

Bacterial growth on a superhydrophobic surface containing silver nanoparticles

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Abstract. The antibacterial effect of silver can be exploited in the food and beverage industry and medicinal applications to reduce biofouling of surfaces. Very small amount of silver ions are enough to destructively affect the metabolism of bacteria. Moreover, superhydrophobic properties could reduce bacterial adhesion to the surface. In this study we fabricated superhydrophobic surfaces that contained nanosized silver particles. The superhydrophobic surfaces were manufactured onto stainless steel as combination of ceramic nanotopography and hydrophobication by fluorosilane. Silver nanoparticles were precipitated onto the surface by a chemical method. The dissolution of silver from the surface was tested in an aqueous environment under pH values of 1, 3, 5, 7, 9, 11 and 13. The pH value was adjusted with nitric acid and ammonia. It was found that dissolution rate of silver increased as the pH of the solution altered from the pH of de-ionized water to lower and higher pH values but dissolution occurred also in de-ionized water. The antimicrobial potential of this coating was investigated using bacterial strains isolated from the brewery equipment surfaces. The results showed that the number of bacteria adhering onto steel surface was significantly reduced (88 %) on the superhydrophobic silver containing coating.

1. Introduction

Nanosized silver has proved its efficiency as antibacterial material. Even low concentrations of silver ions (Ag^+) have a significant effect on bacterial growth on surfaces. Due to the negative impact of antibiotics to the environment and the restriction of law, the applications of silver have attracted more attention in research and development [1]. Antimicrobial activity of silver is exploited to reduce infections and prevent bacteria colonization in various applications, for instance dental materials, prostheses, stainless steel materials and textile fabrics. Silver can be used for water treatment and in medicine in burn treatment and for several non-medical applications such as antimicrobial protection of toilet seats, children's toys, telephone receivers and sports fabrics [2–6].

Superhydrophobic surface has a high water contact angle and a low roll-off angle. Water forms pearl-like drop on a superhydrophobic surface and rolls off easily when the surface is tilted.

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Superhydrophobic surface requires high surface roughness and low surface energy. When a water droplet rolls on a superhydrophobic surface, it removes dust and dirt particles and the surface is cleaned. This property is known as self-cleaning effect or 'lotus effect'. In some cases superhydrophobicity seems to weaken bacterial attachment to surface which can be exploited in applications that require bacteria free environment. Together superhydrophobic surface and silver added on the surface could form a coating that is highly antibacterial and easy to clean [7,8].

2. Experimental

2.1. Preparation of the surfaces

Superhydrophobic surfaces were prepared using similar method as Zhang et al. have used in their work [9,10]. At first 3 g aluminium tri-sec-butoxide ($C_{12}H_{27}AlO_3 > 97\%$, VWR) and 30 ml isopropyl alcohol ($C_3H_7OH > 99\%$, VWR) were mixed and stirred at ambient conditions for 1 h. After that 2 ml ethyl acetoacetate ($C_6H_{10}O_3 > 98\%$, VWR) was added and the solution was stirred for 1 h. Then the mixture of 1 ml de-ionized water and 5 ml isopropyl alcohol was introduced for the hydrolysis. After that the solution was stirred for 2 h and then coated on the AISI 304 type austenitic stainless steel plates. Before coating the substrates were rinsed first with acetone, then ultrasonicated in ethanol and water and finally wiped with isopropyl alcohol. After the cleaning, spin coating was carried out at 1500 rpm for 20 s. The samples were dried for 10 minutes in air and then heat treated at 400 °C for 15 min after which they were immersed into boiling water for 10 min and dried in air followed by heat-treatment at 600 °C for 30 min to obtain γ -alumina phase structure.

Silver particles were added on the surface by Tollens process. Firstly, 1 % silver nitrate solution was prepared and 1 ml sodium hydroxide (NaOH, 65 %) was added and silver oxide precipitation was formed. Then ammonia (NH_3 , 34 %) was added to form diamminesilver complex ions until the precipitation solved and the solution was clear. Finally, 1 g glucose dissolved into 10 ml de-ionized water was added to reduce the diamminesilver complex ions to metallic silver. The as-prepared solution was spin-coated on the prepared flaky γ -alumina surface and heat-treated at 80 °C for 15 min to ensure the reduction of silver.

