





Antibacterial properties of photochemically prepared AgTiO₂ membranes

Azzah Nazihah Che Abdul Rahim ^a, Hisashi Hoshida ^a, Sergio Mestre ^b and Izumi Kumakiri ^{a,*}

^a Graduate School of Sciences and Technology for Innovation, Faculty of Engineering, Yamaguchi University, 2-16-1 Tokiwadai, Ube, 755-8611, Japan

^b University Institute of Ceramic Technology/Chemical Engineering Department, Universitat Jaume I, Avda. Vicent Sos Baynat, 12071, Castellón, Spain

*Corresponding author. E-mail: izumi.k@yamaguchi-u.ac.jp

 ANCAR, 0000-0002-7847-6776; HH, 0000-0003-2613-232X; SM, 0000-0002-7799-8927; IK, 0000-0001-5804-5277

ABSTRACT

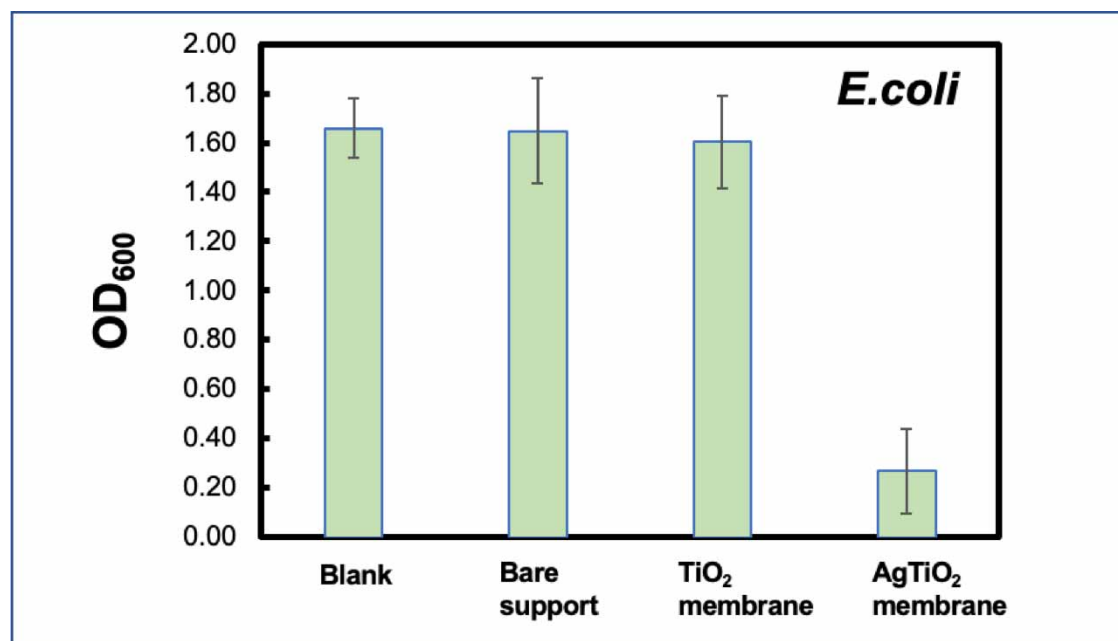
Biofouling reduces the membrane performance and has become a problem in many applications. One of the strategies to reduce biofouling is to apply antibacterial materials to the membrane surface, which prevents the attachment and growth of microorganisms. In this study, the surface of flat ceramic supports was covered with TiO₂ powder, and silver was applied by photoreduction using a CH₃COOAg solution at room temperature. After the photoreduction, AgO_x and metallic silver were found on the TiO₂ as analyzed by XPS. While a negligible amount of silver was released from the prepared AgTiO₂ membranes into water, the dissolution of silver was enhanced in a 0.09 M NaCl solution. The AgTiO₂ membranes inhibited the growth of *Escherichia coli* in dark conditions. The inhibition cannot be explained only by the concentration of silver ions released from the membranes. Microscopic observation showed that direct contact with AgTiO₂ kills *E. coli*. The results showed the possibility of improving the antibacterial activity of membranes by applying an AgTiO₂ coating.

