Journal of Agricultural Sciences
Vol. 54, No 3, 2009
Pages 269-275

UDC: 66.099.7:663.86
Preliminary communication

EFFICIENCY EVALUATION OF THE DISINFECTANT BASED ON SILVER AND HYDROGEN PEROXIDE

Srđan Tasić[1]

Abstract: Contemporary science has confirmed that silver is one of the most powerful natural antimicrobial agents which can destroy more than 650 species of known pathogen microorganisms. Very high technological demands in the production of colloids and ionized particles are responsible for the fact that silver is used for water disinfection only in the most developed countries. Therefore, proverb in the west says "silver is not the privilege of wealth, but of health".

The aim of the research was to examine the efficiency of the disinfectant SANOSIL SUPER 25® used for 0.5 liter PET bottles washing in the process of the bottling of the carbonated soft drink "Orange ROSA". This multi-component disinfectant (based on silver and hydrogen peroxide) was used in concentrations of 40 ppm. After the washing of the PET bottles in the machine "Bluestar-Simonazzi" a standard bacteriological examination of 192 bottle samples was performed. In none of the examined samples could the presence of bacteria be discovered. The results of the examination of the chemical content of bottled orange soft drink indicated that the concentration of silver was within lawful limits.

Based on all acquired results the conclusion is that SANOSIL SUPER 25®, with concentrations of 40 ppm, may successfully be used as a disinfectant used in the preventive washing of *PET* bottles to be used for the bottling of the carbonated soft drink "Orange ROSA".

Key words: disinfectant, silver, PET bottles, carbonated soft drink, SANOSIL SUPER 25®.

Introduction

Science has shown that silver is one of the most powerful natural antimicrobial agents which destroys more than 650 types of known pathogen microorganisms (Searle, 1919).

[1] Srđan Tasić, School of Applied Studies, Vranje, Filipa Filipovića 20, Serbia

The antiseptic characteristics of silver were known back in ancient times (Russell, 1994). The Phoenicians, for example, used to maintain the freshness of water during their long voyages at sea by keeping their water in containers made of silver. In medieval Europe, at the wealthiest courts, silver cutlery and dishes were used. Silver particles entered the body of medieval people via the silver cutlery and dishes. The result was weak bluish pigmentation on their skin, now known as "argyria" (Hill *et al.*, 1939). The church used the antiseptic characteristics of silver to maintain the freshness of water so that ordinary people would believe to be "holy water". Up to the invention of antibiotics, silver nitrate was often used for the disinfection of the mucous membrane.

Colloidal silver is a water solution, without taste containing silver particles of about 0.001 μm in the diameter (Freundlich, 1922).

Scientific findings and the latest technology have conquered the production of colloidal at acceptable prices enabling silver to enter usage that is more massive and making it more accessible. Unfortunately, high technological demands in the production of real colloidal and ionised particles have made the return of silver possible only for more developed countries.

Silver ions as positive ions and they form electrostatic connections with negative parts in the cell walls of microorganisms, thus leading to changes in their cell wall porosity. In the cell proteins, silver bonds with those amino acids which contain sulphur blocking thus the metabolism. This process is so fast that a microorganism has difficulty in attaining its resistance to colloidal silver (Silver, 2003).

Colloidal silver in *in vitro* conditions with a concentration of just 15 ppm completely destroys *Staphylococcus* (population of 10×10^6) in 4 min, *Salmonella* (population of 10×10^6) in 7 min, *Pseudomonas* (population of 13×10^6) in 10 min, *Candida* (population of 1×10^9) in 2 min (Farinha, 2003).

Hydrogen peroxide as an oxidising agent has a high affinity to those organic compounds which it can oxidise. Latest research has shown that the cause of a higher sensitivity of resistant bacteria is a reduced level of catalysed enzymes in them. Hydrogen peroxide oxidises lipids, proteins and DNA, thus causing chemical modifications in them which result in the death of the bacteria cell[2].

