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Antimicrobial and anti-biofilm activity and biological decontamination efficiency of ED-1 emulsion

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Abstract: Antimicrobial and antibiofilm activity, as well as biological decontamination potential of emulsion ED-1, highly efficient in radiological decontamination of metal surfaces contaminated with uranium isotopes, was assessed. The antimicrobial potency of ED-1 was evaluated against 10 different microorganisms including four Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NCTC 9001, *Klebsiella pneumoniae* ATCC 13803 and *Pseudomonas aeruginosa* NCTC 10662), four Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057, *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* NCTC 6571) and two fungi (*Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019). Although without strong bactericidal and fungicidal properties in standard agar diffusion assays, ED-1 effectively inhibited the growth of *P. aeruginosa* cells in liquid culture and more importantly, showed high potential to disperse *P. aeruginosa* biofilms. ED-1 was also capable to efficiently remove *Bacillus subtilis* ATCC 6633 spores in quantitative and a semi-quantitative biological decontamination tests on metal surfaces. Antimicrobial and antibiofilm activity and biological decontamination efficiency of ED-1 was comparable to and better than that of calcium hypochlorite solution or commercial decontaminant BX-24. This study highlighted the possibility to use ED-1, with up to 5-fold reduced amounts of calcium hypochlorite in comparison to currently used methodology, for both biological and radiological decontamination, resulting in both environmental and financial benefits.

Keywords: biological warfare agents; emulsion; decontaminant; decontaminants based on activated chlorine; biofilm dispersion.

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INTRODUCTION

In the case of chemical, biological, radiological or nuclear (CBRN) warfare hazards, special armed forces are involved in CBRN mitigation to minimize the risk for humans or the environment. Today, research efforts in developing new methodologies and materials for efficient CBRN decontamination are ongoing and it is estimated that the worldwide governments' spending on CBRN defense products and services would be over $13 billion by the year 2023.[1–3]

The threat of biological warfare agents and their possible use in bioterrorist attacks remains a leading cause of concern in the global community. Highly infectious and profoundly virulent diseases may be caused in combat personnel or in civilian populations by the appropriate dissemination of microorganisms or their toxins. Special attention should be focused on antibiotic-resistant, virulent strains of common microorganisms because no security screening is in effect for common species, so they could serve as potentially near-ideal biological weapons. One of the most common bacteria, *Staphylococcus aureus* has developed resistance to *β*-lactams (known as methicillin-resistant *S. aureus* or MRSA) and has become a major problem in hospitals in the United States.[4] Other species, such as *Pseudomonas aeruginosa* or *Streptococcus pyogenes*, are highly virulent and systemic infection can result in death in times as short as 48 h. There are many species of *Pseudomonas* that are widespread in the environment and commonly occurring in soil and water. Usually, these bacteria are not listed among classical biological agents that could be used as biological weapons.[5] Still, *P. aeruginosa* is a very important species for public health considerations because it can produce serious nosocomial infections if it gains access to the body. *Pseudomonas spp.* in general can grow as planktonic bacteria, but more often as biofilms, a form that resist antibiotic treatment, causing considerable problems not only in medical but also in industrial and environmental settings.[6]

Countermeasures against microorganisms used as biological weapon may be directed toward destroying or neutralizing these bacteria before entering human body. The first step in neutralizing biological warfare agents is disinfection, or in other words, treatments that can destroy or neutralize the agent in a wide range of inorganic, organic, or living environments before the agent has had a chance to come into contact with human beings in a sufficiently large dose to cause infection of harm.[7] Decontamination, aimed at eliminating the hazard biological warfare agents, is required on the battlefield as well as in laboratories, and biological agent production, storage, and destruction sites. Many current decontaminating formulations are effective, but they require the use of strong oxidizing agents or organic solvents that have deleterious effects on human health and the surrounding environment. Among them are chlorine-releasing agents, predominantly used as hard-surface disinfectants.[8] Chlorine-releasing agents are used for double decontamination (biological and chemical) with ASH (active sodium hypochlorite solution)

and SLASH (self-limiting ASH) systems that rely on turning "bactericidal inactive" hypochlorite anions into the "bactericidal active", although unstable, hypochlorous acid that evaporates.[9,10]

