Asian Journal of Chemistry; Vol. 33, No. 2 (2021), 371-375

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23002

Antibacterial Action and Efficacy of Silver and Copper Exchanged Na-Y Zeolite from Fly Ash on 304 Stainless Steel Coupons by Agar Diffusion Method

RASHMI RANI PADHY and SANGEETA TIWARI*

Amity Institute of Applied Sciences, Amity University, Noida-201303, India

*Corresponding author: E-mail: stiwari2@amity.edu

Received: 26 September 2020; *Accepted*: 21 November 2020; *Published online*: 15 January 2021; AJC-20223

Na-Y Zeolite synthesized from fly ash was exchanged with Ag^+ and Cu^{2+} ions by ion exchange process. The antibacterial action and efficacy of silver and copper exchanged Na-Y zeolite on *Escherichia coli* and *Staphylococcus aureus* were investigated. *Escherichia coli* and *Staphylococcus aureus* was suspended in broth media and exposed to silver and copper exchanged zeolite Y (ranging from 25-1000 µg) for a period of 24 h. The minimum bactericidal concentration (MBC) of silver and copper loaded zeolite-Y powder was 50 and 250 µg for *Escherichia coli*. *Staphylococcus aureus* was destroyed at a powder concentration of 100 µg for both Ag-Y and Cu-Y zeolite. Further, the present work also focuses to incorporate 5% silver and copper exchanged zeolite pigment in coating and study the antibacterial effect on the coated 304 stainless coupons. Paint containing copper exchanged zeolite shows antibacterial activity compared to paint with silver containing zeolite.

Keywords: Na-Y zeolite, Antibacterial study, Reactive oxygen species.

INTRODUCTION

The study in the discipline for developing various materials, which have high antibacterial properties is a emerging topic among scientists and researchers. Biomaterials infused with various types of antibacterial pigment have been applied for many years in various applications in medicinal science [1-4]. The different types of antibacterial compounds that have been used as conventional antibiotics are triclosan, benzalkonium chloride and few inorganic compounds containing silver [5-9] and other metals. Previous workers [10-14] also reported the use of various antimicrobial materials for non-medical applications, such as used in carpets, hand lotions, gloves, wallpaper adhesives, pavement marking materials and window cleaners.

There are 600-700 different types of compounds consisting of some form of antimicrobial or antibacterial pigment added to them [15]. An estimated of around 76 million people get affected by foodborne sickness in USA every year. So to reduce the illness caused by the microorganism, food industry is always fascinated for the incorporation of antibacterial agents in coating on the various surfaces which can lower the growth of microbial specimen [16-18]. The usage of antibacterial compounds has many significance in construction industry as well. Engineers and purchasers of both commercial and non-commercial properties are always exploring different techniques to inhibit growth of fungus in the construction raw materials, as that may cause many potential health hazards [19]. The air quality, in those specific surrounding because of the inhabit of microbe is harmful to human health [20]. Broadly silver is the integral part of all the frequently used antimicrobial substances. The antimicrobial mechanism suggested for silver include various aspect like intervene with electron transport system, hindering the bacterial enzymes growth and binding to DNA of the bacteria [21].

Zeolites are the example of crystalline alumina silicates, which consist of silicon, aluminium and oxygen in their frame work. The open framework structure of zeolite may contain cations and water molecules. The cation present within the zeolite can be exchanged with monovalent or divalent ions [22]. The biological applications of zeolite are gradually gaining as a research interest in recent years. It can be suggested that zeolites will be used extensively in different biological product because of their unique framework with definite shape and size, alterable binding with tiny biological entities that eventually will control the immune system of the microorganism [22]. The antibacterial action of different zeolites exchanged with few metals like silver and copper have been explained in several reports [23-26].

The present work aims to study the antibacterial study of silver and copper exchanged Na-Y zeolite from fly ash with *Escherichia coli* and *Staphylococcus aureus* bacteria for a period up to 24 h. Further, the antibacterial efficacy of these synthesized pigment was evaluated by incorporating them in paints coated on 304 stainless steel coupons by agar diffusion method. This work also aims to understand the mechanism suggested by silver and copper ions on the antibacterial activity on relevant bacteria.

EXPERIMENTAL

All the chemicals used were of analytical grade and were obtained from Sigma-Aldrich.

