37°C for 1.5 h under a class two laminar flow/fume hood (H.J. Engineering ApS, Galten, Denmark) before 100 µl of chemical test solution (or distilled water; control) was spread over the dried suspension for a contact time of 5 min at 20°C. Coupons were then immediately transferred into sterile 'blue-cap' glass bottles (base diameter = 5 cm) containing 10 ml of appropriate neutralizing broth as described above and 5 g of borosilicate glass beads (2.5 to 3.5 mm diameter; BDH, Poole, UK) and shaken on an orbital shaker (model KS 260, IKA, Staufen, Germany) at 300 RPM for 10 min. The number of live bacteria from the surface after exposure to the test solution was then determined by serial dilution and plating on TSA according to the European standard (Anonymous, 2009). The bactericidal efficacy of the chemical test solutions on the test bacteria was calculated as the difference between the log transformed number of live bacteria exposed to distilled water (control) and test solution (Anonymous, 2009); a > 4 log reduction in the CFU of the test bacteria meant the test solution had the required bactericidal efficacy on the test bacteria attached to the test surface according to the standard (Anonymous, 2009). It was confirmed according to the test requirements in the DS/EN 13697 standard that the test bacteria were removed from the surface coupons by the glass beads 'scrubbing' process (Anonymous, 2009).

Accelerated corrosion tests

Accelerated corrosion or degradation tests were done on coupons of the three test materials. For the aluminium and stainless steel, coupons of the two materials were cleaned as above, allowed to dry at room temperature under a fume hood, and further dried at 60°C for 30 min. These were placed immediately (one coupon per tube) in previously acetone-rinsed and dried glass test tubes. The test tubes were labelled and 5 ml of chemical test solution or distilled water used as control was added per tube. The tubes were incubated for 30 days. The experiments were done at two incubation temperatures; 20 and 60°C. At the end of incubation, coupons were removed from tubes with clean forceps, thoroughly rinsed with distilled water to remove any loosely hanging material, dried under a fume hood at room temperature, and at 60°C for 1 h. This accelerated corrosion assay was according to ASTM G31-72 (ASTM, 2004) and US Forestry Service test Method 5 (Anonymous, 2000)

with modifications. Tests were done in duplicates for each test solution/test surface pairing; two independent repeat experiments were conducted. Visual observation of changes in the material surfaces after the tests were made and coupon were graded on a 1 - 3 - 5 scale for compatibility of disinfectant with lining material by comparing with the control samples of the materials as follows: for aluminium 1 = no observable change in metal compared to the control sample, 3 = observed discolouration and minor smudges or etching along edges of metal, 5 = dissolution of the metal, uniform etching or presence of see-through holes; for stainless steel 1 = no observable change in metal compared to the control sample, 3 = minor discolouration, isolated pit at edge or minor etching along the edge, 5 = uniform corrosion observed as deep pits and etching of the entire surface. This was according to a modification of the grading system used by Green and Thickett (1995). The accelerated degradation test performed on the FRP coupons using the test solutions was according to ASTM S543-06 (ASTM, 2006) with some modifications. The assays were similar to those described above for aluminium and stainless steel. Tests were done in duplicates for each test solution/ test surface pairing; two independent repeat experiments were conducted. Compatibility grading for using the test solutions on the FRP were made according to a modification of the Green and Thickett (1995) system as above, with 1 = no observable change in surface compared to the control sample, 3 = isolated crack or minor smudges along edges, 5 = major deep cracks over the entire surface.

Practical usability index

A novel index for the practical usability of the test solutions for use in equipment containing the three test surfaces was created. First, variable *D*, the composite bactericidal efficacy, was calculated by assigning a number from 0 - 7 to each test solution for the number of the tested strains for which it produced the required > 4 log CFU reduction on all three test surfaces (based on Table 2), hence for example peracetic acid (PAA) had *D* = 7.00 while sodium hypochlorite (NaOCl) had *D* = 5.67. Secondly, a variable *C*, the average composite corrosiveness compatibility, was calculated assigning a number from 1 - 5 to each of the test solution by averaging the ratings at the two accelerated corrosion test temperatures (Table 3), hence for example PAA had *C* = 1.33; while NaOCl had *C* = 4.67. The practical usability index *M*, was calculated as $M = D/C$.

Table 2. Effect of disinfectants against seven bacteria strains (\log_{10} reduction) surface dried on three surfaces, tested according to the European standard DS/EN 13697 test¹.