Over the past few decades, a lot of effort was implemented in the development of new decontamination methods that would produce less damage to the environment, equipment and would not be harmful to people. However, hypochlorite solutions are still widely used disinfectants, easily combined with some other cleaning elements and detergents. ED-1 emulsion, developed by Serbian Armed Forces, was proven efficient in radiological decontamination of old and new metal surfaces contaminated with fission products and uranium isotopes.[11] In this study, we investigated the antimicrobial and anti-biofilm effect of the multipurpose emulsion ED-1. The potency of ED-1, prepared under the realistic applicative conditions, to reduce the number of viable *Bacillus subtilis* spores from contaminated metal plate, was also evaluated.

EXPERIMENTAL

Microorganisms

A total of 11 microorganisms consisting of 9 bacteria (4 Gram-negative: *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NCTC 9001, *Klebsiella pneumoniae* ATCC 13803, *Pseudomonas aeruginosa* NCTC 10662 and 5 Gram-positive: *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057, *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* NCTC 6571, *Bacillus subtilis* ATCC 6633) and 2 fungi (*Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019) were used in this study. Test organisms for the antibacterial assays were obtained from the American Type Culture Collection (ATCC) or National Collection of Type Cultures (NCTC).

Preparation of emulsion ED-1

Emulsion for decontamination ED-1 is composed of calcium hypochlorite ($Ca(ClO)_2$; Merck KGaA, Darmstadt, Germany), tetrachloroethylene (C_2Cl_4; Sigma–Aldrich, Munich, Germany) and Meriten FN-10 (poly(oxy-1,2-ethanediyl), *α*-(nonylphenyl)-*ω*-hydroxy-, a low molecular-weight polymer, emulsifier with CAS registry number: 9016-45-9; Henkel, Kruševac, Serbia) in a final proportion: 20 g L^{-1}, 10 ml L^{-1} and 5 g L^{-1}, respectively.[12] ED-1 was freshly prepared before each experiment by dissolving components in sterile water and mixing the components by sonication for 10 min at the maximum amplitude (SONIC 1G ultrasonic bath, Sonic, Niš, Serbia). In parallel with ED-1, water solution of each individual component was prepared in the same way.

Agar well diffusion method

The activity of ED-1 emulsion against different microorganisms was estimated using standard agar well diffusion assay. Briefly, the agar plate surface was inoculated by spreading 1 mL of the microbial suspension diluted to optical density OD_{600} = 0.5 over the entire surface of agar plates (Luria-Bertani (LB) consisting of tryptone 10 g L^{-1}; sodium chloride (NaCl) 10 g L^{-1}; yeast extract 5 g L^{-1}; pH 7, for bacteria and Sabouraud dextrose agar consisting of glucose 40 g L^{-1}; peptone, 10 g L^{-1}; agar, 15 g L^{-1}; pH 5.6, for *Candida* spp.). A well with a diameter of 5 mm was made aseptically in the agar with a sterile Pasteur pipette and the same volume (25 μL) of the freshly prepared ED-1 emulsion or the control solutions was introduced

into the well. Subsequently, agar plates were incubated overnight at 30 °C and the inhibitory effect of the tested solutions was estimated by measuring the zones of inhibition (zones around wells where no bacterial growth occurred).

Antimicrobial susceptibility tests for planktonic cells

The minimum inhibitory concentration (*MIC* value) of freshly prepared ED-1 was determined according to standard broth microdilution assays recommended by the National Committee for Clinical Laboratory Standards (M07-A8) for bacteria and Standards of European Committee on Antimicrobial Susceptibility Testing (EDef7.1.) for fungi. Water solution of $Ca(ClO)_2$ (20 g L^{-1}) was used as a control. The highest concentration used was 10 vol.% of the ED-1 and $Ca(ClO)_2$ solution and the inoculums were 10^5 colony forming units, CFU mL^{-1}. The *MIC* value corresponds to the lowest concentration that inhibited the growth after 24 h at 37 °C for *P. aeruginosa*. The bacterial growth was estimated *via* optical density at 600 nm (OD_{600}) using a Tecan Infinite 200 Pro multiplate reader (Tecan Group Ltd., Männedorf, Switzerland).