The surface of the sample was modified by (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane ($CF_3(CF_2)_7CH_2CH_2Si(OCH_3)_3$), denoted as FAS, ABCR GmbH & Co. KG, Karlsruhe, Germany) to obtain low surface energy. A mixture of 1 ml FAS and 50 ml ethanol (EtOH) was stirred for 1 h at room temperature. Finally, the samples were immersed into the solution for 1 h and then heat-treated at 180 °C for another 1 h for the condensation reaction between the surface and FAS molecules.

2.2. Characterization of the superhydrophobic silver containing coatings

Superhydrophobic coatings were characterized with a high resolution field emission scanning electron microscope (FESEM ZeissULTRAplus). Energy dispersive spectroscopy (EDS, INCA Energy 350) analysis was used to determine the existence of silver particles on the surface.

2.3. Measurement of dissolved silver from superhydrophobic silver containing coatings

Solutions with pH values of 1, 3, 5, 7, 9, 11 and 13 were prepared and superhydrophobic silver containing coatings were immersed into the solutions. The pH values were adjusted using nitric acid and ammonia. Closed plastic containers were used to prevent vaporization of the solutions. Dissolution of silver was measured with atomic absorption spectrophotometer (Philips PU 9200X) during eight weeks.

2.4. Antibacterial activity of the coating

Antibacterial and antiadhesive properties were examined with a method based on cultivation. Samples were placed on a Petri dish and bacterial species were introduced to the samples. A biofilm was formed and it was then rinsed. The bacteria were removed by ultrasonication and vortexing. The

solution with bacteria was diluted and cultivated to determine the amount of viable bacteria. The used bacterial strains were *Lactobacillus paracasei*, *Serratia marcescens* and *Pseudomonas fluorescens*.

2.5. Change in functionality and morphology of the coating after exposure to solutions with different pH values

Morphology changes on the surfaces that were immersed into solutions with different pH values were characterized with field emission scanning electron microscope (FESEM Zeiss ULTRApplus). Functionality of the surface was studied by measuring water contact angles. The water contact angle measuring system is composed of an optical system that is connected to a computer. A water droplet of 5 μ l was released to the front of the camera from a microliter scale pipette and the water contact angle was measured with the computer. The water contact angle was measured from five different positions and the average of the five measurements was calculated.

3. Results and discussion

3.1. Morphology of the superhydrophobic silver containing coatings

Microstructure of the superhydrophobic silver containing surface is shown in Figure 1a. In Figure 1 silver particles appear as white agglomerated spheres of approximately 200 nm. Figure 1b presents a water droplet on the superhydrophobic silver containing surface. The water contact angle of the surface was measured to be 154 $^{\circ}$.

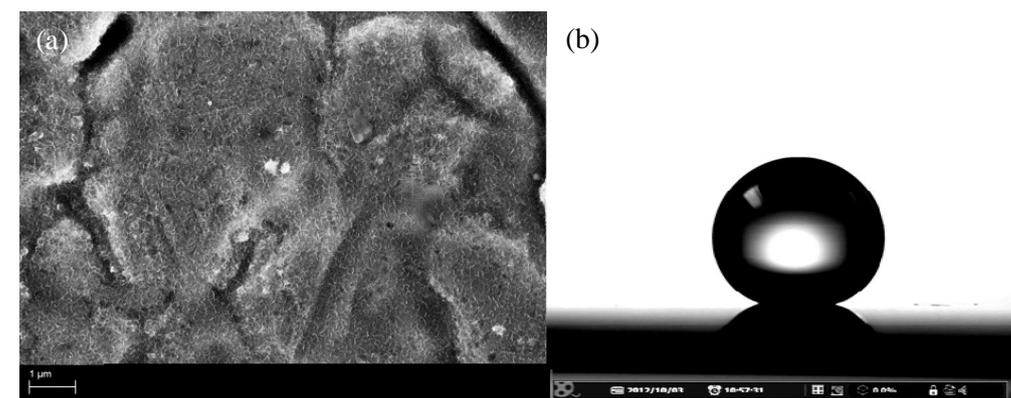


Figure 1. (a) Microstructure of the superhydrophobic silver containing surface (b) Water droplet on superhydrophobic silver containing surface.

3.2. Dissolution of silver from superhydrophobic coatings

Figure 2 presents the concentration of dissolved silver in the solutions into which the superhydrophobic silver containing samples were immersed. Silver dissolved in all tested environments during eight weeks. The rate of the dissolution was highest with low pH values partly due to the degradation of the coating in nitric acid. Lowest dissolution rates were detected near neutral pH values.