| Bacteria | Surface | Log ₁₀ reduction for various test solutions | | | | | | | | |
|---------------------------------|-----------------|--|------|-------|------------------|------|-----|------|------|------|
| | | ² QAS | DSM | NaOCl | HNO ₃ | NaOH | PAA | LEV | ATS | SDS |
| <i>E. coli</i> K12 | AL ³ | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.5 | 4.0 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.7 | 4.0 |
| | FRP | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.7 | 4.0 |
| <i>E. coli</i> DSM 682 | AL | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 3.4 | 1.24 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.8 | 4.0 |
| | FRP | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.1 | 3.2 | 3.2 |
| <i>S. Typhimurium</i> P6 | AL | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.1 | 0.88 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 0.81 | 0.46 |
| | FRP | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 1.3 | 0.32 |
| <i>S. Senftenberg</i> DSM 10062 | AL | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.3 | 2.5 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 1.6 | 2.0 |
| | FRP | 4.0 | 4.0 | 4.0 | 3.9 | 4.0 | 4.0 | 4.0 | 1.7 | 2.8 |
| <i>L. innocua</i> P577 | AL | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.8 | 2.7 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.9 | 2.6 |
| | FRP | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 2.8 | 2.6 |
| <i>L. monocytogenes</i> Scott A | AL | 0.15 | 0.33 | 0.83 | 0.36 | 0.20 | 4.0 | 0.50 | 0.55 | 0.02 |
| | SS | 0.12 | 0.22 | 0.74 | 0.36 | 0.10 | 4.0 | 0.61 | 0.40 | 0.02 |
| | FRP | 0.08 | 0.59 | 0.78 | 0.14 | 0.17 | 4.0 | 0.63 | 0.66 | 0.14 |
| <i>P. aeruginosa</i> DSM 939 | AL | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 1.7 |
| | SS | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 1.8 |
| | FRP | 4.0 | 3.6 | 3.4 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 1.3 |

¹Efficacy of test solutions on the test bacteria attached to the surfaces in this test requires a > 4 log CFU reduction. Log reductions > 4.0 are shown as 4.0. Calculated standard deviations have not been shown.

²Test solutions were: QAS - benzalkonium chloride, DSM - Disinfect Maxi, NaOCl - sodium hypochlorite, HNO₃ - nitric acid, NaOH - sodium hydroxide, PAA - peracetic acid, LEV - levulinic acid, ATS - AT Special, SDS - sodium dodecyl sulphate.

³Surfaces used were: AL - aluminium (alloy 5754); SS - stainless steel (alloy 304); FRP - fibre re-enforced plastic [Q-Liner[®]; Maersk Container Industry (MCI), Tinglev, Denmark]

Statistical analysis

Statistical significance of differences in the various efficacy assays were tested using one-way analysis of variance (ANOVA) test and means were separated by Tukey's error rate (MINITAB v15 Minitab Ltd., Coventry, UK). Probabilities less than 0.05 ($p < 0.05$) were considered significant. Assays were performed in two independent repeat experiments. For each repeat, assays were done in duplicates.

RESULTS

Effect of test solutions on bacteria in suspension

Results from the suspension tests are shown in Figure 1A to G. Sodium dodecyl sulphate (SDS) could not produce the > 5 \log_{10} CFU reduction required to show bactericidal or disinfection efficacy according to the DS/EN 1267 standard on all bacteria strains tested, while AT special (ATS) only had efficacy for *P. aeruginosa* in

the suspension test. Only peracetic acid (PAA) showed disinfection efficacy on all test bacteria in suspension (Figure 1). The other test solutions such as benzalkonium chloride (QAS), Disinfect Maxi (DSM), sodium hypochlorite (NaOCl) and sodium hydroxide (NaOH) showed disinfection efficacy for six of the test bacteria strains in suspension, but did not cause the required reduction in the log CFU count of *L. monocytogenes* Scott A according to the DS/EN 1267 standard (Figure 1).

Effect of test solutions on bacteria dried on surfaces

Results from the surface tests are shown in Table 2, with standard deviations omitted for clarity of table presentation. The bactericidal efficacy of the chemical test solutions varied considerably in the DS/EN 13697 assay where efficacy was adjudged by a > 4 log reduction on initial bacteria population on the test surface. Reductions more than 4 \log_{10} CFU are shown with a value of 4.

Table 3. Accelerated corrosion test results for lining surface materials and general usefulness of disinfectant for surfaces.