Biofilm dispersion assay

P. aeruginosa biofilms were developed on cover slips and the effect of ED-1 on their dispersal was assessed by microscopy.[13] Briefly, an overnight culture of *P. aeruginosa* was diluted to 5×10^7 CFU mL^{-1} in LB broth and 3 mL was added per well of six well microtiter plate containing plastic cover slips. After 24 h, non-adherent cells were removed, biofilms were washed with NaCl (9 g L^{-1}), further incubated for another 24 h in 3 mL of freshly added LB with ED-1 or water solution of $Ca(ClO)_2$. After the second incubation, the biofilms were washed again with NaCl (9 g L^{-1}) and stained with 2.5 μM SYTO9 green fluorescent dye and 2.5 μM propidium iodide (PI) red fluorescent dye of Live/Dead staining kit (LIVE/DEAD® BacLight™ bacterial viability kit, Thermo Fisher Scientific, Waltham, MA, USA). Cells were observed under a fluorescence microscope (Olympus BX51, Applied Imaging Corp., San Jose, CA, USA) under 20,000× magnification.

Quantitative emulsion test with Bacillus subtilis

Decontamination capability of ED-1 emulsion against *Bacillus subtilis* was estimated according to guidelines for accreditation of disinfectants developed by German Society of Hygiene and Microbiology (Deutsche Gesellschaftfür Hygiene und Mikrobiologie (DGHM) method).[14] Briefly, 0.1 mL of the bacterial suspension (1×10^8 CFU mL^{-1}) was mixed with 8.9 mL of the test product (ED-1 emulsion, water solution of $Ca(ClO)_2$ (20 g L^{-1}) or BX-24 commercial decontaminant (Cristanini S.p.A., Rivoli Veronese, Italy). BX-24 formulation is based on sodium dichloroisocyanurate, with the addition of different components like surfactants, corrosion inhibitors, complexing and thickening agents. After the exposure time an aliquot of 1 mL of the mixture was transferred into 9 mL of TSB (tryptone 17 g L^{-1}; soytone 3 g L^{-1}; glucose 2.5 g L^{-1}, NaCl 5 g L^{-1}; dipotassium phosphate 2.5 g L^{-1}; pH 7), further incubated for another 20 min and subsequently 4 successive 1:10 dilutions of this mixture in distilled water were prepared. Aliquots of 1 mL from all samples were plated on TSA plates. Untreated control (test organism in distilled water) was processed in the same manner. Plates were incubated for 12 to 48 h at a temperature of 37 °C and colonies enumerated. The reduction of microorganisms was calculated as the difference between the log of the untreated control (no disinfectant) and the log of the disinfectant-treated sample after the exposure time.

Semi quantitative surface disinfection test

Capability of ED-1 emulsion to decontaminate surfaces covered with *Bacillus subtilis* was tested according to previously established methodology.[15,16] The surface of a metal plate (100 mm×100 mm) was coated with *B. subtilis* spore emulsion (1×10^8 CFU mL^{-1}) and subsequently dried at 50 °C. Decontamination was performed in the operating conditions for the chemical decontamination, which provides adequate dispersion of decontaminant. Metal plate was completely covered with decontaminant and incubated for 30 min at ambient temperature ranging from 14 to 18 °C. After exposure, metal plate was washed and the number of surviving cells in eluate was determined. Semi-quantitative reduction in a number of *B. subtilis* spores was estimated by comparing the number of surviving cells in eluate with the control (eluate from plate that was not decontaminated). The number of surviving cells on the metal plate was determined by printing with RODAC plates (RODAC: Replicate Organism Detection and Counting; BD, Heidelberg, Germany) subsequently incubated for 12 h at 30 °C.