The disinfectant based on silver and hydrogen peroxide shows a much stronger bactericidal effect in comparison to the effect caused by a 3% H_2O_2 (Nikolic–Bujanovic, 2006). In certain cases, the combination of silver and hydrogen peroxide may show a very synergetic influence which can be a

[2] Refer to: [EC] European Commission, [DG] Directorate-General. (1999): Consumer Policy and Consumer Health Protection. Opinion of the Scientific Steering Committee on antimicrobial resistance (Report of the European Commission, DG XXIV, Consumer Policy and Consumer Health Protection; Brussels: European Commission.

thousand times stronger than the individual influences of either agent put together (Pedahzur, 2000).

The Regulations covering the quality and other demands referring to soft drinks, syrups and soft drink powders and soda water (Official Gazette Sl. list SRJ, issues 03, 10, 22, 35, 53, 58/ 2002) do not prescribe maximum silver concentrations. Silver is not toxic to the human body except in extreme cases of overdose[3].

Official confirmation about the intoxicant characteristic of silver may be found in the Recommendations for Drinking Water Quality of the World Health Organisation (WHO) saying: "It is not necessary to prescribe the maximum amount of silver allowed in water because silver is not dangerous for the health of human beings." In accordance with these recommendations, the new standards of the European Union referring to drinking water do not prescribe a maximum level of silver allowed in drinking water. On the contrary, the WHO, the European Union as well as the United States Environmental Protection Agency especially recommend colloidal silver as the most powerful disinfectant for drinking water with a concentration of 80 ppm.

The Serbian Regulations Covering Hygienic Demands of Drinking Water (Official Gazette *Sl. list SRJ*, issue 42/98) state that the maximum silver concentration allowed in water is 0.01 mg/l.

The aim of the research was to evaluate the efficiency of the multi-component disinfectant based on colloidal silver and hydrogen peroxide, SANOSIL SUPER 25®, for the washing purpose of *PET* bottles of 0.5 l in the filling process of the soft drink containing orange fruit juice, "Orange ROSA".

Material and Methods

Standard bacteriological methods were used (Regulations about Methods for Microbiological Analyses and Super-analyses of Food Products, Official Gazette Sl. list SFRJ, issue 25/80).

The microbiological mediums used were those produced by "Merck" KgaA – Germany, "Biolife" S.r.l. – Italy.

The total number of viable microorganisms in 1 ml was determined after sowing a nutrient agar (Nutrient agar – Biolife S.r.l.) with the material. The detection and enumeration of the total number of coliform bacteria were performed by means of the membrane filtration method on an Endo agar (Biolife Sr.l.). The detection of *Pseudomonas aeruginosa* was performed by means of the standard membrane filtration method by applying 0.2 μm of filter disc membrane

[3] Refer to: [USEPA] United Stated Environmental Protection Agency, R.E.D. Facts: Silver. Office of Prevention, Pesticides And Toxic Substances, U.S. Environmental Protection Agency, Washington D.C., H-7508W, (1992).

("PALL-Gelman") and the highly selective medium *Cetrimide agar* (Merck KgaA). The determination of the presence of faecal streptococci was performed by means of the standard membrane filtration method and the medium used was the highly selective medium *KF Streptococcus Agar Base* (Merck KgaA) to which we added 10 ml/l 1% solution TTC (2,3,5-triphenyltetrazolium chloride) immediately prior to dispersion. The detection of the presence of sulphite-reducing clostridia was performed by applying *Sulphite Polymyxin Sulphadiazine Agar* (Biolife S.r.l). The determination of the presence of yeast and moulds was performed by means of Sabouraud maltose agar (Biolife Sr.l.).

The bacteriological control of the disinfectant success was performed after the washing had been done. 24 bottles were sampled for each procedure. The water content in those bottles (about 50 ml) was examined by means of the membrane filtration method. After the filtration, the filter disc membranes were placed onto the nutrient mediums Nutrient agar, Endo agar, Cetrimide agar *(*for the *Pseudomonas aeruginosa)* and the *KF Streptococcus Agar Base*. The detection of presence of sulphite-reducing clostridia was performed by pouring 50 ml of the water content from the treated *PET* bottles into a large test tube and then it was poured over with 50 ml of double concentrated *Sulphite Polymixin Sulphadiazine Agar* (Biolife Sr.l.) cooled down to 50ºC.