Metal-loaded zeolite: Fly ash was used as a raw material for the synthesis of Na-Y zeolite as described earlier [27]. Fly ash (5 g) was grounded with finely powdered 6 g NaOH pellets and fumed silica (20% wt.) and mixed well. The mixture was transferred to silica crucible and blended at 590 ± 5 ºC for 3 h in a muffle furnace. Distilled water 1:5 (solid to water ratio) was mixed with the crushed fused mass. The mixture was left for 18 h with constant stirring in a magnetic stirrer. The liquid mixture was transferred to an autoclave with a Teflon lining to carry out the hydrothermal process in a preheated oven for 6 h at 90 ºC. The synthesized product was filtered, rinsed with distilled water several time to remove excess NaOH and air dried. The Na-Y zeolite sample was than suspended in 0.05 M $Cu(NO_3)_2$ and 0.01 M $AgNO_3$ solutions at a solid to solution ratio of 1:100 and finally stirred the mixture for 48 h. The resulting sample was filtered, rinsed with distilled water and dried at room temperature. The above procedure was performed in dark by covering the glassware and sample with aluminium foil, when silver was used. The synthesized zeolites were denoted as Cu-Y and Ag-Y. The Ag-Y zeolite was transferred to an air-tight bottle and kept in the dark. Different concentrations of both zeolites (Cu-Y and Ag-Y) *viz.* 25, 50, 100, 250, 500 and 1000 µg were screened for antibacterial assay.

Preparation of coatings: Epoxy resin (aralditeXIN-100) and hardener (araldite XIN-900) was used to formulate the paint. The stainless steel coupons of 304 grade were employed as metallic substrate for the antibacterial studies of paint. Paint 1 was constituted of 5% of synthesized Na-Y (v/v), paint 2 comprised of 5% of the Cu-Y zeolite, while paint 3 contained Ag-Y as antibacterial pigment. All pigment contents were referred to the total pigment content. The other components like TiO_2, fly ash and silica, were also incorporated to complete the paint formulation. The pigment volume concentration was kept 30% and the coating formulations are shown in Table-1. The stainless-steel coupons 2 × 2 inches were cleaned by gentle rubbing with isopropyl alcohol and allowed to air dry prior to coating. Single coupon painted with a brush to a thickness of 40 ± 5 µm. Painted stainless coupons were kept indoors for 7 days before being tested.

TABLE-1
PAINT COMPOSITIONS (% BY VOLUME)

Material	Paint 1	Paint 2	Paint 3
Titanium dioxide	2.5	2.5	2.5
Fly ash	2.0	2.0	2.0
Silica	1.5	1.5	1.5
Epoxy resin	7.0	7.0	7.0
Hardener	3.5	3.5	3.5
Na-Y	1.0	–	–
Ag-Y	–	–	1.0
Cu-Y	–	1.0	–
Toluene	6.5	6.5	6.5

Characterization: The phase purity of zeolite Y particles as well as the exchanged zeolite were analyzed with X-ray diffraction. The surface morphology of both zeolites Y with and without metal was examined by scanning electron microscopy (SEM) using a Zeiss EVO18. The elemental composition of both zeolite Y and metal loaded zeolite Y was determined by EDAX.

Antibacterial efficacy assays by agar diffusion method: Two microorganisms, *viz. E. coli* and *S. aureus* were used for antibacterial studies. The stock cultures of bacteria were received from Bio-Genics, Hubli, India. Media used for the growth of bacteria was consist of peptone 10 g, NaCl 10 g and yeast extract 5 g, agar 20 g in 1000 mL of distilled water. Metal loaded zeolite Cu-Y and Ag-Y powders with different concentrations were used for antibacterial studies. Each plate was inoculated with 18 h old cultures (100 µL, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control wells with dil. HCl were also prepared. All the plates were incubated at 37 ºC for 24 h and the diameter of inhibition zone were noted.

Incubation on coated 304 stainless steel coupons: Antibacterial efficacy for coated 304 stainless-steel coupons were studied by agar diffusion method. Each plate was inoculated with 18 h old cultures (100 µL, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the coated stainless steel coupons with different antibacterial pigment were placed in the centre of petri dish by exposing the coated side to the culture containing the bacteria. All the plates were incubated at 37 ºC for 24 h and the diameter of inhibition zone were noted.