RESULTS AND DISCUSSION

Preparation and characterization of ED-1 emulsion

Historically, decontaminants based on activated chlorine have been effectively employed in surface decontamination against chemical warfare agents and they are standard decontaminants in military applications worldwide.[17] This also applies to the Serbian Armed Forces where sanitation, chemical and biological decontamination are carried out with $Ca(ClO)_2$ water solutions (40-100 g L^{-1}). The ED-1 emulsion, composed of $Ca(ClO)_2$, C_2Cl_4 and Meriten FN-10 in a final proportion: 20 g L^{-1}, 10 ml L^{-1} and 5 g L^{-1}, respectively, proved its efficiency in chemical and radiological decontamination.[11,12] More specifically, ED-1 showed efficient decontamination activity on metal surfaces contaminated with nerve agents (S-yperite or Mustard gas ($C_4H_8Cl_2S$: 1-chloro-2-[(2-chloroethyl)sulfanyl]ethane), Soman ($C_7H_{16}FO_2P$: 3,3-dimethylbutan-2-yl-methylphosphonofluoridate) and VX ($C_{11}H_{26}NO_2PS$: ethyl ({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate) and uranium isotopes, respectively. The main decontamination component was $Ca(ClO)_2$, known to release active chlorine, reactive species with chlorinating, oxidizing and catalytic activity. The addition of the organic solvent C_2Cl_4, widely used for dry cleaning of fabrics, contributed to the cleaning and decontaminating power of the emulsion, while Meriten FN-10 (nonionic surfactant) was used as emulsifier. In order to analyze antimicrobial activity of ED-1, the emulsion was freshly prepared by mixing the constitutive components with water (in a specified order) in both laboratory and realistic applicative conditions (Supplementary material to this paper, Fig. S-1). In a laboratory conditions, mixing of the water and organic phase was improved by sonication, giving homogeneous solution (Fig. S-1a). Under the realistic applicative conditions, ED-1 was prepared by mixing of all three components and water with continuous stirring and applied by spraying (concentric or splashing stream) with SANIJET C.921 decontamination system (Cristanini S.p.A., Rivoli Veronese, Italy, Fig. S-1b).

Antimicrobial activity of emulsion ED-1

The antimicrobial activity of ED-1 was tested against a panel of different microorganisms using standard agar well diffusion assay. The emulsion for decontamination ED-1 had antibacterial properties against *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *E. faecium* and *S. aureus*, with zones of growth inhibition ranging from 1.5 up to 3 mm in diameter when 25 μL of the freshly prepared ED-1 emulsion, or its individually prepared constituents, was applied into the well (Table I). No zones of growth inhibition occurred when *E. coli*, *L. monocytogenes* or fungi were used as test organisms. Water solution of C_2Cl_4 (10 ml L^{-1}) itself inhibited the growth of three bacteria (*K. pneumonia*, *P. aeruginosa* and *S. aureus*) but in the case of *K. pneumonia* and *P. aeruginosa* it was only a slight inhibition of growth (turbid zone) while emulsion ED-1 completely inhibited the growth (clear zone) in both cases, suggesting that ED-1 has improved antimicrobial potency. In the case of *S. aureus* growth inhibition was more pronounced with water solution of C_2Cl_4 (10 ml L^{-1}), compared to the emulsion of ED-1. Notably, growth inhibition was not observed with water solution of $Ca(ClO)_2$ (20 g L^{-1}), widely used as disinfectant. This is probably due to the fact that the activity of decontaminants based on activated chlorine decreases rapidly upon solubilization in water and exposure to air and heat, while the results of the agar diffusion assay were observed after incubation of 12 h at 30 °C. Under the tested conditions ED-1 emulsion was not active against fungi.

TABLE I. Antibacterial properties of ED-1 emulsion and ED-1 individual components dissolved in water, presented as zones of growth inhibition (mm) in agar diffusion assay against the panel of microorganisms

Test organism	ED-1	$Ca(ClO)_2$ 20 g L^{-1} (aq)	C_2Cl_4 10 ml L^{-1} (aq)	Meriten FN-10 5 g L^{-1} (aq)
A. baumanni ATCC19606	1.5	–[a]	–	–
E. coli NCTC9001	–	–	–	–
K. pneumoniae ATCC13803	2	–	2[b]	–
P. aeruginosa NCTC10662	2	–	2[b]	–
E. faecalis ATCC29212	3	–	–	–
E. faecium ATCC6057	2	–	–	–
L. monocytogenes NCTC11994	–	–	–	–
S. aureus NCTC6571	2	–	5	–
C. albicans ATCC10231	–	–	–	–
C. parapsilosis ATCC22019	–	–	–	–