Key words: AgTiO₂, antibacterial activity, *Escherichia coli*, immobilization, photoreduction

HIGHLIGHTS

- AgTiO₂ membranes were prepared by photoreduction using the CH₃COOAg solution.
- Silver oxide was formed by photoreduction with a light intensity of 3.3 mW/cm².
- Silver leaching from the AgTiO₂ membranes was negligible in water but enhanced in 0.09 M NaCl solution.
- AgTiO₂ membranes inhibited the growth of *E. coli* under dark conditions.
- The results showed potential antibacterial activity of silver oxide.

GRAPHICAL ABSTRACT



INTRODUCTION

Clean water and renewable energy are essential but ensuring access to these resources is one of the major challenges of today. Membrane separation processes are regarded as a promising option for a sustainable water production, which has a simple configuration and enables treating wastewater with low or no chemical additives (Obotey Ezugbe & Rathilal 2020). Membrane technology can also be used in generating electricity by a forward osmosis process, where semi-permeable membranes separate seawater and brackish water (Bogler *et al.* 2017). While various types of membranes have been intensively studied and developed, one of the challenges is the biofouling caused by the growth of microorganisms under practical conditions that reduces the membrane performance (Nguyen *et al.* 2012; Bogler *et al.* 2017).

One way to prevent the growth of microorganisms is to apply disinfectants such as chlorine and bromine gas. However, they react with substances present in water and simultaneously produces harmful, carcinogenic disinfection byproducts (Pandian *et al.* 2022). Chemical cleaning can be applied to recover the membrane performance. The major drawbacks of chemical cleaning are the creation of wastewater formed by the treatment and the increase in the operational cost (Bogler *et al.* 2017).

Applying coatings with antibacterial activity to the membrane surfaces is another approach to prevent the biofouling. Incorporating silver nanoparticles having antibacterial property into membranes is one example that reduces membrane biofouling by inhibiting bacterial growth. The most common method to immobilize silver on membranes is mixing silver nanoparticles into a polymeric matrix (Zodrow *et al.* 2009). For example, Jeon and Lee incorporated silver nanoparticles into polymeric membranes via interfacial polymerization and reported an enhanced anti-biofouling performance (Jeon & Lee 2020). One of the challenges of this method is the poor compatibility between silver particle and polymer matrix that reduces the stability of the membrane. To overcome this problem, Tan *et al.* anchored silver in metal organic framework (Ag-MOF) and mixed the Ag-MOF nanoparticles in a polyamide layer (Tan *et al.* 2022). They reported outstanding antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*.

Silver and silver composite materials are also immobilized on inorganic membranes by various methods. For example, Li *et al.* prepared Ag nanosheets and mixed them into titania sol to fabricate membranes by a sol-gel method. They reported increased antibacterial activity (Li *et al.* 2020). Fan *et al.* prepared carbon nanotubes modified with silver nanoparticles and immobilized them on ceramic membranes by vacuum filtration followed by annealing. They also reported enhanced anti-fouling and antimicrobial properties (Fan *et al.* 2019).

Silver nanoparticles can be deposited on membranes by reducing silver salts, such as silver nitrate (AgNO_3) (Guin *et al.* 2007) and silver acetate (CH_3COOAg) (Wodka *et al.* 2010). Among various reducing methods, photoreduction is attractive because it does not require additional reducing chemicals (Iravani *et al.* 2014). In photoreduction, electron-hole pairs are generated on the TiO_2 membrane surface by UV irradiation. The formed electrons reduce silver ion and deposit silver on the surface as nanoparticles (Naoi *et al.* 2004). The photochemically deposited silver can be in a metallic state (Wodka *et al.* 2010) or in a silver oxide state (Li *et al.* 2015). The state can affect its antibacterial activity and stability in different media. For example, Ag_2O is known to dissolve into media containing chloride ion faster than metallic silver (Levard *et al.* 2013).