RESULTS AND DISCUSSION

Mineralogical properties: The EDAX graph of Na-Y and metal loaded zeolite is given in Fig. 1a, which shows the formation of NaY zeolite from fly ash. The EDAX graph of metal loaded zeolite sample (Fig. 1b) shows the Ag Peak confirming the incorporation of Ag in the Na-Y zeolite frame work.

XRD analysis: Fig. 2 displays the XRD patterns for synthesized Na-Y zeolite and exchanged zeolites (Cu-Y and Ag-Y). The XRD patterns of all the samples showed the characteristic peaks at 2θ values of a well crystallized NaY zeolite [28]. The XRD image of Cu-Y and Ag-Y samples also shows the peaks at 2θ values of a Na-Y zeolite, which is indicative that after ion-exchange with Ag^+ and Cu^{2+}, while the zeolitic framework remains unaltered. The XRD results are also supported by SEM images of metal loaded zeolite.

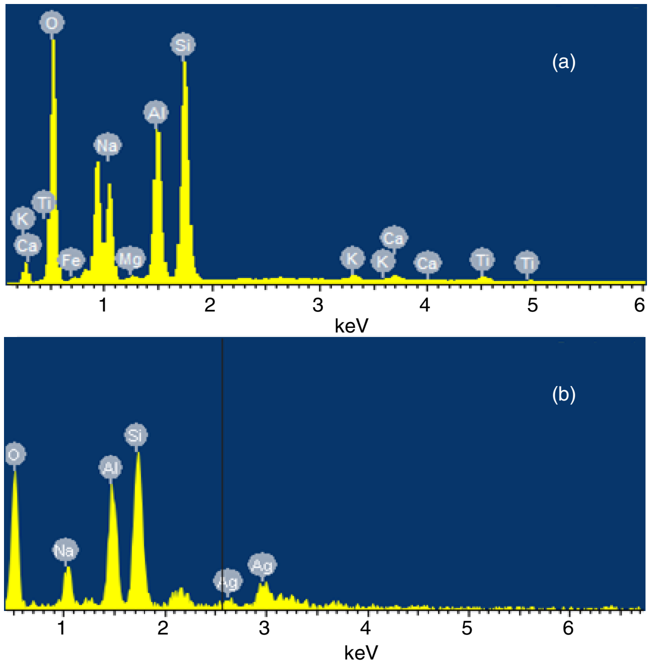

Fig. 1. (a) EDAX showing Si and Al peak in Na-Y zeolite, (b) EDAX showing incorporation of Ag in Ag-Y sample

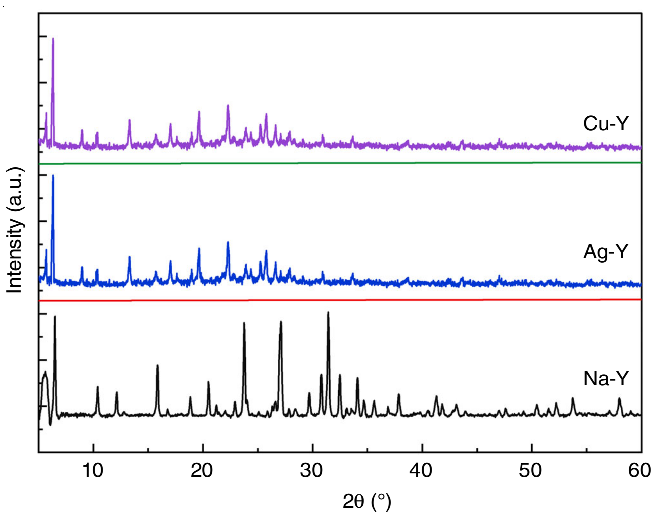

Fig. 2. XRD patterns of Na-Y zeolite and metal loaded zeolite

SEM studies: SEM images of Na-Y zeolite sample is shown in Fig. 3, which validates the formation of regular octahedral morphology related with NaY zeolite [27,28]. SEM images of Na-Y zeolite with and without silver and copper indicated that particles closely similar in size and appearance suggesting the loading of silver and copper ions have no significant effect on the morphology of the zeolites. As the size of the metal loaded zeolite particles are less than 1 μm, they can be easily incorporated in the paint preparation without altering the properties of other constituents in the paint.