[a]No zone of growth inhibition; [b]turbid zone of growth inhibition

Antimicrobial activity of ED-1 emulsion against planktonic cells and biofilms of Pseudomonas aeruginosa

P. aeruginosa is very important species for public health considerations because it is resistant to many antibiotics and can produce serious nosocomial

infections if it gains access to the body. The efficiency of freshly prepared ED-1 to inhibit growth of planktonic *P. aeruginosa* was compared to the effect of $Ca(ClO)_2$, ordinarily used as decontaminant by Serbian Armed Forces (Fig. 1). During the 17 h long incubation period, untreated *P. aeruginosa* shows typical bacterial growth with distinct phases: lag phase, the delay before the start of exponential growth; exponential phase, where cell division proceeds at a constant rate and a stationary phase, when conditions become unfavourable for growth and bacteria stop replicating. When the comparable amounts of two decontaminants were used, in respect to the amount of $Ca(ClO)_2$ present (2 g L^{-1} in both ED-1 and water solution of $Ca(ClO)_2$), the effect was generally comparable, although bactericidal effect was not observed. Upon treatment with ED-1 (containing 0.2 g L^{-1} of $Ca(ClO)_2$) the bacterial growth was delayed for 5 h, but eventually reaching the same OD600 as the untreated control. The water solution of $Ca(ClO)_2$ (0.2 g L^{-1}) did not cause significant effect in comparison to the untreated control. The bacteriostatic effect of ED-1 emulsion was more pronounced in comparison to the effect of water solution with the same amount of $Ca(ClO)_2$, verifying its improved antimicrobial effectiveness.

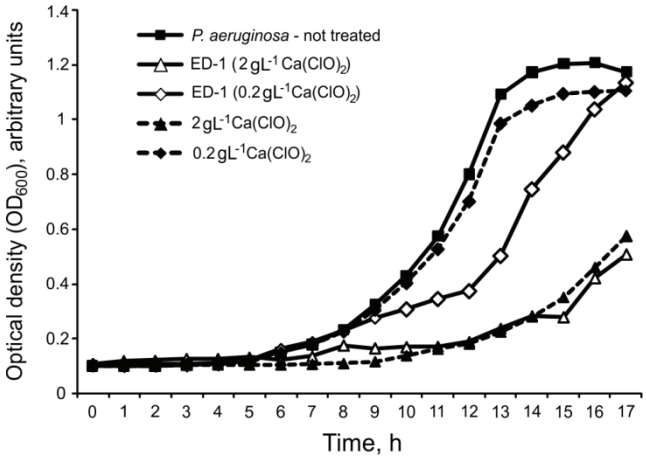

Fig. 1. Growth curves of *P. aeruginosa* in LB broth in the presence of ED-1 and $Ca(ClO)_2$.

P. aeruginosa can grow as planktonic bacteria, but also in a form of biofilms and biofilm formation causes considerable problems in medical, industrial and environmental settings, since bacteria in biofilms can resist antibiotic treatment, host immune responses, and biocide treatment.[18] *P. aeruginosa* may readily colonize spa pools and lead to wound infections if persons with open wounds or sores use them. Various strategies, relying on activated chlorine based decontaminants, have been developed and employed for the efficient biofilm control.[19] It was also showed that a combination of sodium hypochlorite and hydrogen peroxide effect-

ively killed *P. aeruginosa* cells and removed biofilms from both stainless steel and aluminium surfaces.[20] In this study we tested the ability of ED-1 emulsion to disperse 24 h-old *P. aeruginosa* biofilms (Fig. 2). ED-1 with comparable amount of $Ca(ClO)_2$ (0.2 g L^{-1}) was more efficient in the dispersion of preformed *P. aeruginosa* biofilm (Fig. 2b) in comparison to water solution of $Ca(ClO)_2$ (0.2 g L^{-1}, Fig. 2c).