In this study, AgTiO_2 membranes were synthesized by a photochemical deposition of silver on TiO_2 membranes using a silver acetate solution (Kumakiri *et al.* 2022). The leaching of silver from the membrane in water and in a NaCl solution was studied. Antibacterial activity of the membranes in dark conditions was also studied using *E. coli* as an example of microorganisms.

METHODS

Preparation of AgTiO_2 nanocomposite powder

Silver was deposited photochemically on commercial TiO_2 powder (P25, Evonik Industries AG, Germany) (Wodka *et al.* 2010). Silver acetate solution (CH_3COOAg , purity 97%, Wako Pure Chemical Industries, Ltd, Japan) was used as a silver source. First, P25 powder (150 mg) was dispersed in 5×10^{-5} or 1×10^{-3} mol/L silver acetate solution (30 g) with stirring at 450 rpm. Then UV light was applied with 3.3 mW/cm^2 intensity at room temperature for 1 h. Black lamps (TBL-08/5N, Toshiba Co., Japan) were used as the light source. Details of the experimental setup can be found elsewhere (Che Abdul Rahim *et al.* 2022). The concentration of silver in the solution before and after applying light irradiation was measured by Inductively Coupled Plasma (ICP, SII Nano Technology Co. Ltd, Tokyo, Japan). The amount of silver deposition on the TiO_2 powder was calculated from the concentration change measured by ICP and the mass of solution and powder used. The size and distribution of silver on TiO_2 were analyzed by TEM (JSM-7600F JEOL Ltd, Tokyo, Japan). The state of silver was analyzed by Thermo Scientific K-Alpha X-Ray Photoelectron Spectroscopy (XPS) using $\text{AlK}\alpha$ radiation of 1,486.6 eV. Charge correction and peak fitting were performed according to C1s binding energy peak of 284.8 eV using Thermo Scientific Avantage software.

Preparation of AgTiO_2 membranes

Porous disk-shaped supports with a diameter of 47 mm were prepared using kaolin (ER, Caobar S.A. Spain), alumina (AR12B5, Aluminium Pechiney, France), and potato starch (Sigma Aldrich Inc. USA), with polyvinyl alcohol (PVA, Mowiol 4-88, Sigma Aldrich Inc., USA) as ligand and sintered at 1,673 K for 4 h (Che Abdul Rahim *et al.* 2022). TiO_2 powder was mechanically deposited on the supports followed by heating at 637 K for 3 h (Kumakiri *et al.* 2011). Then, silver was photochemically deposited as described above using 5×10^{-5} and $1 \mu\text{mol}$ CH_3COOAg solutions. The AgTiO_2 membranes obtained after the photoreduction were named as low Ag and high Ag depending on the concentration of the silver acetate solution. The amount of silver deposited on a TiO_2 membrane was estimated from the decrease of silver concentration in the solution measured by ICP and the mass of the solution used for the photoreduction. After the photoreduction, membranes were washed with water and dried at room temperature.

Silver dissolution study

AgTiO_2 membranes were immersed in 40 g of water or 0.09 M sodium chloride aqueous solution (NaCl , purity 99.5%, Wako Pure Chemical Industries, Ltd, Japan) at room temperature. All the experiments were carried out in dark with magnetically stirred at 450 rpm. After immersing a membrane for a predetermined duration, the membrane was removed from the solution and the silver concentration in the solution was measured by ICP. The removed membrane was washed with water and immersed into a new solution. Membranes were soaked in solutions for different periods with no particular order. For these dissolution study, two types of AgTiO_2 membranes having different amount of silver deposition, low-Ag and high-Ag membranes, were used as described in the preparation section. A few membranes were prepared under the same conditions and used to check the reproducibility. In parallel, porous disks were soaked into a 5×10^{-5} mol/L CH_3COOAg solution and dried to deposit CH_3COOAg . These samples were named as 'Support-Ag'.