| Agent | Surface ¹ | Corrosion test observation and compatibility rating ² | | | | Practical Usability ³ (Disinfection efficacy/ Corrosiveness) |
|------------------|----------------------|--|---------------------------|--|---------------------------|---|
| | | Appearance after exposure 20°C | Compatibility rating 20°C | Appearance after exposure 60°C | Compatibility rating 60°C | |
| QAS | AL | No change | 1 | Little darkening | 3 | 3.60 |
| | SS | No change | 1 | No change | 1 | |
| | FRP | No change | 1 | Few cracks | 3 | |
| DSM | AL | Uniform corrosion; pitting at edge | 5 | Uniform corrosion; salt deposition | 5 | 1.89 |
| | SS | No change | 1 | No change | 1 | |
| | FRP | Clear deep cracks | 5 | No change | 1 | |
| NaOCl | AL | Uniform corrosion; salt deposition | 5 | Uniform corrosion; salt deposition | 5 | 1.22 |
| | SS | A little smudge | 3 | Clear deep pitting | 5 | |
| | FRP | Major deep cracks with salt deposition | 5 | Major deep cracks with salt deposition | 5 | |
| HNO ₃ | AL | Uniform corrosion; pitting at corner | 5 | Uniform corrosion; scattered pits | 5 | 1.42 |
| | SS | No change | 1 | Tiny smudge | 3 | |
| | FRP | Clear pits | 5 | Clear deep pits | 5 | |
| NaOH | AL | Metal mostly dissolved; see-through holes | 5 | Metal mostly dissolved; almost non recovered | 5 | 1.38 |
| | SS | Little scraping | 3 | Spot corrosion | 5 | |
| | FRP | Tiny crack | 3 | Major crack; salt deposition | 5 | |
| PAA | AL | No change | 1 | Slight colouring | 3 | 5.25 |
| | SS | No change | 1 | No change | 1 | |
| | FRP | No change | 1 | No change | 1 | |
| LEV | AL | Few pits at edges | 5 | Uniform corrosion; salt deposition | 5 | 1.55 |
| | SS | No change | 1 | Pitting | 5 | |
| | FRP | No change | 1 | Cracks; discoloration | 5 | |
| ATS | AL | Little etching | 3 | Uniform corrosion; salt deposition | 5 | 0.43 |
| | SS | No change | 1 | Thin salt deposition/ corrosion at edge | 3 | |
| | FRP | No change | 1 | No change | 1 | |
| SDS | AL | Slight corrosion at edge | 5 | Little smudge | 3 | 0.67 |
| | SS | No change | 1 | No change | 1 | |
| | FRP | No change | 1 | No change | 1 | |

¹Surfaces used were: AL, aluminium (alloy 5754); SS, stainless steel (alloy 304); FRP, fibre re-enforced plastic [Q-Liner[®], Maersk Container Industry (MCI), Tinglev, Denmark]; full names of disinfecting agents are in Table 1.

²Compatibility classifications were according to modified form of that used by Green and Thickett (1995). Individual descriptions of the observed basis for the grading are shown (see Figure 2).

³Practical usability matrix was calculated by: (a) assigning a number (0 - 7) to each test solution for number of the tested strains for which it produced the required > 4 log CFU reduction on all three test surfaces (based on Table 2) (*D*), and (b) assigning a number from (1 - 5) for average composite corrosion rating (*C*). Usability matrix (*M*) was calculated as $M = D/C$ as shown. The higher the value of *M*, the better it is for use on equipment with composite materials.

