The Evaluation of Commercially Available Disinfectant Combinations on Biofilms for Use in Slaughter Houses

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Background

Microbial Biofilm is a cohesive matrix of microorganisms, mucopolysaccharides (slime), and extracellular constituents that exist in virtually every natural environment. Biofilms form in an environment in response to the presence of a solid surface as well as other factors, such as shear force (flow), as a mechanism to avert being removed from that environment (Costerton *et al*., 1995). Microbial biofilms demonstrate recalcitrance towards a wide range of antimicrobial treatments and have been reported to be 100-1000 less susceptible than their planktonic counterparts (McBain and Gilbert, 2001). This resistance is due to the presence of extracellular polysaccharide matrix, the physio-chemical heterogeneity developed within such consortia, acquiring of multi-antimicrobial resistance genes and the presence of cells of highly recalcitrant physiology (persisters) (Gilbert *et al*., 2002). Disinfectants and protocols for their use have been developed and deployed on the basis of eradicating planktonic forms of bacteria, and not their biofilm counterparts. This may explain common failures of disinfectant products in various agri-food industries, which has caused disease transmission and seriously affected the agriculture markets (Sharma, M. and Anand, S. K., 2002). For this reason, biofilms have been identified as a major issue in Hazard Analysis and Critical Control Point (HACCP) programs (Sharma, A. and Anand, S. K., 2002). USDA Economic Research Service statistics showed that Food borne illness and food spoilage associated with bacterial infections have an annual cost of $ 600 million up to $ 6 billion. Our data suggest that current disinfectant products available to the beef, dairy, hog and poultry industries are not fully effective against biofilms. Moreover, we have identified several disinfectants and decontamination protocols that are safe and effective against biofilms.

Objective

To evaluate commercially available food and feed area disinfectant formulations based on MBC (minimum bactericidal concentration) and MBEC (minimum biofilm eradication concentration) values using MBEC assay ™ (Innovotech Inc., Edmonton, Canada) at three different time exposure; 10 minutes, 30 minutes and 16 hours.

Bacterial Strains

Table 1. list of the tested bacterial strains, the growth media, the growth conditions and the average biofilm biomass grown on the growth control pegs.

Bacterial Strain	Code	Growth Media	Growth Condition	Storage conditions	Average Biofilm Growth control
Pseudomonas aeruginosa ATCC 27853	PA	Trypticase Soya broth TSA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	2.95E+07
Escherichia coli ATCC 25922	EC	Trypticase Soya broth TSA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	9.75E+06
Staphylococcus aureus ATCC 29213	SA	Trypticase Soya broth TSA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	2.26E+06
Campylobacter jejuni (clinical isolate)	CJ	Blood-Free Selective Agar Base CBFSA (Oxoid, UK)	microaerophilic 37°C (90% N_2, 5% O_2 and 5% CO_2)	Microbank™ cryovials at – 70°C	6.86E+05
Escherichia coli 0157:H7(clinical isolate)	EC H7	Trypticase Soya broth TSA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	6.25E+04
Salmonella choleraesuis ATCC 10708	SC	Trypticase Soya broth TSA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	1.56E+05
Listeria monocytogenes ATCC 19114	LM	Brain Heart Infusion Agar BHIA (Becton Dickinson, USA)	37°C incubator for 24 hours	Microbank™ cryovials at – 70°C	1.60E+07

Tested Biocides

Among the wide range of commercially available biocides, Virkon®, Environ LpH®, 1-Stroke®, Oxonia Active®, SterBac KQ-12®, 400 Sanitizer®, XY-12® and BevroKlene®, Vortexx® and Vantocil® were the biocide products that were approved by the Canadian Food Inspection Agency CFIA and used for sanitizing purposes in the food and feed processing areas.

Table 2. list of the tested biocides, the Brand name, the active ingredients, the biocide classifications.