Antibacterial study

For the antibacterial study, the porous disks were cut into smaller pieces before the deposition of AgTiO₂. The surface area of membranes was $0.52 \pm 20\%$ cm² measured by ImageJ software (National Institutes of Health (NIH), United States). About 3–4 times higher amount of silver was deposited for this study compared to the membranes used in the dissolution study. The membranes used for the antibacterial study were labeled as AB. All membranes were autoclaved before the antibacterial tests. Besides fresh membranes prepared as above, a few prepared AgTiO_{2-low Ag} membranes were soaked in a 0.09 M NaCl solution for over 50 h in incubator at 37 °C. During this soaking period, the soluble part of silver deposited onto the membrane will be dissolved into the solution as silver ion. Then, the membranes were dried in a vacuum oven at 120 °C for 20 min and kept in a nitrogen atmosphere to avoid a formation of an oxidized layer before the antibacterial study. These treated membranes were named as ‘Treated AgTiO_{2-low Ag} (AB)’.

An *E. coli* strain DH5 α was maintained on Luria–Bertani (LB) agar plate. The bacterial cells were inoculated in LB medium made using 0.5% yeast extract, 1% peptone (Kyukuto, Japan), 0.5% (0.09 M) NaCl (Sigma Aldrich Inc., USA) and incubated at 37 °C overnight (around 21 h) with agitation at 150 rpm. One milliliter LB medium was put into the wells of a 24-well plate, and membrane samples were soaked in the medium. The growth of *E. coli* was analyzed by the optical density at 600 nm (OD₆₀₀) wavelength using a spectrophotometer (Genesys 10uv, ThermoFisher, Tokyo). Ten microliters of pre-culture (OD₆₀₀ of pre-cultures were 3–4) were inoculated. After around 21 h incubation at 37 °C, the culture was diluted and OD₆₀₀ was measured. 5×10^{-3} mol/L of L-ascorbic acid (Vitamin C, C₆H₈O₆, purity 99.6%, FUJIFILM Wako Pure Chemical Corporation, Japan) was added to some of the media. The tests with Vitamin C were indicated with ‘+ VC’.

In another tests, CH₃COOAg and sodium acetate (CH₃COONa·3H₂O, purity 99%, FUJIFILM Wako Pure Chemical Corporation, Japan) solutions were added to the LB medium at a final concentration of 0 to 5×10^{-5} mol/L, then *E. coli* cells were missed and the solutions were kept at 37 °C for overnight (around 21 h). After the incubation, OD₆₀₀ values of the solutions were measured.

AgTiO₂ particles were deposited on glass plates to perform microscopic observation. Glass plates without any particles were also used as a blank. DH5 α cells transformed with a red-fluorescence protein (RFP) expression plasmid (Nakamura *et al.* 2018) was cultured in LB medium as described above. The cells were washed with sterilized water and suspended in water containing 0.5 mM Sytox Green. Water-dispersed *E. coli* cells were dripped onto the plates, and the plates were kept for 26 h at room temperature. An Axio Imager A1 fluorescence microscope (Carl Zeiss, Jena, Germany) equipped with the filter set 38HE and 43HE was used to observe green and red fluorescence.

RESULTS AND DISCUSSION

AgTiO₂ nanocomposites and membranes

The concentration of silver ion in the starting silver acetate solution decreased more than 85% after the photoreduction as a result of the silver deposition on TiO₂. Changing the starting concentration from 5×10^{-5} and 1×10^{-3} mol/L resulted in about 9.8 and 170×10^{-8} mol/cm² silver deposition on the TiO₂ membranes, respectively. The color of a TiO₂ membrane changed from white to brownish after the photoreduction when a CH₃COOAg solution of lower concentration was used. The membrane color changed to dark purple color with higher CH₃COOAg concentration. As a comparison to the photoreduction method, porous supports were soaked into the CH₃COOAg solution and dried without rinsing with water. These samples were named as ‘Support-Ag’. The silver concentration in the solution decreased about 35% by the soaking. Different from membranes prepared by photoreduction, the color of these supports did not change and kept white, for silver acetate powder, which is white in color, deposited on the support surface.

Figure 1 shows TEM image of the AgTiO₂ powder prepared by the photoreduction method. Spherical silver particle of 1–2 nm was found (red arrow) on TiO₂ crystalline structure (Figure 1(a)). Larger number of particles were found when a silver acetate solution of 20 times higher concentration than Figure 1(a) was used (Figure 1(b)). Figure 1(c) and 1(d) shows elemental mapping (EDS-TEM) of titanium and silver. The EDS images of silver show that both the size and the size distribution of silver particles got larger with higher silver precursor concentration.