Bactericidal activity of metal loaded zeolite: The minimal bactericidal concentration (MBC) of silver and copper exchanged zeolite-Y powder was 0.05 mg/50 μg and 0.25 mg/250 μg, respectively for *E. coli* and *S. aureus* was killed at a powder concentration of 0.1 mg/100 μg for both Ag-Y and Cu-Y zeolites. Zone of inhibition for Na-Y zeolite shows similar values for both the bacteria. Diameter of zone of inhibition in mm for *E. coli* and *S. aureus* for metal exchanged zeolite powder are shown in Table-2.

Mechanism: The metal loaded zeolite Ag-Y/Cu-Y when in contact with the broth medium shows the following reactions:

$$C\left(\frac{Ag}{Cu-Y}\right) + \text{Broth media} \xrightarrow{\text{Release of Ag/Cu ions}}$$

$$X\left(\frac{Ag^{+}}{Cu^{2+}}\right) + (C-X)\left(\frac{Ag}{Cu-Y}\right) + \text{Broth media}$$

where, C is the initial concentration of silver and copper ions in the zeolite framework, X is the concentration of silver and copper ions released from the zeolite framework. After release from the zeolite framework, Ag^{+}/Cu^{2+} ions move closer to the vicinity of bacterial cell membrane [29-31]. Although oxygen is the terminal electron acceptor of the aerobic respiration and it is essential for all aerobic organisms. But however, oxygen has toxic effects on *E. coli* and *S. aureus* organisms. Under aerated conditions intracellular Ag^{+} /Cu^{2+} ions generate reactive oxygen species (ROS). In presence of large number of oxidases like xanthine oxidase, aldehyde oxidase and NADPH oxidase, superoxide anion (O_2^{-}) is produced by one electron reduction of molecular oxygen within the cell [32]. The superoxide anion reacts with H_2O_2 to produce hydroxyl radicals. The hydroxyl

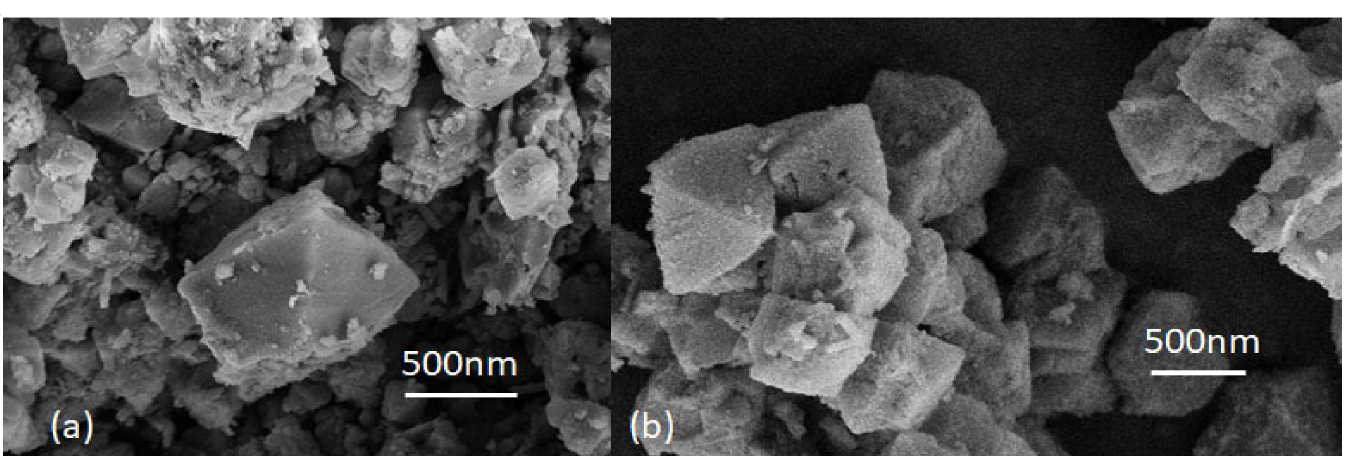

Fig. 3. SEM images of (a) Na-Y zeolite (b) metal loaded zeolite

TABLE-2
DIAMETER OF ZONE OF INHIBITION IN MM FOR *E. coli* AND *S. aureus* BACTERIA

Sample	*E. coli*						*S. aureus*					
	25 µg/0.025 mg	50 µg/0.05 mg	100 µg/0.1 mg	250 µg/0.25 mg	500 µg/0.5 mg	1000 µg/1 mg	25 µg/0.025 mg	50 µg/0.05 mg	100 µg/0.1 mg	250 µg/0.25 mg	500 µg/0.5 mg	1000 µg/1 mg
Na-Y	0	0	0	2	6	11	0	0	0	2	5	10
Cu-Y	0	0	0	11	17	25	0	0	8	15	19	26
Ag-Y	0	7	11	15	21	27	0	0	6	11	16	22
Standard (ciprofloxacin)	26	29	32	34	38	*	25	28	31	34	36	*