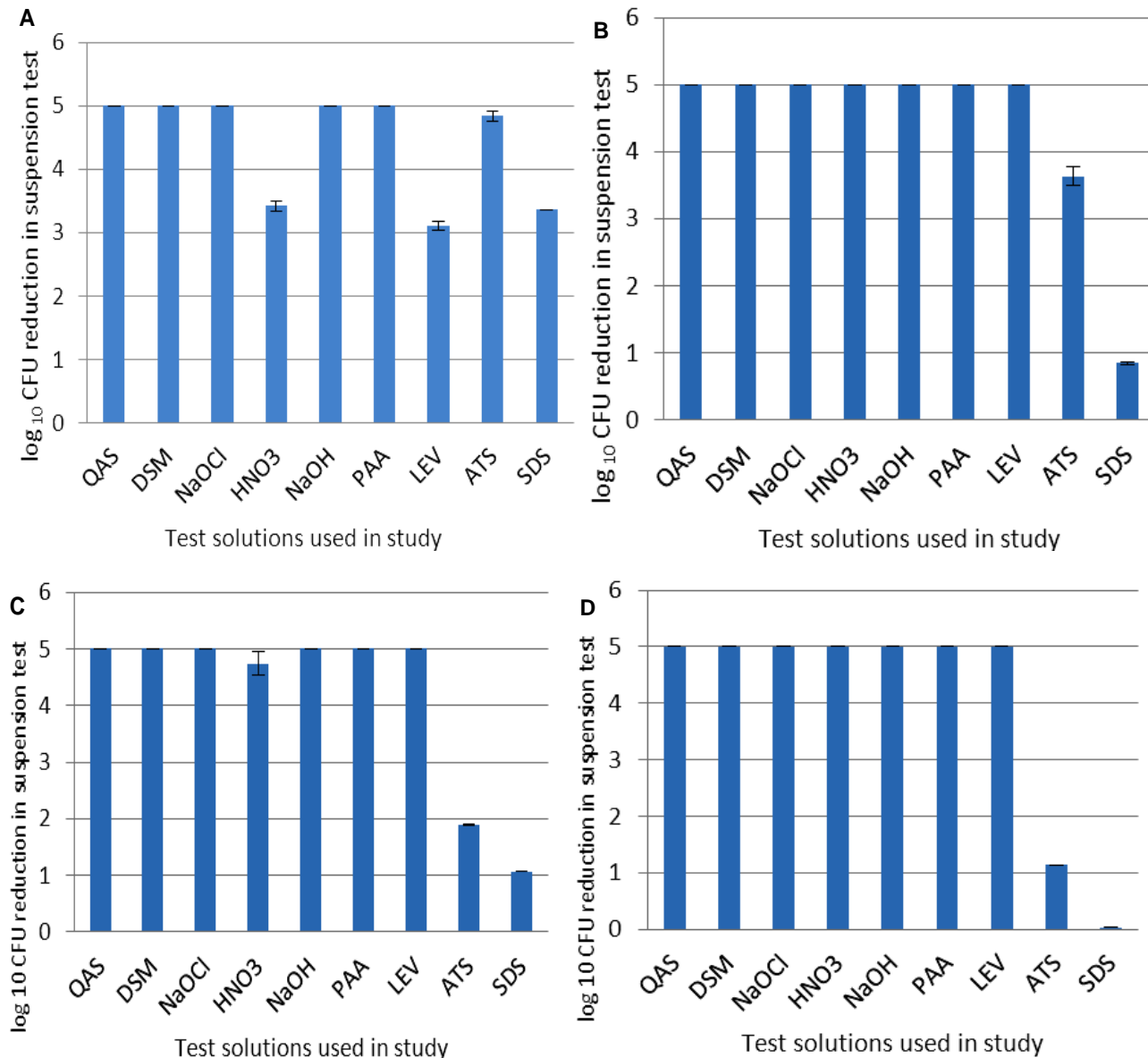


Figure 1. Effect of disinfectant test solutions against seven test bacteria in suspension, tested according to the European standard DS/EN 1276 test. Efficacy of test solution on the test bacteria requires a $> 5 \log_{10}$ CFU reduction in the test. Reductions above $5 \log_{10}$ are shown as 5. Error bars are standard deviations. Test solutions were: QAS - benzalkonium chloride, DSM - Disinfect Maxi, NaOCl - sodium hypochlorite, HNO₃ - nitric acid, NaOH - sodium hydroxide, PAA - peracetic acid, LEV - levulinic acid, ATS - AT Special, SDS - sodium dodecyl sulphate. A - *E. coli* K12, B - *E. coli* DSM 682, C - *S. Typhimurium* P6, D - *S. Senftenberg* DSM 10062, E - *L. monocytogenes* Scott A, F - *L. innocua* P577, G - *P. aeruginosa* DSM 939.

SDS only had efficacy for *E. coli* K12 for all three test surfaces, while ATS only had efficacy for *P. aeruginosa* for all three surfaces. Other test solutions such as QAS, DSM, NaOCl, HNO₃ and NaOH showed disinfection efficacy for 6 of the test bacteria strains on all three surfaces, but did not cause the required reduction in the log CFU count of *L. monocytogenes* Scott A on any of the three test surfaces according to the DS/EN 13697 standard (Table 2). As with the suspension tests, only peracetic acid (PAA) showed disinfection efficacy on all

test bacteria adhered to all three test surfaces (Table 2). There was no general trend to suggest an effect of the surface type on the efficacy of any of the test solutions ($p < 0.05$). However, there was significantly higher ($p > 0.05$) bactericidal reduction for both *Salmonella* spp. serovars tested by ATS on aluminium compared to same test solution on stainless steel and FRP. Levulinic acid (LEV) also showed a significantly lower bactericidal efficacy against *E. coli* DSM 682 on FRP than for the same bacteria attached to aluminium and stainless steel

