THE EFFICACY OF ANOLYTE AS AN ENVIRONMENTALLY FRIENDLY DISINFECTANT ON *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* CONTAMINATED COTTON, POLYESTER/COTTON AND POLYESTER

Jana F Vermaas*, Celia J Hugo, Hester JH Steyn & Robert Schall

OPSOMMING

Tekstielstowwe word gewoonlik gesteriliseer deur dit te behandel met chemikalië soos natriumhipochloried en met temperature so hoog soos kookpunt. Dit lei tot twee potensiële probleme naamlik: (1) dit het 'n negatiewe effek op die sterkte eienskappe van die tekstielstof; en (2) die skadelike chemikalië word in die omgewing vrygestel.

Elektro-chemies geaktiveerde water word geproduseer deur 'n anode-katode sisteem. Water en verdunde NaCl is die enigste rou produkte. Die Anolyte bestaan in 'n meta-stabiele toestand en bevat vrye radikale asook 'n verskeidenheid molekules, en het 'n hoë oksidasie-reduksie potensiaal. Die Anolyte keer egter terug na 'n stabiele toestand na 48 uur en word weer onaktief, en is dus nie 'n bedreiging vir die omgewing wanneer dit vrygestel word na gebruik nie. Anolyte word daarom as omgewingsvriendelik geag.

Katoen, poliëster en poliëster/katoen monsters is afsonderlik besmet met *E.coli* en *S. aureus*, en daarna behandel volgens die AATCC 61-2009 toetsmetode. Wasmiddels wat ondersoek is, sluit in gefiltreerde water, fosfaat bevattende verwysings detergent B, natrium hipochloriet en Anolyte. Temperature was behou by 24 °C, 30 °C of 60 °C. Verdunnings was voorberei en oppervlak geplaat op nutriënt agar en geïnkubeer teen 28°C en 37°C (afsonderlik) vir *E. coli* en *S. aureus.* Bakteriese tellings is gerapporteer as hoeveelheid bakterieë of kolonie vormende eenhede (cfu) per ml.

Anolyte was die suksesvolste behandeling, aangesien daar geen oorlewing was na behandeling, ongeag die was temperatuur. Daarom kan Anolyte as 'n omgewingsvriendelike alternatief gebied word tot die huidige steriliseermiddels.

— ***Dr JF Vermaas****
Department of Consumer Science, University of the Free State
Tel: +27 (51) 401 9750
e-mail: neljf@ufs.ac.za
*Corresponding author

— ***Prof CJ Hugo***
Department of Microbiology, Biochemistry and Food Biotegnology, University of the Free State
Tel: +27 (51) 401 2692
e-mail: hugocj@ufs.ac.za

— ***Prof HJH Steyn***
Department of Consumer Science, University of the Free State
Tel: +27 (51) 401 2304
e-mail: steynhj@ufs.ac.za

— ***Prof R Schall***
Department of Mathematical Statistics and Actuarial Science, University of the Free State
Tel: +27 (51) 401 2945
e-mail: schallr@ufs.ac.za

INTRODUCTION

Control of microorganisms on textile fabrics is important in health care settings and in the food industry. In these settings, where moisture and nutrients are readily available, textiles are exposed to microorganisms. The textiles then act as disease vectors by transmitting infectious diseases (Lee *et al*, 2003; Thiry, 2010a). Textiles are sources of cross-contamination, because they have large surface areas, retain moisture, and are difficult to clean or disinfect (Thiry, 2010a). Antimicrobial treatments used for cleaning must be strong enough to kill the infectious agents, and efficacy must be proven to the satisfaction of government regulatory standards and hospital administration personnel (Thiry, 2010b).

Although a wide range of disinfectants is available, the number of pathogens resistant to liquid chemical germicides is increasing. Recurrent replacement of one biocidal agent with another does not solve the problem. The ideal disinfectants should have a high bactericidal activity and a long shelf life, but should not have a negative effect on the environment (Bakhir *et al,* 2003). Therefore, a disinfectant must have long-term broad spectrum biocidal activity. Practical disinfectants with effective antimicrobial treatments are needed (Venkitanarayanan *et al*, 1999b).

Chlorine has been among the most frequently used chemical disinfectants for laundry. Unfortunately, disinfection with high doses of chlorine is undesirable, because it can lead to the formation of mutagenic chlorinated by-products (Lehtola *et al,* 1999).