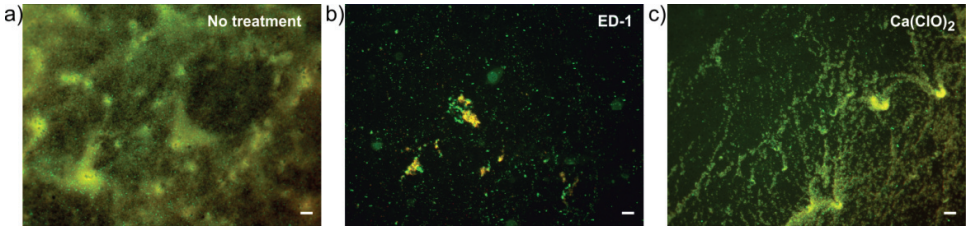

Fig. 2. Dispersion of *P. aeruginosa* biofilms pre-formed on plastic surfaces: a) without treatment, b) treated with ED-1 (containing 0.2 g L^{-1} of $Ca(ClO)_2$) and c) treated with water solution of $Ca(ClO)_2$ (0.2 g L^{-1}). Biofilms were stained with Syto9 (green) and PI (red), scale bars represent 10 μm.

Antimicrobial activity of emulsion ED-1 against Bacillus subtilis *in quantitative and a semi-quantitative test*

B. anthracis, *Bacillus cereus* and *Bacillus thuringiensis* are Gram-positive bacteria that produce robust endospores that can be easily disseminated and thus infect larger areas in bioterrorism actions.[21] *B. subtilis*, non-pathogenic species naturally found in soil and vegetation is model organism for studying endospore formation. Antimicrobial activity of freshly prepared emulsion ED-1 against *B. subtilis* was tested in laboratory conditions, using the quantitative emulsion test. Bactericidal activity of ED-1 was compared to the activities of water solution of $Ca(ClO)_2$ (20 g L^{-1}) and BX-24,commercial decontaminant widely used for a Chemical, Biological, Radiological and/or Nuclear (CBRN) decontamination.[12] Generally, disinfectant is considered efficient enough if log of reduction factor is equal to or greater than 5.[22] In quantitative emulsion test all three compositions showed acceptable and comparable bactericidal effect (Table II).

In order to test decontamination efficiency of ED-1 emulsion in the realistic conditions, out of a laboratory, semi-quantitative test with *B. subtilis* was performed. ED-1 was prepared by mixing of all three components and water with continuous stirring and applied using SANIJET C.921 decontamination system.

Following disinfection, the semi-quantitative reduction in a number of *B. subtilis* spores from contaminated metal plate was estimated in two ways, counting surviving cells eluted from the plate and those that remained on a plate. In the semi-quantitative metal surface disinfection test, the efficiency of decontamination was as follows ED1>BX-24>$Ca(ClO)_2$ (Table III). It can be assumed that

greater efficiency of ED-1 emulsion is a result of a better dissolution of the organic material to the aqueous solutions and improved stability leading to better germ killing power.

TABLE II. Efficiency of ED-1 to reduce *B. subtilis* spores given as mean $\log_{10}$-reduction factor in comparison to activated chlorine based decontaminants

Decontaminant	log of reduction factor
ED-1	5.39[a]
$Ca(ClO)_2$ 20 g L^{-1} (*aq*)	5.89
BX-24	5.89

[a]Results are mean of two independent measurements

TABLE III. The bactericidal effects of ED-1 in semi-quantitative surface disinfection test using metal panel contaminated with *B. subtilis* spores

Decontaminant	Number of CFU per carrier	
	Eluate	Smear
ED-1	10[a]	0
$Ca(ClO)_2$ 20 g L^{-1} (aq)	106	160
BX-24	51	8
Control	–[b]	–

[a]Results are mean of two independent experiments; [b]number of CFU was not determined due to the massive overgrowth (CFU>> 10000)