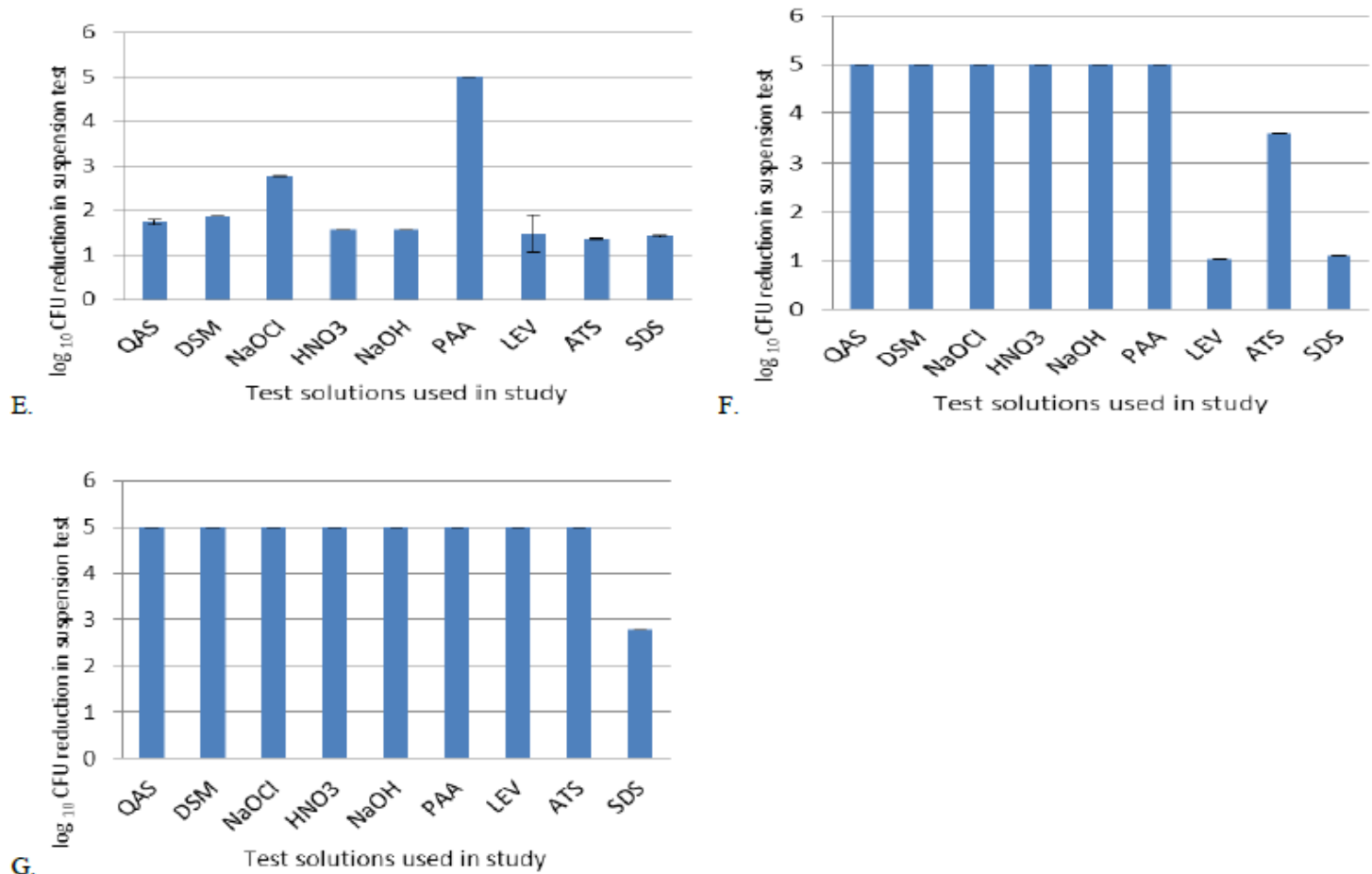


Figure 1. Continue.

($p > 0.05$). Both DSM and NaOCl could not attain the required > 4 log reduction of *P. aeruginosa* on FRP, though the required reduction was attained for the same organism on aluminium and stainless steel (Table 2).

Accelerated corrosion tests and usability matrix

Classification of compatibility between disinfectants and surface material was made using a modification of that by Green and Thickett (1995). Examples of the three surfaces after assay showing the best case or grade 1 and worst case or grade 5 in the classification scheme are shown in Figure 2. The original three point scale of Green and Thickett (1995) has also been used for simplicity, though the authors are aware of the expanded 5-point grade system from 0 to 4 used by Robinet and Thickett (2003). The modification in the classification took into account the possibility of test solutions producing no corrosiveness at either temperature on test surfaces, hence a scale starting from 1, to avoid the denominator in the calculation of M being zero.

The accelerated tests were performed using standard protocols with modifications for all three surface types.

There were generally differences in the effect of temperature on the outcome of the test, with more corrosive damage observed at 60°C than at 20°C (Table 3). Adverse corrosion on the surfaces included partial or complete dissolution of the aluminium metal coupons in NaOH and see-through holes in NaOCl; while in stainless steel deep pit uniformly distributed all over the coupon surface was observed with both NaOCl and NaOH. Adverse degradation of FRP was observed as deep cracks on the entire surface with both NaOCl and NaOH (Figure 2; Table 3). Generally PAA was the least corrosive across all three surfaces while NaOCl was the most corrosive based on the calculated values of C , the average composite corrosiveness compatibility (results not shown).

The results of the practical usability index calculations are shown in Table 3. The higher the value of M , the better it is for use on equipment with all three tested materials in the long term. The specific interpretation of the M -value for a test solution should be based on the parameters used (number of strains tested, number of surfaces tested). PAA had the highest M -value of 5.25 while ATS had the lowest value of 0.43.