The potential contamination risks were eliminated by controlling the quality of the prepared medium. Additional control levels were the so-called "negative controls" (transferring sterile disk membrane filters, without any sample, onto the nutrient medium) and the so-called "positive controls" (inoculating "wild type" strains of known bacteria species: *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Clostridium sp.).*

Results and Discussion

The bacteriological examination of *PET* bottles of 0.5 l, after leaving the blowing machine "Sidel SBO-10", proved their sterility.

While transporting them in a 100-meter-long lift to the washing machine, the bottles may be contaminated by microorganisms under the influence of the circulation of infectious air used for the transportation of the bottles. The total number of microorganisms, determined in the isotonic solution added to the bottles before washing amounted approximately to 140 microorganisms per bottle.

For the washing of the *PET* bottles, the machine "Bluestar- Simonazzi" was used. Swiss made 40-ppm-concentrated SANOSIL SUPER 25® was used for the disinfection during the first washing procedure. During the second washing procedure, the disinfectant residue was rinsed by sterile water. Every washing

lasted for about 10 seconds at a temperature of about 10ºC and under a pressure of 2 bars.

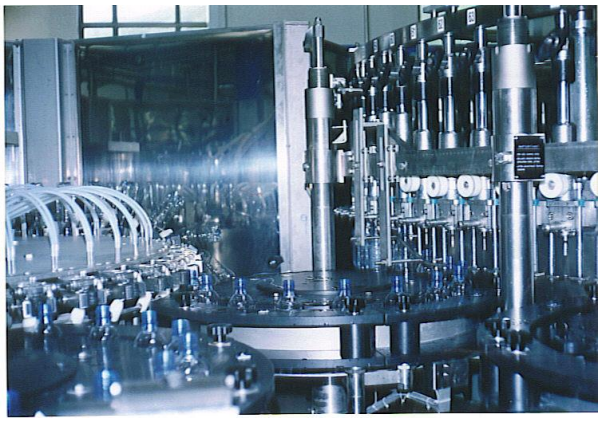

Fig. 1. – The bottles were machine washed by „Bluestar“ 100-140-24, Rinser, Filler, Capper, „Simonazzi“

Tab. 1. - The germicide effect of SANOSIL SUPER 25® (40 ppm)

Tested microorganism	Concentration (McFarland standard)	SANOSIL SUPER 25® impact time (min)					
		2	5	10	15	20	control
Escherichia coli	6 x 10^8	-	-	-	-	-	+
Pseudomonas aeruginosa	6 x 10^8	-	-	-	-	-	+
Streptococcus faecalis	6 x 10^8	+	-	-	-	-	+
Clostridium sp.	6 x 10^8	+	+	-	-	-	+
Candida albicans	6 x 10^8	-	-	-	-	-	+

SANOSIL SUPER 25® is a patented (CH673225, GB4915955, USA2189394) multi-component disinfectant, based on silver and hydrogen peroxide. It is, like water, a transparent fluid. When concentrated, it has a very weak odour. When prepared, the solution has no smell and no taste. The pH value is about 1.2. It does not form any film or similar layers. When used with the given concentration and at the mentioned temperature it does not cause corrosion.

In laboratory conditions, the combined disinfecting SANOSIL SUPER 25®, because of its synergetic influence of silver and hydro peroxide, in the concentration of 40 ppm, at a temperature of 10ºC and without the presence of protein burdening, destroys the blastospores *Candida albicans* and all *Gram* negative bacteria after only two minutes. *Streptococcus faecalis*, being an extremely resilient non-sporulated species, can be destroyed after five minutes. In the same conditions, after a ten-minute contact, ability of growth couldn’t be

noticed, also not any of *Clostridium sp.* The concentration of the examined microorganisms was $6x10^8$ McFarland standards.

The results of the examinations performed on all sampled bottles after the washing did not indicate the presence of any bacteria.

As based on the results of the chemical content examination of the soft drink "Orange ROSA", obtained in referential laboratories in Serbia (Institute of Health Protection in Serbia "Dr Milan Jovanovic-Batut" and the Institute of Health Protection in Vranje), the conclusion may be drawn that in the examined samples of the final products there was no residue of this disinfectant. Moreover, in all the examined samples of water, the silver concentration was far below the maximum concentration determined by the law (< 0.01 mg/l).