According to Kerwick *et al.* (2005) electrochemical disinfection is one of the alternatives to chlorination. Gao and Cranston (2008) noted that most of the biocides used on textiles induce bacterial resistance, especially in clinical use. Anolyte is a potential environmentally friendly broad-spectrum microbial decontaminant with strong bactericidal activity for the inactivation of many pathogens (Fabrizio & Cutter, 2005).

Anolyte, which is acidic electrolyzed water, is generated by the electrolysis of a dilute salt solution. Anolyte has a high oxidation potential and a pH between 2 and 9 (Marais & Williams, 2001); it contains high concentrations of dissolved chloride and oxygen and functions as a bactericide (Nakae & Inaba, 2000). After use, Anolyte degrades without the formation of toxic substances and does not require neutralization before discharge. Anolyte is activated during a period of relaxation, the time during which spontaneous change of its chemical characteristics, catalytic and biocatalytic activity takes place. The mixture of metastable active agents eliminates microbes' ability to adapt to the bactericidal effect of the Anolyte (Bakhir *et al,* 2003). Anolyte has been successfully used as a disinfectant in different fields such as agriculture, dentistry and medicine (Ayebah *et al*, 2005).

The advantages of using Anolyte are: (1) it is a non-thermal treatment for microbial inactivation; (2) no chemicals except NaCl are required; (3) it has a strong antimicrobial effect to prevent cross -contamination of processing environments; (4) it can be produced on site and on demand at the concentration required for direct use so that no dilution from concentrated chemicals is needed; (5) it is a smaller health hazard to the worker than alternative agents (Park *et al*, 2002). Huang *et al.* (2008) indicate that the most important advantage of the Anolyte is its safety. Although it is a strong acid, it is not corrosive to skin, mucous membranes or organic material.
Anolyte is more effective as a material disinfectant than peracetic acid and sodium hypochlorite (eWater Systems, 2009). Water is conserved, because there is no need to rinse after sanitizing. It is easy to use, fast acting and therefore, requires less contact time. There are no residues, so products are not tainted by chemicals and the process is virtually odour free. Since Anolyte returns to ordinary water after some time, it is not a threat to the environment.

A need is recognized for a new disinfectant for textile products, which is effective against pathogenic microorganisms, while it is not harmful to the environment and can be used at a lower temperature in order to conserve energy. In the hospital environment and in the food industry, the two pathogens occurring most frequently are Gram-positive *Staphylococcus aureus* and Gram-negative *E. coli* (Garbutt, 1997:165,169). This study, therefore, investigated the efficacy of Anolyte as a disinfectant against *E. coli* and *S. aureus*. The efficacy of the Anolyte was compared to that of filtered water, detergent and a combination of detergent and sodium hypochlorite. The effect of temperature on the antimicrobial action of these agents was determined at 24 °C, 30 °C and 60 ° C.

METHODOLOGY

Textile fabrics

All fabrics were purchased from Test fabrics, Inc., West Pittston, Pennsylvania. The weft and warp yarns were machine spun and a plain weave was used to create the fabric.

Fabric 1: 100% Cotton (Style 400)
The fabric consists of 36 weft yarns and 30 warp yarns per 10 mm². The fabric weighed 0,33 grams per 50 mm².

Fabric 2: 100% Dacron (polyester)
The fabric consists of 23 weft yarns and 20 warp yarns per 10 mm². The fabric weighed 0,40 grams per 50 mm².

Fabric 3: 50/50 Polyester/Cotton blend
The weft and warp yarns were machine spun from 50% cotton fibres and 50% polyester fibres. The fabric consists of 38 weft yarns and 23 warp yarns per 10 mm². The fabric weighed 0,30 grams per 50 mm².

Study design

For each textile (cotton, polyester and polyester/ cotton) the study was carried out as a full 2 x 4 x 3 factorial design, with factors microorganism (*E.coli* and *S.aureus*) treatment (Anolyte, sodium hypochlorite, detergent, water) and temperature (24 °C, 30 °C and 60 °C). For each textile and each of the 24 °C combinations of factor levels per textile, three replicate measurements of microorganism survival (cfu/ml) were taken

Bacterial strains and culture media

For evaluation of the survival of microorganisms in this study, *S. aureus* (ATCC 25923) was used to represent the Gram-positive skin microflora and *E. coli* (ATCC 25922) to represent Gram-negative organisms from faecal contamination. The organisms were grown in 10 ml nutrient broth (Oxoid CM0001) for 24 °C hours at 37 ^oC and 28 ^oC respectively, streaked out on nutrient agar (Oxoid CM0003) and checked for purity by Gram-staining within 24 hours. Pure cultures were streaked out on nutrient agar slants, incubated at the respective temperatures for 24 hours and stored at 4 ^oC until used.