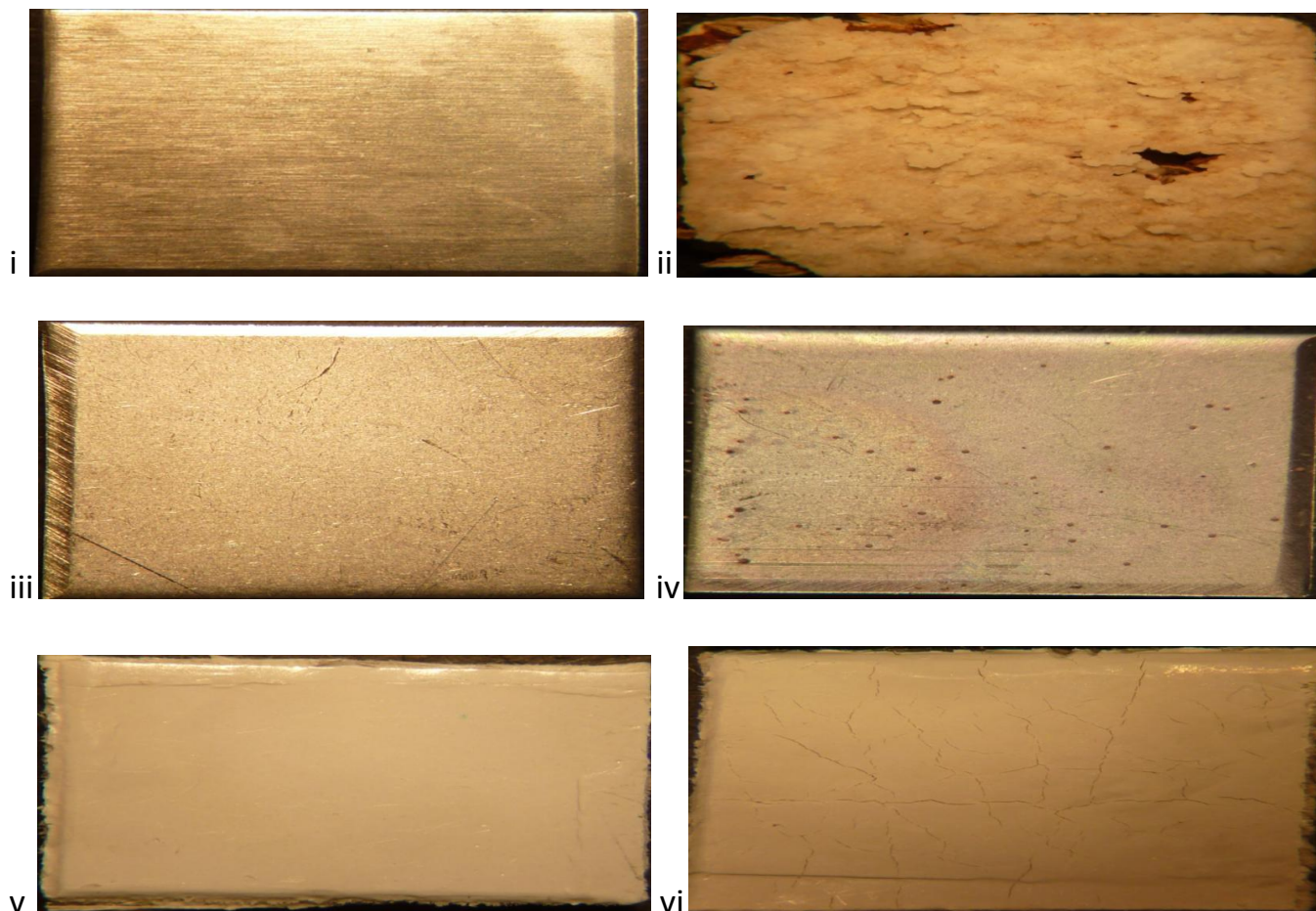


Figure 2. Examples of corrosion grading (compatibility for use, according to a modification of the grading system used by Green and Thickett (1995)) for the three studied materials after modified accelerated corrosion tests showing best case or grade 1 = no observable change in surface (all three) compared to control sample, and worst case or grade 5 = dissolution of the metal, uniform etching or presence of see-through holes (aluminium); uniform corrosion observed as deep pits and etching of the entire surface (stainless steel); or major deep cracks over the entire surface (FRP). i - aluminium, grade 1; ii - aluminium, grade 5; iii - stainless steel, grade 1; iv - stainless steel, grade 5; v - FRP, grade 1; vi - FRP, grade 5.

DISCUSSION

The trends in effectiveness of the disinfectants both on bacteria in suspension and dried on surfaces were similar in the way that test solutions were ineffective at reducing counts of *L. monocytogenes* Scott A, with the exception of PAA. The disinfectants used were those reported in the literature to have desirable qualities of effective bacteria reduction and or low corrosive potential on surfaces (Campdepadros et al., 2012; Deza et al., 2005; Dvorak, 2008; Espigares et al., 2003; Zhao et al., 2010). Though suspension test are common for evaluating the disinfecting efficacy of various chemicals, surface tests represent a more useful tool for assessing true usefulness of disinfectants in food environments as most bacteria targeted during disinfection are attached to a surface rather than in a suspension (Møretrø et al., 2007). A general effect of test surface type on the bactericidal efficacy of the test solutions was not

observed; however, there were few observations of efficacy on FRP being lower than on aluminium and stainless steel. Gelinas and Goulet (1983) observed that concentration of some disinfectants needed for disinfection of *Pseudomonas aeruginosa* on polypropylene was 10 times higher the concentration required on stainless steel, and explained this by the production of extracellular material produced by the *Pseudomonas* on the polypropylene. The authors however found similar concentration required on both polypropylene and aluminium (Gelinas and Goulet, 1983). Rogers et al. (1961) also reported that the concentrations of a phenolate disinfectant required for 99% reduction of two *Salmonella* spp. was over 10 times higher for plastic fortified rubber surfaces than for stainless steel and ceramic surfaces. These are consistent with our observations.