*Zone of inhibition could not be measured due to merging

radicals are exceptionally reactive species. Superoxide anions damages nucleic acids, lipids and DNA because they cannot pierce into the cell membrane. Among the ROS (reactive oxygen species) hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) are more powerful oxidizing agents. They directly enter into the cell membrane and cause damages to the bacteria [33]. The formation of superoxide anion and the hydroxyl radicals are shown as follows:

$$O_2 + 1e^- \longrightarrow O_2^- \quad (1)$$

$$O_2^- + H_2O_2 + H^+ \longrightarrow O_2 + H_2O + {}^{\bullet}OH \quad (2)$$

It is also reported that in addition to these oxidases, some metal ions also generate ROS [34] for example, under aerobic conditions Cu^{2+} ion also generate ROS (eqns. 3 and 4):

$$Cu^{2+} + O_2^- \longrightarrow Cu^+ + O_2 \quad (3)$$

$$Cu^+ + H_2O_2 \longrightarrow Cu^{2+} + OH^- + {}^{\bullet}OH \quad (4)$$

Therefore, the reactive oxygen species generated by Ag^+/Cu^{2+} are highly reactive and they bind with the mercapto groups present in the tertiary and quaternary structure of the proteins. The tertiary structure of protein is a result of the interactions between all side chains and groups of polypeptide chain, which gives a specific conformation to the protein. The quaternary structure of protein involves the intermolecular interactions between the different polypeptide chains to form an aggregate structure. The functional -SH group present in the proteins are blocked by this binding. Because of the binding with -SH groups the tertiary and quaternary structure of the proteins are distorted and degenerated. These metal ions deactivate the proteins structure and that will mainly cause the death of the microorganism due to the reduction in the membrane penetrability [35].

The observed differences in the antibacterial effect of Ag-Y sample on the Gram-positive and Gram-negative bacteria may be due to the much thicker peptidoglycan layer (20-80 nm) in the cell walls of Gram-positive bacteria.

Antibacterial activity of coated steel coupons against broth cultures: Cultures of *S. aureus* and *E. coli* were effectively killed by exposure to metal loaded zeolite powder. In order to study the anti bacterial effect of Ag-Y and Cu-Y zeolite in coating, the coated stainless-steel coupons were placed in the centre of the petri dish containing bacterial cultures. Table-3 shows the diameter of zone of inhibition in mm for coated stainless steel coupons with *E. coli* and *S. aureus*. No clear zone of inhibition is shown for paint-1, suggesting that Na-Y zeolite pigment has no antibacterial effect in coating. Even Na-Y powder also does not show any antibacterial effect. The differences observed in zone of inhibition for paint-2 and 3 might be due to differences in the release of Ag^+ ions. The diffusing Ag^+ may be confined within the zeolite structure in paint 3, which slows down the silver ion release to the broth media.

TABLE-3
DIAMETER OF ZONE OF INHIBITION IN mm FOR COATED STAINLESS-STEEL COUPONS WITH *E. coli* AND *S. aureus* BACTERIA

Sample	*E. coli*	*S. aureus*
Paint-1 (Na-Y)	Not recognizable	Not recognizable
Paint-2 (Cu-Y)	5	2
Paint-3 (Ag-Y)	Not recognizable	Not recognizable

Fig. 4 shows the schematic representation of the antibacterial mechanism by the copper and silver exchanged zeolite. When the positively charged metal ions from the respective paints, come into contact with the negatively charged cell membrane of the bacteria, they attract each other. The proteins present on the bactericidal cell deactivated by the metal ions and this will cause the killing of the microorganism [35].