The results of quantitative and a semi-quantitative decontamination tests on metal surfaces pointed out that the ED-1 is as effective in biological decontamination as BX24, multi-functional, environmentally friendly and non-corrosive disinfectant (Cristanini, Verona, Italy). Both formulations are based on activated chlorine, as well as numerous formulations and methods for detoxifying both chemical and biological warfare agents, patented and used in The United States Armed Forces.[23] These disinfectants are highly active oxidizing agents, with hypochlorous acid (HOCl) being the active moiety. At present, it can be summarized that the primary effect of HOCl is either or both: *i*) the oxidation of sulfhydryl groups of essential enzymes and antioxidants and *ii*) the harmful effects on DNA synthesis. Also, the increased level of reactive oxygen species (ROS) is detected under exposure to HOCl, contributing indirectly to its bactericidal effect.[24] If antioxidants are exhausted or the activities of these enzymes are once inactivated by HOCl, O_2^- and H_2O_2 would accumulate. On the other hand, free iron is found to be released from microbial iron centers, *i.e.*, heme and non-heme iron proteins, during exposure to HOCl which leads to the production of highly reactive $^{\bullet}OH$ *via* the Fenton reaction. Thus, it is likely that the endogenously formed $^{\bullet}OH$ is also responsible for the potent bactericidal activity of HOCl.

CONCLUSIONS

Although effective decontamination materials are utilized worldwide, there is a need for continuous effort in finding new universal decontaminants that would be more efficient and with the least possible threat to the environment and to CBRN personnel.[25] Different formulations based on activated chlorine are very effective in CBRN decontamination and despite significant side effects they are still irreplaceable in final decontamination. Besides previously shown efficiency in radiological decontamination, powerful antimicrobial and antibiofilm activity has been demonstrated for the emulsion-based formulation ED-1. Its efficiency in eliminating different microorganisms from the surface was comparable or higher in comparison to pure $Ca(ClO)_2$ or even commercial CBRN decontaminant BX-24, suggesting that ED-1 has the potency to be successfully used for biological decontamination. Taking into account that ED-1 is effective for both biological and radiological decontamination, that the concentration of its active component (hypochlorite) is reduced up to 5 fold in comparison to decontamination methodology currently used in Serbian Armed Forces, thus resulting in both financial and environmental benefits, it can be concluded that the introduction of the emulsion ED-1 into the equipment of CBRN units will result in the increase of their operational and functional capabilities.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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И З В О Д

АНТИМИКРОБНА АКТИВНОСТ, АНТИБИОФИЛМ ЕФЕКАТ И ЕФИКАСНОСТ ED-1 ЕМУЛЗИЈЕ У БИОЛОШКОЈ ДЕКОНТАМИНАЦИЈИ

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У овом раду су испитивани антимикробна активност, антибиофилм ефекат, као и потенцијал за биолошку деконтаминацију емулзије ED-1, врло ефикасне у радиолошкој деконтаминацији металних површина контаминираних изотопима урана. Антимикробна активност ED-1 је тестирана на 10 различитих микроорганизама, укључујући четири Грам-негативне бактерије (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NCTC 9001, *Klebsiella pneumoniae* ATCC 13803 и *Pseudomonas aeruginosa* NCTC 10662), четири Грам-позитивне бактерије (*Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057, *Listeria monocytogenes* NCTC 11994 и *Staphylococcus aureus* NCTC 6571) и 2

гљиве (*Candida albicans* ATCC 10231 и *Candida parapsilosis* ATCC 22019). Иако ED-1 емулзија у стандардном диск-дифузионом есеју није показала изражен антимикробни ефекат, ефикасно је инхибирала раст бактерије *Pseudomonas aeruginosa* у течној култури и још значајније, показала висок потенцијал за нарушавање претходно формираних биофилмова *Pseudomonas aeruginosa*. ED-1 се показала ефикасном и у уклањању спора *Bacillus subtilis* ATCC 6633 у квантитативним и полуквантитативним биолошким тестовима деконтаминације металних површина. Антимикробни ефекат и ефикасност у биолошкој деконтаминацији са ED-1 су били упоредиви или чак и бољи од дејства раствора калцијум-хипохлорита или BX-24, комерцијалног раствора за деконтаминацију. Наши резултати су указали да би ED-1 емулзију, која садржи до пет пута мању количину калцијум хипохлорита у поређењу са тренутно коришћеном методологијом, било могуће применити не само за радиолошку, већ и за биолошку деконтаминацију, а то би сем значајног смањења трошкова имало и позитивног ефекта са становишта очувања животне средине.

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