L. monocytogenes Scott A is a 4b serovar clinical isolate of the species which is widely distributed and used as a model organism in laboratory investigations to repre-

sent 4b serovars important as pathogens in the human food chain (Brier et al., 2011). *L. monocytogenes* serovars are known to persist and are difficult to remove from various food environments/ surfaces (Carpentier and Cerf, 2011). Various authors have found different disinfectant groups including some of those tested in this study (QAS, NaOCl, NaOH) either alone or in combination to be ineffective for disinfection of this organism, either in suspension or on surfaces (Aarnisalo et al., 2007; Best et al., 1990). This is especially so in the presence of residue, which was also true for this study as it was done under 'dirty' conditions with bovine serum albumin. The general observation in this study that *L. monocytogenes* Scott A was the most difficult organism to disinfect is thus in agreement with observation reported in the literature.

Accelerated corrosion tests are useful when performed under the right conditions and can yield beneficial data in selecting the most appropriate combination of surface material and chemical disinfection for various applications in the long term (Guthrie et al., 2002). Corrosion of the surface/contact materials can be expected over time as the materials are bound to react on some level with the food environment it is exposed to. The results of the corrosion tests were mostly expected given the chemical reactivity of the active components of the disinfectants. Per the results from the chosen test parameters, only PAA will be suitable for all three surfaces in containers containing the materials. The visual observation grading was used in place of the calculated weight change method described in the standard (Anonymous, 2000) as it was observed in earlier trials that there were instances of weight gain due to salt deposition from test solution dehydration, even in situations where clear corrosion was visible (unpublished information).

The calculated practical usability index created in this work is a novel way of taking into accounts the two important parameters of bactericidal efficacy and material corrosion for permanent or temporary applications especially regarding value-for-money choices for given equipment and environments. The index is useful especially for environments where different materials are present as food contact surfaces, as can be found in cargo containers for food transportation (Abban et al., 2013).

Peracetic acid (dissolved in acetic acid) was found to be the most cost-effective disinfectant both in suspension and on surfaces. Peracetic acid has been registered since 1985 for use as broad spectrum indoors disinfectant, sanitizer and sterilant in the US (Anonymous, 2007) and is an ideal antimicrobial due to its high oxidizing potential. It also breaks down in food to safe, environmentally friendly products (acetic acid and hydrogen peroxide) and can thus be used in minimal rinse and non-rinse applications (Anonymous, 2007). Though it is known to be corrosive to some metals (Dvorak, 2008), it had negligible estimated long term effect on the three studied surfaces. However, its long term use should be

decided alongside the effects on exposure for the handlers/users, since it causes irritation to the skin, eyes and respiratory system (Anonymous, 2010). General acute exposure for up to 8 h has been set at 0.52 mg/m³ (Anonymous, 2010).

Conclusion

In the present study, we have shown that though several disinfectant types may be useful both in suspension and surface disinfection for several bacteria of pathogenic significance, peracetic acid is the most effective of those tested, especially against *L. monocytogenes*. The effect of the three surfaces aluminium, stainless steel and FRP on the effectiveness of disinfection was negligible. However, accelerated corrosion tests have indicated differences in corrosiveness of the evaluated chemicals on the three test surfaces. A usability index was created to take into account disinfection effectiveness and low corrosiveness; its use can be very informative in choosing appropriate disinfectant for composite environments with several contact surface materials. Peracetic acid was the most appropriate disinfectant both for low corrosion effect on test surfaces and high levels of disinfection on all tested strains in this study.

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REFERENCES

- Aarnisalo K, Lunden J, Korkeala H, Wirtanen G (2007). Susceptibility of *Listeria monocytogenes* strains to disinfectants and chlorinated alkaline cleaners at cold temperatures. *LWT- Food Sci. Technol.* 40:1041-1048.
- Abban S, Jakobsen M, Jespersen L (2012). Attachment behaviour of *Escherichia coli* K12 and *Salmonella* Typhimurium P6 on food contact surfaces for food transportation. *Food Microbiol.* 31:139-147.
- Abban S, Jakobsen M, Jespersen L (2013). Assessment of interplay between UV wavelengths, material surfaces and food residues in open surface hygiene validation. *J. Food Sci. Technol.* DOI: 10.1007/s13197-013-0927-9.
- Abban S, Tano-Debrah K (2011). Automatic teller machines (ATMs) as potential sources of food-borne pathogens - a case from Ghana. *Nat. Sci.* 9(9):63-67.
- Anonymous (2000). Corrosion tests (Uniform Corrosion) - Test method 5. USDA Forest Service, Washington DC, USA.
- Anonymous (2001). Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas — test method and requirements (phase 2, step 1), DS/EN-1276. The Danish Standards Association DS, Copenhagen, Denmark.