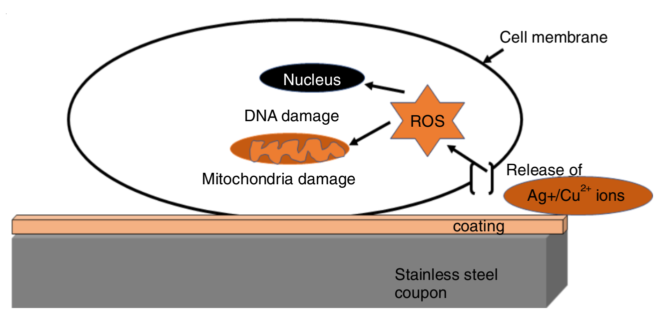

Fig. 4. Schematic representation of antibacterial mechanism by the coating

Conclusion

The antibacterial properties of fly ash based Na-Y zeolite with copper and silver ions were examined. The minimum bactericidal concentration (MBC) of silver and copper loaded zeolite-Y powder was 50 and 250 µg for *E. coli* bacteria. For *S. aureus*, both Ag-Y and Cu-Y zeolite shows similar antibacterial effect. The fly ash-based zeolites showed antibacterial activity after introduction of Cu and Ag into the framework. Reactive oxygen species (ROS) generated by Ag^+/Cu^{2+} bind with the mercapto groups present in the tertiary and quaternary structure of the proteins. Because of the binding with -SH groups the tertiary and quaternary structure of the proteins are changed and fragmented. Paint containing copper exchanged zeolite shows better antibacterial activity compared to paint with silver containing zeolite. The difference of antibacterial effect of paint-2 and paint-3 may be due to the slow diffusion of silver ions from the paint-3. So, the results in this work indicate the antibacterial application of copper and silver ion exchanged fly ash-based Na-Y zeolite.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. W.-K. Loke, S.-K. Lau, L.L. Yong, E. Khor and C.K. Sum, *J. Biomed. Mater. Res.*, **53**, 8 (2000); https://doi.org/10.1002/(SICI)1097-4636(2000)53:1<8::AID-JBM2>3.0.CO;2-3
2. P. Malassiney, O. Goeau-Brissonniere, M. Coggia and J.-C. Pechere, *J. Antimicrob. Chemother.*, **37**, 1121 (1996); https://doi.org/10.1093/jac/37.6.1121
3. H. Nikawa, T. Yamamoto, T. Hamada, M.B. Rahardjo, H. Murata and S. Nakanoda, *J. Oral Rehabil.*, **24**, 350 (1997); https://doi.org/10.1046/j.1365-2842.1997.d01-297.x
4. J.M. Schierholz, A.F.E. Rump, G. Pulverer and J. Beuth, *Anticancer Res.*, **18**, 3629 (1998).
5. B.D. Kalyon and U. Olgun, *J. Infect. Control.*, **29**, 124 (2001); https://doi.org/10.1067/mic.2001.113229
6. E.N. Rees, S.E. Tebbs and T.S.J. Elliott, *J. Hosp. Infect.*, **39**, 323 (1998); https://doi.org/10.1016/S0195-6701(98)90298-5
7. C.N. Kraft, M. Hansis, S. Arens, M.D. Menger and B. Vollmar, *J. Biomed. Mater. Res.*, **49**, 192 (2000); https://doi.org/10.1002/(SICI)1097-4636(200002)49:2<192::AID-JBM6>3.0.CO;2-C
8. D.J. Stickler, *Curr. Opin. Infect. Dis.*, **13**, 389 (2000); https://doi.org/10.1097/00001432-200008000-00011
9. S. Subramanyam, A. Yurkovetsiky, D. Hale and S.P. Sawan, *J. Endourol.*, **14**, 43 (2000); https://doi.org/10.1089/end.2000.14.43
10. M.A. Ruggiero, P. Magan, T.E. Robitaille, R.P. Roth and J.J. Irovando, Biocide Plus Aurfactant for Protecting Carpets, US Patent US 1211487 (1998).
11. H. Nakashima, I. Matsunaga, N. Miyano and M. Kitagawa, *J. Health Sci.*, **46**, 447 (2000); https://doi.org/10.1248/jhs.46.447
12. G.C. Mixon, Antimicrobial Gloves and a Method of Manufacture Thereof, US Patent US 8782755 (1998).
13. G.M. Buccellato, R.R. Robideau, K.M. Hoffmann and G.F. Jacobs, Addition of Antimicrobial Agents to Pavement Marking Materials, US Patent US 1209321 (1998).