Company name	Product name	Active ingredient (s)	Biocide family
ECOLAB	SterBac KQ-12	Quaternary ammonium compounds, benzyl-c12-c16-alkyldimethyl, chlorides 7 – 13 % Ethanol 1 – 5 %	QUAT
Steris	1-Stroke	ortho-phenylphenol 10%-ortho bezyl-para-cholorophenol 8.5%	Phenols
Ecolab	XY-12	Sodium hypoclorite 8.4%	Chlorine oxidizer
Ecolab	400 Sanitizer	Phosphoric acid 30 – 60 % propylene glycol 7 – 13 % dodecylbenzenesulfonic acid 7 – 13 %	Acid sanitizer
Vétoquinol NA Inc.	Virkon	Potassium Peroxymonosulfate 21.4%	Oxidizing agents
Ecolab	Oxonia Active	Hydrogen peroxide sol. 15-40% Acetic acid 7 – 13 % Peracetic acid 5 - 10%	Acetic/Peroxide
Ecolab	BevroKlene	citric acid 3 – 7% aluminum sulfate 1 – 5% poly(oxy-1,2-ethanediyl), .alpha.-(nonylphenyl)-.omega.-hydroxy-, compd with iodine 1 – 5%	Iodophore
Brenntag International	Vantocil IB	PHMB (Polyhexamethylene biguanide)	Biguanides
Steris Corporation	Environ LpH	*o*-Benzyl-*p*-chlorophenol 6.4% *p*-Tertiary-amylphenol 3.0% *o*-Phenyl phenol 0.5% Hexylene glycol 4.0% Glycolic acid (hydroxyacetic) 12.6% Isopropanol 8.0%	phenols
Ecolab	Vortexx	Caprylic acid 3% Hydrogen peroxide 6.9% Peroxyacetic acid 4.4%	Peroxide

Table 3. Concentrations range of each tested biocide that were used in the challenge plates against the tested bacterial strains.

Product name	Concentrations range (diluting the biocide product in D.D. water)						
	1	2[α]	3		9	10	11
SterBac KQ-12®	0.4%	**0.2%**	0.1%		0.00157%	0.000785%	0.0004%
1-Stroke®	0.8%	**0.4%**	0.2%		0.00313%	0.00157%	0.000785%
XY-12®	0.6%	**0.3%**	0.15%		0.0023%	0.0012%	0.00059%
400 Sanitizer®	0.3%	**0.15%**	0.075%		0.0012%	0.00059%	0.0003%
Virkon® β	3%	**1.5%**	0.75%		0.012%	0.0059%	0.003%
Oxonia Active®	0.6%	**0.3%**	0.15%		0.0023%	0.0012%	0.00059%
BevroKlene®	0.3%	**0.15%**	0.075%		0.0012%	0.00059%	0.0003%
Vantocil IB®	0.6%	**0.3%**	0.15%		0.0023%	0.0012%	0.00059%
Environ LpH®	1.56%	**0.78%**	0.39%		0.00625%	0.00313%	0.00157%
Vortexx®	0.5%	**0.25%**	0.125%		0.002%	0.001%	0.0005%

α: Manufacturer Recommended concentrations. β: Virkon Concentrations are in w/v.

Neutralizing agents

This universal neutralizer recipe (Innovotech Inc., Edmonton AB) consists of 1.0 g L-Histidine (Sigma, USA), 1.0 g L-Cysteine (Sigma, USA), 2.0 g Reduced glutathione (Sigma, USA) in 20 ml double distilled water. This solution was sterilized through filtration through 0.22 µm diameter pore size filter (Corning Inc., Germany). This solution was stored at -20°C. The surfactant supplemented growth medium recipe contains 1 litre of cation adjusted Muller Hinton Broth (Becton Dickinson, USA), supplemented with 20.0 g per litre of saponin (Sigma, USA) and 10.0 g per litre of Tween-80 (Sigma, USA). This solution was adjusted with diluted sodium hydroxide to the correct pH (7.0 ± 0.2 at 20° C). 500 µL of the universal neutralizer was added to each 20 ml of the surfactant supplemented growth medium used for recovery plates.

Methodology

Figure 1 shows the detailed procedure for MBEC assay, this includes growing the bacterial biofilms, the antimicrobial challenge, and the recovery. The *Campylobacter jejuni* biofilm were incubated for 48 hours in the anaerobic incubator. Other strains were incubated for 24 hours for biofilm growth.