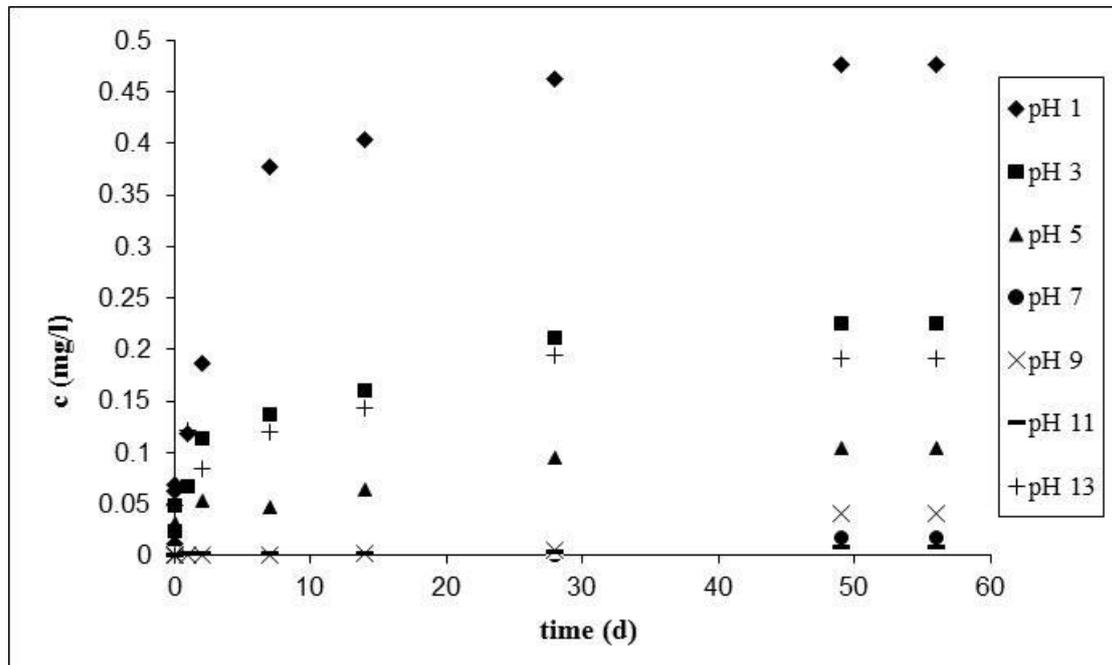


Figure 2. Concentration of silver ions dissolved from superhydrophobic coatings in solutions with different pH values.

3.3. Antibacterial activity of superhydrophobic silver containing plates

In the antibacterial activity test 88 % of the bacteria that were adhered on the superhydrophobic silver containing coating were killed. Figure 3 shows the bacterial cell count (cm^{-2}) of biofilm formed on superhydrophobic coating with silver particles and AISI 304 stainless steel reference plate.

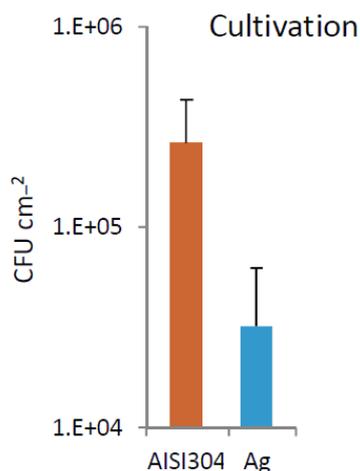


Figure 3. Bacterial cell count (cm^{-2}) of biofilm formed on superhydrophobic coating with and without silver and AISI 304 stainless steel reference plate.

3.4. Chemical durability of the superhydrophobic silver containing plates

Superhydrophobic silver containing coatings were imaged with field emission scanning electron microscope after being immersed to solutions with different pH values. Figure 4 illustrates surfaces after 1, 5 and 8 weeks of exposure to the solutions of pH 1 and 13.