Preparation of inocula

Test organism growth from a 24 hour nutrient agar slant culture was inoculated loop for loop in 5 ml sterile 1 N phosphate buffer until a density comparable to a McFarland 1 standard (Difco 0691326) and representing 10^6-10^7 organisms/ml. The standardized culture was used to inoculate the various test materials.

Inoculation of textiles

Textile swatches (5 cm x 5 cm) were placed one-by-one in a glass Petri-dish and autoclaved for 60 minutes. Using a microliter pipette, 1 ml of the inoculum was applied carefully onto each swatch, ensuring even distribution. The swatches were left to dry in the opened Petri-dish in a safety cabinet for 45 minutes.

Treatment of fabrics

The AATCC Test Method 61-2009 procedures 2A and 5A (AATCC Technical Manual, 2009), were followed with the LaunderOmeter (Atlas). The stainless steel canisters were autoclaved for 60 minutes. Each stainless steel canister contained a single material swatch aseptically transferred from a Petri-dish, 50 sterilized stainless steel balls and 150 ml wash liquid (wash liquids were prepared according to the test method). This solution was preheated to the prescribed temperature (24 °C, 30 °C or 60 °C) and laundered for 45 minutes. Canisters were aseptically opened and each specimen removed with sterile pliers from the canister to glass beakers containing 150 ml sterile distilled water, and rinsed for 1 minute. Each specimen was aseptically moved with sterile pliers from the glass beaker and placed in a sterile WhirlPak™ bag.

Determination of effectiveness of wash liquids

Each of the rinsed swatches was aseptically weighed in a sterile WhirlPak™ bag. The appropriate amount of phosphate buffer was added to ensure a 10^{-1} dilution. Each swatch was homogenized in a stomacher (Lab Blender 400, ART Medical Equipment) for 2 minutes. Further dilutions were prepared in 9 ml phosphate buffers to obtain dilutions to 10^{-4}, which were surface plated on nutrient agar and incubated. Bacterial counts were reported as number of bacteria or colony forming units (cfu) per ml. For statistical analyses, the bacterial counts were transformed to log cfu/ml. This method was repeated five times with each test fabric, laundering temperature and wash liquid.

Statistical Analysis

The data to be analysed were the counts of surviving microorganisms, namely 3 replicate counts for the 24 combinations of factor levels in the 2 × 4 × 3 factorial design. The data for the three types of textile fabrics (cotton, polyester, and polyester/cotton) and two types of microorganism, *E. coli* and *S. aureus*, were analysed separately.

First, it was noted that there was no survival of microorganisms for treatment with Anolyte for any textile at any temperature, and similarly, there was no survival of microorganisms at 60 ° C temperature, for any textile and any treatment (that is, all microorganism counts were zero under those conditions).

After taking the logarithm to the base 10 of the counts of surviving organisms, the data was analysed using two-way analysis of variance (ANOVA) fitting the factors treatment (sodium hypochlorite, detergent, water), temperature (24 °C and 30 °C), and the treatment×temperature interaction term (the zero counts for the Anolyte treatment, and for 60 °C temperature were excluded from this analysis). Based on these ANOVA, treatment with sodium hypochlorite was compared to treatment with detergent and water, respectively, and the associated p-value is reported. Similarly, laundering at 24 °C was compared with laundering at 30 °C, and the associated P-value is reported.

Furthermore, the treatment with Anolyte was compared to treatment with sodium hypochlorite using Fisher's exact test for the proportion of zero microorganism counts under the two treatments. Similarly, laundering at 60 °C temperature was compared to laundering at 30 ° C temperature using Fisher's exact test for the proportion of zero microorganism counts under the two temperatures.

The statistical analysis was carried out using SAS Version 9.2, Proc GLM and FREQ.

RESULTS AND DISCUSSION

The effects of laundering with water, detergent, Anolyte and sodium hypochlorite solution on the survival of *E. coli* and *S. aureus* on cotton, polyester and polyester/cotton fabrics are summarised in Table 1, where mean $\log_{10}$ survival in cfu/ml is reported for each textile, laundering agent, and laundering temperature. Furthermore, P-values are reported associated with between-treatment and between-temperature differences in survival.