14. S.B. Levy, *Emerg. Infect. Dis.*, **7**, 512 (2001); https://doi.org/10.3201/eid0707.017705
15. Silver News, Germ-killing Silver Showing up in Hundreds of Everyday Products: Japanese Leading the Charge for Silver Based Hygienic Products, April/May:1–2 (1997).
16. A.G.M. Scannell, C. Hill, R.P. Ross, S. Marx, W. Hartmeier and E.K. Arendt, *Int. J. Food Microbiol.*, **60**, 241 (2000); https://doi.org/10.1016/S0168-1605(00)00314-7
17. L. Vermeiren, F. Devlieghere and J. Debevere, *Food Addit. Contam.*, **19(sup1)**, 163 (2002); https://doi.org/10.1080/02652030110104852
18. CDC, *MMWR Morb. Mortal. Wkly. Rep.*, **50**, 241 (2001).
19. L.D. Stetzenbach, *Microbiologica*, **25**, 51 (1998).
20. R.B. Thurman, C.P. Gerba and G. Bitton, *CRC Crit. Rev. Environ. Contam.*, **18**, 295 (1989); https://doi.org/10.1080/10643388909388351
21. S. Demirci, Z. Ustaoglu, G.A. Yilmazer, F. Sahin and N. Bac, *Appl. Biochem. Biotechnol.*, **172**, 1652 (2014); https://doi.org/10.1007/s12010-013-0647-7
22. Y. Inoue, M. Hoshino, H. Takahashi, T. Noguchi, T. Murata, Y. Kanzaki, H. Hamashima and M. Sasatsu, *J. Inorg. Biochem.*, **92**, 37 (2002); https://doi.org/10.1016/S0162-0134(02)00489-0
23. A. Top and S. Ulku, *Appl. Clay Sci.*, **27**, 13 (2004); https://doi.org/10.1016/j.clay.2003.12.002
24. B. Kwakye-Awuah, C. Williams, M.A. Kenward and I. Radecka, *J. Appl. Microbiol.*, **104**, 1516 (2008); https://doi.org/10.1111/j.1365-2672.2007.03673.x
25. P. Lalueza, M. Monzon, M. Arruebo and J. Santamaria, *Chem. Commun.*, **47**, 680 (2011); https://doi.org/10.1039/C0CC03905E
26. R.R. Padhy, R. Shaw, S. Tiwari and S.K. Tiwari, *J. Porous Mater.*, **22**, 1483 (2015); https://doi.org/10.1007/s10934-015-0029-3
27. L. Tosheva, A. Brockbank, B. Mihailova, J. Sutula, J. Ludwig, H. Potgieter and J. Verran, *J. Mater. Chem.*, **22**, 16897 (2012); https://doi.org/10.1039/c2jm33180b
28. Q.L. Feng, J. Wu, G.Q. Chen, F.Z. Cui, T.N. Kim and J.O. Kim, *J. Biomed. Mater. Res.*, **52**, 662 (2000); https://doi.org/10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3
29. Y. Matsumura, K. Yoshikata, S. Kunisaki and T. Tsuchido, *Appl. Environ. Microbiol.*, **69**, 4278 (2003); https://doi.org/10.1128/AEM.69.7.4278-4281.2003
30. I. Sondi and B. Salopek-Sondi, *J. Colloid Interface Sci.*, **275**, 177 (2004); https://doi.org/10.1016/j.jcis.2004.02.012
31. H.G. Schlegel, General Microbiology, University Press: Cambridge, edn 7, pp. 271-272 (1986).
32. M.M. Naik, H.S.B. Naik, G. Nagaraju, M. Vinuth, K. Vinu and S.K. Rashmi, *J. Mater. Sci. Mater. Electron.*, **29**, 20395 (2018); https://doi.org/10.1007/s10854-018-0174-y
33. H.J. Park, J.Y. Kim, J. Kim, J.H. Lee, J.S. Hahn, M.B. Gu and J. Yoon, *Water Res.*, **43**, 1027 (2009); https://doi.org/10.1016/j.watres.2008.12.002
34. K. Karthik, S. Dhanuskodi, S.P. Kumar, S. Sivaramakrishnan and C. Gopinath, *Mater. Lett.*, **206**, 217 (2017); https://doi.org/10.1016/j.matlet.2017.07.004
35. L. Tosheva, A. Brockbank, B. Mihailova, J. Sutula, J. Ludwig, H. Potgieter and J. Verran, *J. Mater. Chem.*, **22**, 16897 (2012); https://doi.org/10.1039/c2jm33180b