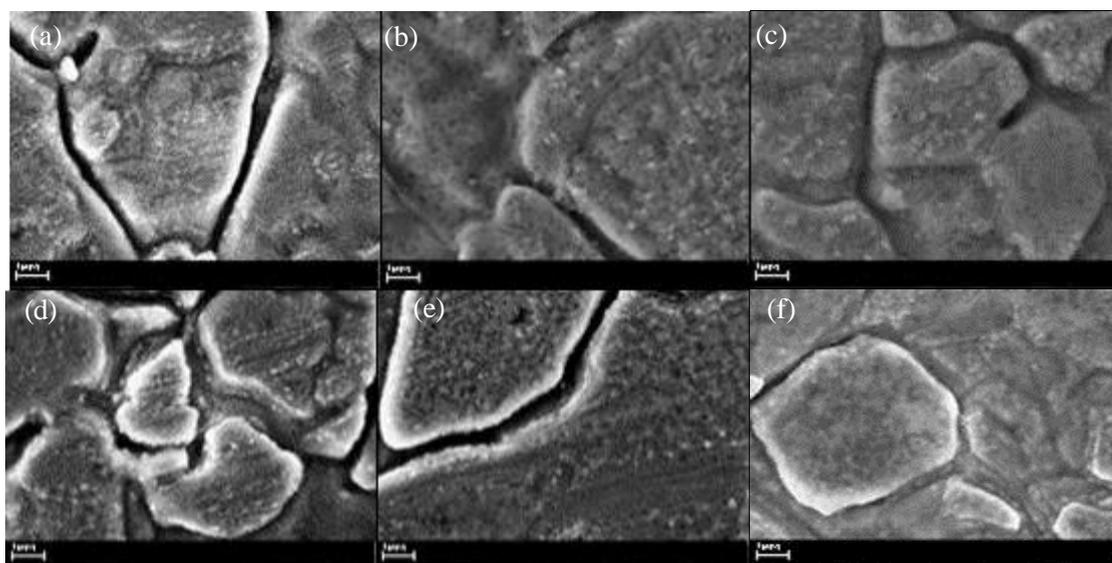


Figure 4. Superhydrophobic silver containing surfaces exposed to nitric acid solution with pH of 1 after (a) 1, (b) 5 and (c) 8 weeks of exposure. Superhydrophobic silver containing surfaces exposed to ammonia solution of pH 13 after (d) 1, (e) 5 and (f) 8 weeks of exposure.

The coating had detached already after 5 weeks of exposure when pH of the solution was 1. No silver could be detected by EDS analysis or from the FESEM images of the surface. Instead, in the solution with a pH of 13 only minor changes in morphology could be observed after 5 weeks of exposure and silver was detected by the EDS analysis. However, after 8 weeks exposure to the solution with a pH of 13 the coating was severely damaged.

In pH 3, 5, 7, 9 and 11 only minor changes in morphology after 8 weeks of exposure could be detected in FESEM images and silver particles were detected from all of the coatings by EDS analysis. Changes in functionality of the coating were investigated by measuring water contact angle of the surface after exposure to the solutions with different pH values. Water contact angles after 8 weeks are listed in table 1. The results show that at pH of 7, 9 and 11 superhydrophobic property has preserved after 8 weeks exposure. Instead, at pH 1 and 13 functionality is lost as it can be predicted from the FESEM images.

Table 1. Water contact angle of superhydrophobic silver containing surfaces after 8 weeks of exposure to solutions with different pH values.

pH	Water contact angle after 8 weeks exposure [°]
1	106
3	123
5	131
7	142
9	143
11	145
13	98

4. Conclusions

The results show that silver dissolves from superhydrophobic coatings at some extent in all aqueous solutions regardless of the pH of the solution. Dissolution rate is at its peak with high and low pH values but silver dissolves also in de-ionized water. The solubility of silver was highest in nitric acid partly due to the high rate of degradation of the coating in acidic environment.

The chemical durability of the coatings was relatively poor in acidic and alkaline solutions. In severe conditions the changes in functionality and morphology of the surface could be seen after one week of exposure to the solutions. In de-ionized water the surface preserved functionality and only minor changes in morphology could be seen after eight weeks exposure to de-ionized water.

Antibacterial test revealed that the superhydrophobic silver containing coating prevents bacterial growth and majority of the bacteria of the tested strains on the coating is killed. The surface was efficient against both gram-positive and gram-negative bacteria.

The results of this study were promising but more study concerning the durability of the coating and the effect of the coating on different bacterial strains is needed to be done. In the future study the adhesion of the bacteria to the surface will be investigated. The study aims also to enhanced chemical and mechanical durability of the coating.

References

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