Escherichia coli survival

Anolyte was the only treatment that completely eradicated *E. coli* (counts of 0 cfu/ml) on all textiles studied, namely polyester, polyester/cotton and cotton. Generally, after Anolyte, the next best treatment was sodium hypochlorite, but Anolyte was statistically significantly more effective than sodium hypochlorite in eradicating *E. coli* for cotton and polyester/cotton textiles. For polyester, Anolyte and sodium hypochlorite did not differ significantly, but both agents were statistically significantly more effective than either detergent or water.

Three distinct characteristics have been suggested to be responsible for the antimicrobial effect of Anolyte: (1) chlorine content and hypochlorous acid, (2) pH, and (3) oxidation-reduction potential (ORP). Kim *et al.* (2000)

TABLE 1: MEAN LOG$_{10}$ SURVIVAL (CFU/ML) OF E. COLI AND *S. AUREUS* ON TEXTILE FABRICS, BY TREATMENT AND LAUNDERING TEMPERATURE

Textile	Design Factor		Organism			
			E. coli		S. aureus	
			Mean	P-value	Mean	P-value
Cotton	**Treatment**			vs. NACIO		vs. NACIO
		Anolyte	zero	0,0022	zero	0,0022
		NaClO	2,08		5,81	
		Detergent	3,66	<0,0001	5,81	0,9927
		Water	3,23	0,0009	6,01	0,2613
	Temperature			vs. 30 °C		vs. 30 °C
		24 °C	2,79	0,0817	5,85	0,6978
		30 °C	3,19		5,90	
		60 °C	zero	<0,0001	zero	<0,0001
Polyester	**Treatment**			vs. NACIO		vs. NACIO
		Anolyte	zero	1,000	zero	0,0022
		NaClO	0,38		3,49	
		Detergent	4,05	<0,0001	4,54	<0,0001
		Water	5,38	<0,0001	5,49	<0,0001
	Temperature			vs. 30 °C		vs. 30 °C
		24 °C	3,53	0,3061	4,79	0,0002
		30 °C	3,01		4,22	
		60 °C	zero	0,0090	zero	<0,0001
Polyester/Cotton	**Treatment**			vs. NACIO		vs. NACIO
		Anolyte	zero	0,0152	zero	0,0022
		NaClO	1,30		4,80	
		Detergent	2,73	0,0124	5,65	0,0002
		Water	3,96	0,0001	5,66	0,0001
	Temperature			vs. 30 °C		vs. 30 °C
		24 °C	2,51	0,4376	5,52	0,0467
		30 °C	2,82		5,23	
		60 °C	zero	<0,0001	zero	<0,0001

suggested that a specific range of ORPs is required for the growth of bacteria. When a solution with high oxidizing capability, such as the Anolyte (1050-1190 mV) in this study, is applied to bacteria, ions are withdrawn and the cellular membrane becomes unstable, which facilitates the entry of antimicrobial agents into the microorganism. Jay *et al.* (2005: 313) concluded that an ORP of 650 mV should result in the immediate destruction of *E. coli* irrespective of the pH or chlorine concentration. Issa-Zacharia *et al.* (2010) stated that ORP plays an important role, in combination with a high proportion of hypochlorous acid (HOCl) in killing *E. coli*. An explanation for the high ORP of Anolyte could be the oxygen released by the rupture of the weak and unstable bond between hydroxy and chloric radicals (Venkitanarayanan *et al,* 1999a).

According to Venkitanarayanan *et al*. (1999b) it is possible that the low pH of the Anolyte sensitizes the outer membrane of bacterial cells, which gives easier entry for the hypochlorous acid into the bacterial cell. White (1999:515) observed that available chlorine was removed through ORP reactions with a variety of materials such as proteins, vitamins, lipids and minerals. It was also indicated that with *E. coli*, there is a significant difference in bactericidal activity between free and combined available chlorines in the Anolyte. Combined available chlorines had lower bactericidal activity than the free form at the same concentration. Cloete *et al.* (2009) reported that the Anolyte killed the *E. coli* immediately upon exposure, by interfering with their protein composition due to oxidative stress. Zinkevich *et al.* (2000) indicated that electrochemically activated water with a pH of 5,l5 and ORP of 1100 mV acts upon *E. coli* cells by damaging double stranded DNA, RNA and proteins. It probably destroys the covalent bonds in the nucleic acid chains and protein chains.