Conclusion

A total number of 192 samples of *PET* bottles were examined after their washing and disinfecting. In all the examined sampled bottles no presence of any bacteria could be noticed nor were there changes in the organoleptic characteristics of the final product. An identical result was obtained during the "negative controls" as well. Unlike that, the results obtained during the "positive controls" indicated a substantial bacterial growth.

Silver was present in the final product in an amount much lower that the allowed maximum concentration.

Because of the combined influence of silver and hydro peroxide, SANOSIL SUPER 25®, behaves like and disinfectant, but also as an antiseptic.

This product, even when used with a concentration of only 40 ppm has a fast initial influence, it does not create toxic residue, it is degradable, it does not harm the material and it can be used for the disinfection of equipment and machines engaged in the preventive washing procedure of PET bottles used for the filling of refreshing non-alcoholic drinks containing orange fruit juice.

REFERENCES

1. Farinha, M. (2003): Time-Kill Study on SilverKare Colloidal Silver, University of North Texas, U.S.A.
2. Freundlich, H. (1922): Colloid & Capillary Chemistry, Translated by H. S. Hatfield. E. P. Dutton and Company, Inc.: New York, p. 740- 742.
3. Hill, W. R., Pillsbury, M. D. (1939): Argyria-The Pharmacology of Silver, Williams & Wilkins Company.
4. Nikolić–Bujanović, LJ., Popović, N., Simičić, M., Čekerevac, M., Rakin, P. (2006): *Ecocute ekološko dezinfekciono sredstvo*, Jugoslov Med Biohem 25 (3): p 263-267.
5. Pedahzur, R., Katzenelson, D., Barnea, N., Lev, O., Shuval. H., Fattal., B., Ulitzur, S. (2000): The efficacy of long-lasting residual drinking water disinfectants based on hydrogen peroxide and silver. Water Science and Technology; 42 (1-2): 293-8.

6. Russell, A. D., Hugo, W. B (1994): Antimicrobial Activity and Action of Silver, Progress in Medicinal Chemistry; Vol. 31.
7. Searle, A. B. (1919): The Use of Colloids in Health and Disease. E.P. Dutton & Company: New York, p. 75.
8. Silver, S. (2003): Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev; 27: 341–53.

Received: December 19, 2008
Accepted: December 14, 2009

ISPITIVANJE EFIKASNOSTI DEZINFICIJENSA NA BAZI SREBRA I VODONIK PEROKSIDA

Srđan Tasić[1]

Rezime

Savremena nauka je potvrdila da je srebro jedan od najmoćnijih prirodnih antimikrobnih sredstava koji uništava više od 650 vrsta poznatih patogenih mikroorganizama. Visoki tehnološki zahtevi u proizvodnji koloida i jonizovanih čestica čine da je korišćenje srebra za potrebe dezinfekcije vode rezervisano samo za najrazvijenije zemlje. Otuda i poslovica na zapadu da „srebro nije privilegija bogatih, već zdravih".

Cilj istraživanja je bio ispitati efikasnost dezinfekcionog sredstva SANOSIL SUPER 25® za potrebe pranja *PET* boca zapremine 0.5 litra u procesu punjenja osvežavajućeg bezalkoholnog pića sa voćnim sokom narandže „Orange ROSA". Ovaj multikomponentni dezinficijens (baziran na srebru i vodonik peroksidu) je korišćen u koncentraciji 40 *ppm.* Nakon pranja *PET* boca, u mašini „Bluestar-Simonazzi", izvršeno je standardno bakteriološko ispitivanje 192 uzoraka boca. Ni u jednom ispitivanom uzorku nije primećeno prisustvo bakterija. Rezultati ispitivanja hemijskog sastava „Orange ROSA" pokazali su da se koncentracija srebra nalazila u zakonski dozvoljenim granicama.

Na osnovu svih dobijenih rezultata zaključak je da se SANOSIL SUPER 25®, u koncentraciji 40 *ppm*, može uspešno koristiti kao dezinfekciono sredstvo za potrebe preventivnog pranja *PET* boca namenjenih punjenju osvežavajućeg bezalkoholnog pića sa voćnim sokom narandže „Orange ROSA".

Primljeno: 19 decembar 2008
Odobreno: 14 decembar 2009

[1] Srdjan Tasić, Visoka škola primenjenih strukovnih studija, Vranje, Filipa Filipovića 20, Srbija