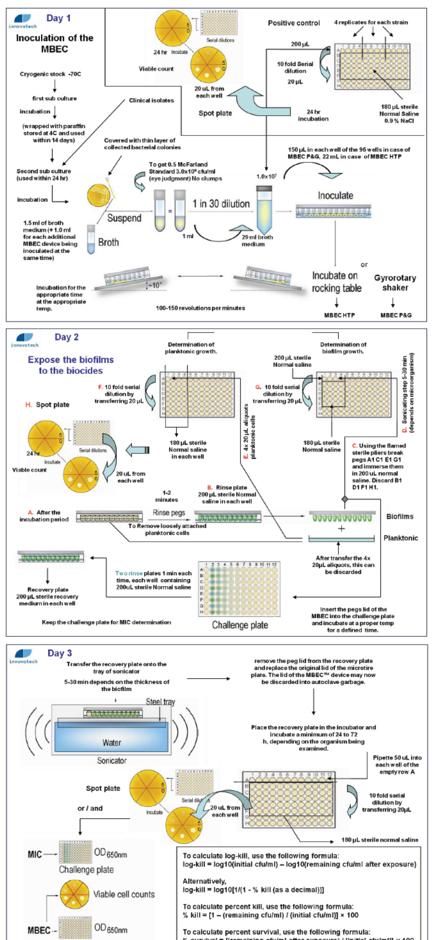

Figure 1. MBEC P&G Assay procedure.

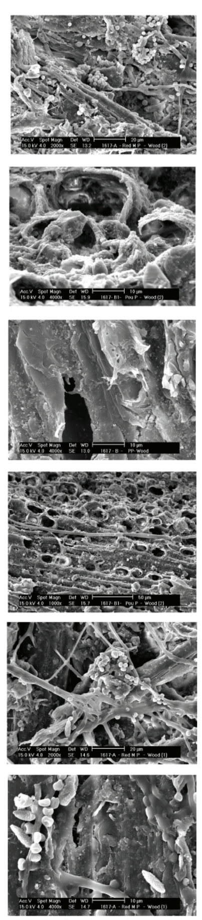

Figure 2. Scanning Electron Microscope images for bacterial biofilms growing on wood surfaces isolated from slaughter houses.

Results

Bacterial strains were exposed to the different tested biocides listed in Table 2 at different concentrations (Table 3) in triplicate, at three different time exposures (10 minutes, 30 minutes and 16 hours). Sterility check and biofilm positive control were performed for each strain (Table 1). All MBC or MBEC percentage value >200% the manufacturer recommended concentration were represented in the graphs as 400%. This study target was to identify the biocide which kills the bacterial biofilm at concentrations only close or lower than the concentration recommended by the Manufacturer.