The sodium hypochlorite solution was more effective at eliminating the *E. coli* than the detergent or the water. Munk *et al.* (2001) also found that when the detergent contained a bleach agent such as sodium hypochlorite, it will kill bacteria more effectively than detergent without bleach. There was still some *E. coli* survival after laundering with the detergent, which was similar to the survival after laundering with water. Removal of bacteria by detergent could be due to the surfactant that is present, which helps to reduce the adhesion of the microbe to the fabric by lowering the surface tension (Ainsworth & Fletcher, 1993). According to Hall *et al.* (2009), laundering with detergents alone is not effective at removing all bacterial contamination or reducing bacterial viability. Bacteria would be released into the laundering water and contaminate other articles in the machine.

Water was associated with the highest *E. coli* survival after laundering. The highest survival was on the polyester fabric and the smallest on the cotton fabric. According to Gerba and Kennedy (2007), dilution of microorganisms into the water is an important factor in microorganism reduction. The mechanical action of the laundering process also has an influence on microorganisms and aids in the disinfection efficacy of both laundering agents and of the water (Scott, 1999).

Staphylococcus aureus **survival**

As with *E.coli,* Anolyte was the only treatment that completely eradicated *S. aureus* on all textiles. After Anolyte, the next best treatment was sodium hypochlorite, but Anolyte was statistically significantly more effective than sodium hypochlorite for all textiles. The antimicrobial effect of Anolyte could be contributed to: (1) chlorine content and hypochlorous acid, (2) pH, and (3) oxidation-reduction potential (Kim *et al,* 2000).

Survival of *S. aureus* after laundering with the water was the largest of all the treatments; the largest survival was found on the cotton fabric and the smallest on the polyester fabric.

The 30 °C temperature is used as a relatively low, economical laundering temperature, but 30 °C is not hot enough to kill *S. aureus* (Hammer *et al,* 2011). According to Bhat *et al.* (2012:419), the optimum temperatures for *S. aureus* survival are between 35 °C - 40 °C, while 44 °C - 48°C is the maximum temperature that they can endure. After laundering with detergent, the smallest *S. aureus* survival was found on polyester fabric and the largest survival on cotton fabric. Laundering with detergent alone is not effective at removing all bacterial contamination or reducing bacterial viability. In fact, bacteria would be released into the laundering water and contaminate other articles in the machine (Hall *et al,* 2009). *Staphylococcus aureus* survived on all the fabrics after laundering with the sodium hypochlorite solution. The largest survival found was on cotton fabric, and the smallest on polyester fabric. Rossoni and Gaylarde (2000) also found that after laundering with a low concentration sodium hypochlorite solution (10%

active chlorine), *S. aureus* was reduced but not eliminated. *S. aureus* is also able to grow in sodium chloride with concentrations up to 25% (Valero *et al,* 2009).

When laundering at 60 °C, there was no survival for any of the treatments. Munk *et al.* (2001) also found that laundering at 60 °C kills all *S. aureus* and *E. coli.* Sterilization by heat treatment is based on the inactivation of proteins in the microorganism (Kitajima *et al,* 2007).

CONCLUSION

There exists a need for a disinfectant for textile products, which is effective against pathogenic microorganisms, while it is not harmful to the environment and can be used at a lower temperature in order to conserve energy. This study investigated the efficacy of Anolyte as a disinfectant against *E. coli* and *S. aureus*.

All treatments studied, reduced the growth of *E. coli* and *S. aureus*, but to different degrees. The Anolyte was found to be the most effective of the treatments in reducing the numbers of the organisms. No *E. coli* or *S. aureus* was found on any of the fabrics after the contaminated fabrics were laundered with the Anolyte. The sodium hypochlorite solution also reduced the number of surviving *E. coli* organisms, while the detergent and the filtered water were not as successful.

Laundering at 60 °C was significantly more effective than laundering at the two lower temperature, 24 °C and 30 °C. Temperature aided in the destruction especially at 60 °C where the temperature was responsible for the destruction of *E. coli* and *S. aureus.*

The results of this study suggest that Anolyte eradicates *E. coli* and *S. aureus* on cotton, polyester/cotton and polyester fabrics, when the textiles are laundered at low temperatures of 24 °C - 30 °C.

It can therefore, be concluded that Anolyte is a viable alternative to chemical disinfectants for the eradication of *E. coli* and *S. aureus* on cotton, polyester/cotton and polyester fabrics, at low temperatures.

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