- Anonymous (2007). Anthrax spore decontamination using hydrogen peroxide and peroxyacetic acid. Pesticides: Topical & Chemical Fact Sheets. U.S. Environmental Protection Agency, Washington, D.C. Available online (06.01.2012) at: http://www.epa.gov/pesticides/factsheets/chemicals/hydrogenperoxide_peroxyaceticacid_factsheet.htm.
- Anonymous (2009). Chemical disinfectants and antiseptics—Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas—test method and requirements without mechanical action (phase 2, step 2), DS/EN-13697. The Danish Standards Association DS, Copenhagen, Denmark.
- Anonymous (2010). Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 8, Chapter 7 - peracetic acid. Committee on Acute Exposure Guideline Levels/ Committee on Toxicology, National Research Council. National Academies Press, Washington, D.C. pp 327-366.
- Anonymous (2011). The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2009. EFSA J. 9(3):2090.
- ASTM (2004). Standard Practice for Laboratory Immersion Corrosion Testing of Metals, ASTM G31 - 72. ASTM International, West Conshohocken, PA, USA.
- ASTM (2006). Standard Practices for Evaluating the Resistance of Plastics to Chemical Reagents, ASTM S543 - 06. ASTM International, West Conshohocken, PA, USA.
- Best M, Kennedy ME, Coates F (1990). Efficacy of a variety of disinfectants against *Listeria* spp. Appl. Environ. Microbiol. 56(2):377-380.
- Brier Y, Klumpp J, Schuppler M, Loessner MJ (2011). Genome sequence of *Listeria monocytogenes* Scott A, a clinical isolate from a food-borne listeriosis outbreak. J. Bacteriol. 193(16):4284-4285.
- Campdepadros M, Stchigel AM, Romeu M, Quilez J, Sola, Rosa S (2012). Effectiveness of two sanitation procedures for decreasing the microbial contamination levels (including *Listeria monocytogenes*) on food contact and non-food contact surfaces in dessert-processing factory. Food Cont. 23:26-31.
- Carpentier B, Cerf O (2011). Review — Persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145:1-8.
- Deza MA, Araujo M, Garrido MJ (2005). Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* on stainless steel and glass surfaces by neutral electrolysed water. Lett. Appl. Microbiol. 40:341-346.
- Dvorak G (2008). Disinfection 101. Centre for Food Security and Public Health, Iowa State University, IA, USA. pp.1-22.
- Espigares E, Bueno A, Fernandez-Crehuet M, Espigares M (2003). Efficacy of some neutralizers in suspension tests determining the activity of disinfectants. J. Hosp. Infect. 55:137-140.
- Gelinas P, Goulet J (1983). Efficacy of 8 disinfectants on 3 types of surfaces contaminated by *Pseudomonas aeruginosa*. Can. J. Microbiol. 29(12):1715-1730. (article in french)
- Green LR, Thickett D (1995). Testing materials for use in the storage and display of antiquities: a revised methodology. Stud. Conserv. 40(3):145-152.
- Guthrie J, Battat B, Grethlein C (2002). Accelerated corrosion testing. AMPTIAC Quart. 6(3):11-15.
- Joseph B, Otta SK, Karunasagar I, Karunasagar I (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Int. J. Microbiol. 64:367-372.
- Jullien C, Benezech T, Carpentier B, Lebret V, Faille C (2003). Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry. J. Food Eng. 56:77-87.
- Klingberg TD, Axelsson L, Naterstad Elsser D, Budde BB (2005). Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages. Int. J. Food Microbiol. 105:419-431.
- Kusumaningrum HD, Riboldi G, Hazeleger WC, Beumer RR (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int. J. Microbiol. 85:227-236.
- Montville R, Schaffner DW (2003). Inoculum size influences bacterial cross contamination between surfaces. Appl. Environ. Microbiol. 69:7188-7193.
- Møretør T, Vestby LK, Nesse LL, Storheim SE, Kotlarz K, Langsrud S (2009). Evaluation of efficacy of disinfectants against *Salmonella* from the feed industry. J. Appl. Microbiol. 106:1005-1012.
- Nyachuba DG (2010). Foodborne illness: is it on the rise? Nutr. Rev. 68(5):257-269.
- Rogers MR, Maher JT, Kaplan AM (1961). A practical approach to evaluation of the germicidal efficiency of a general purpose military disinfectant. Appl. Microbiol. 9(6):497-501.
- Robinet L, Thickett D (2003). A new methodology for accelerated corrosion testing. Stud. Conserv. 48(4):263-268.
- Rusin P, Maxwell S, Gerba C (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram positive bacteria, gram negative bacteria and phage. J. Appl. Microbiol. 3:585- 592.
- Sharma M, Anand SK (2002). Biofilms evaluation as an essential component of HACCP for food/dairy processing industry - a case. Food Cont. 13:469-477.
- Verran J (2002). Biofouling in food processing - biofilm or biotransfer potential? Food Bioproducts Process. 80:292-298.
- Whitehead KA, Verran J (2006). The effect of surface topography on the retention of microorganisms. Food Bioprod. Proc. 84(C4):253-259.
- Zhao T, Zhao P, Doyle MP (2010). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on alfalfa seeds by levulinic acid and sodium dodecyl sulfate. J. Food Protect. 73(11):2010-2017.