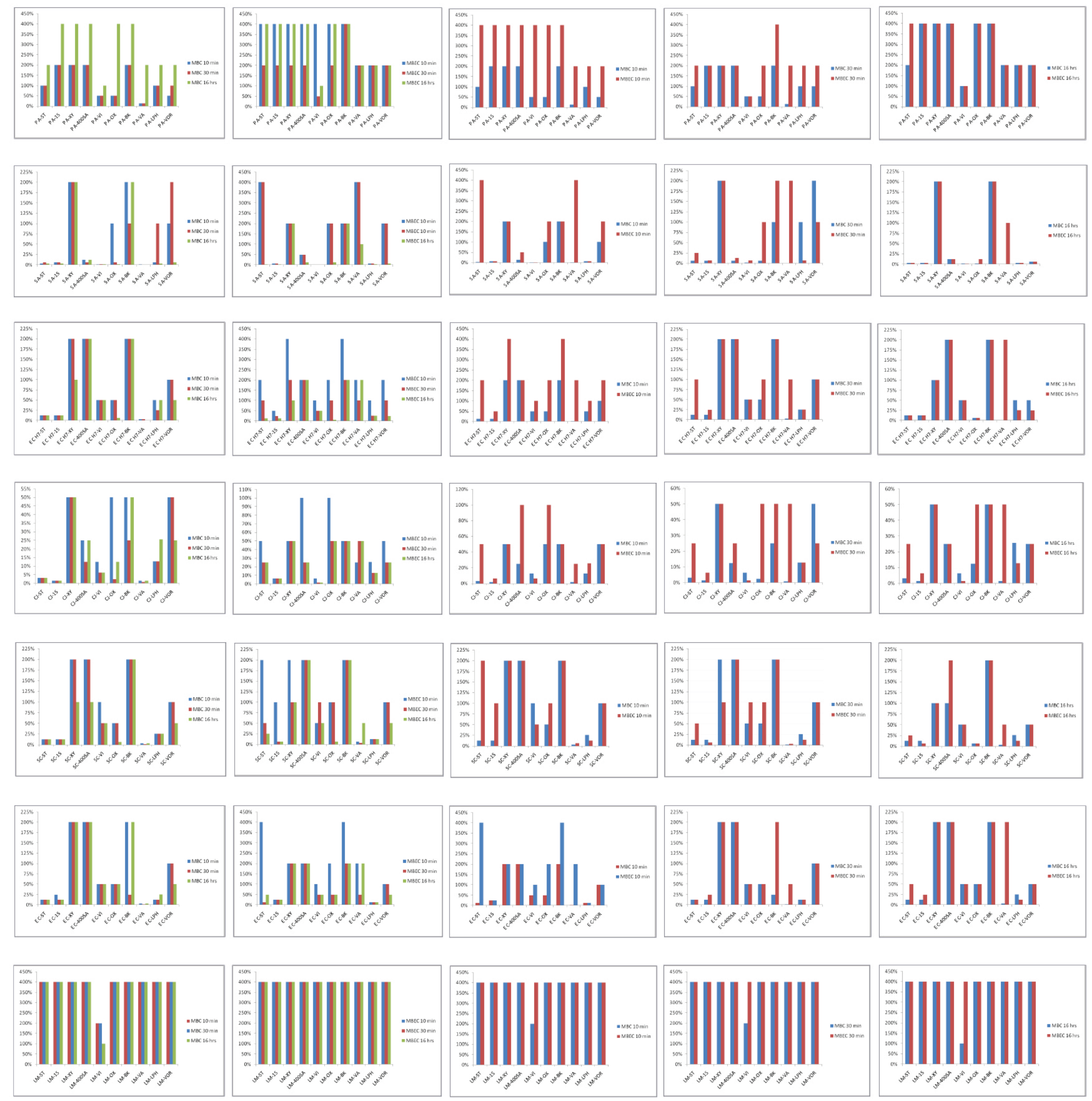

Figure 3. These graphs show the MBEC and MBC values for the tested biocides (Table 2) against the tested strains (Table 1) at three different time exposure; 10 minutes, 30 minutes and 16 hours. VI; Virkon®, LPH; Environ LpH®, 1S; 1-Stroke®, OX; Oxonia Active®, ST; SterBac KQ-12®, 400SA; 400 Sanitizer®, XY; XY-12® and BK; BevroKlene®, VOR; Vortexx® and VA; Vantocil®

Discussion and Conclusions

- Though most of the tested biocides were able to kill the planktonic cells at even lower levels than the concentrations recommended by the manufacturer, only Virkon® was able to kill the biofilm cells at 50-100% of the manufacturer recommended concentration. BevroKlene®, 400 Sanitizer® and XY-12® were only able to kill *E. coli* ATCC 25922 and *S. choleraesuis* , *S. aureus*, *E. coli* 0157:H7, *Listeria monocytogenes* ATCC 19114 and *P. aeruginosa* biofilm cells at concentrations not less than twice the manufacturer recommended concentrations.
- The clinical isolate of *E. coli* 0157:H7 was found to be more resistant than *E. coli* ATCC 25922 and most of the other tested strains; being exposed to disinfectant and antibiotics might be the reason behind the resilience of this clinical strain.
- 10 minutes exposure time was not efficient for biofilm eradication. Instead, 30 minutes was found to be the optimum exposure time for all tested biocides.
- The tested biocide concentrations to kill the bacterial biofilms were 2-8 times higher than the ones killed their planktonic counterparts. 400 Sanitizer®, XY-12® and BevroKlene® (Acid sanitizers, chlorine oxidizer and Iodophores) were the weakest biocides among the tested list, whereas Virkon® is the strongest biocide followed by Environ LpH®, 1-Stroke®, Oxonia Active®, SterBac KQ-12®, Vortexx® and Vantocil® (oxidising agents, QUATs and phenols).

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