Figure 2 shows XPS results of AgTiO₂ powders prepared with solutions of two different Ag concentrations. Figure 2(a) shows the survey scan. The existence of silver was more apparent in the AgTiO_{2-high Ag} sample, because the amount of silver was over 10 times compared to the AgTiO_{2-low Ag} sample. Figure 2(b) shows O1s core-level spectrum with the main peak at 529.6 eV showing lattice O²⁻ present in many metal oxides (Le *et al.* 2018), which is related to TiO₂ lattice and

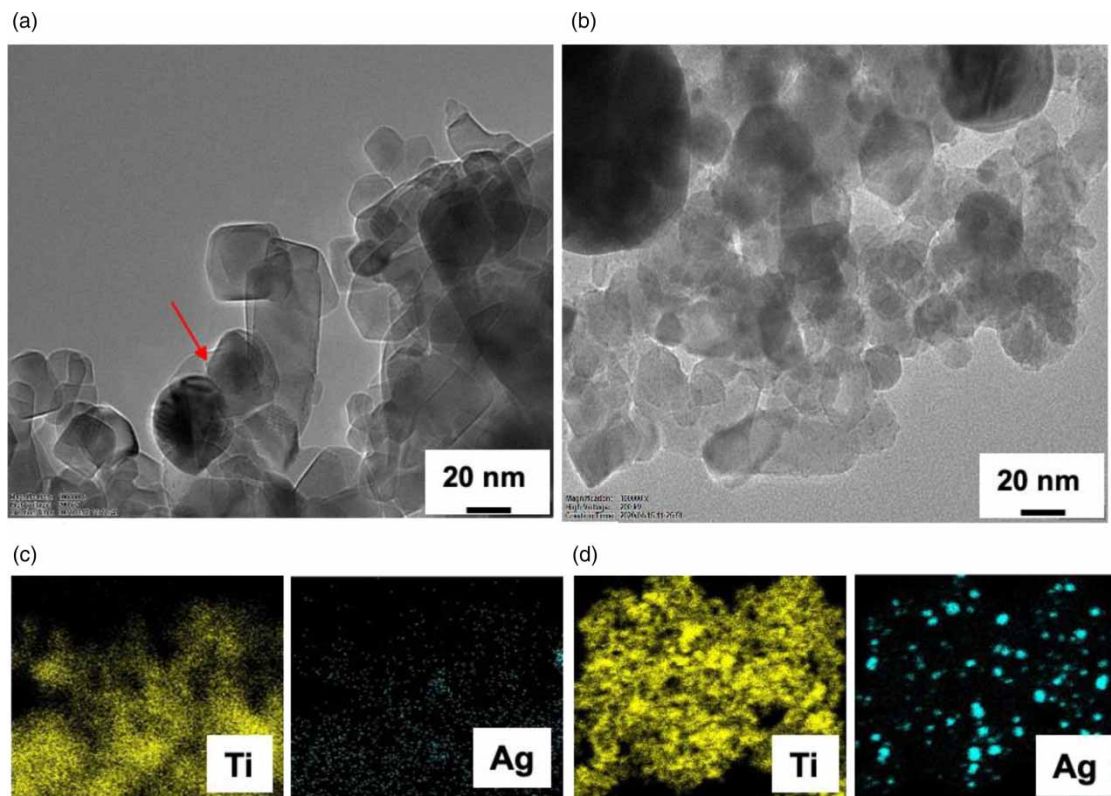


Figure 1 | TEM image (Magnification: 100k \times) of AgTiO₂ nanocomposites: (a) AgTiO_{2-low} Ag and (b) AgTiO_{2-high} Ag; and elemental mapping using EDS-TEM of (c) AgTiO_{2-low} Ag and (d) AgTiO_{2-high} Ag nanocomposites. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wst.2023.003>.

silver oxides. Fitting the peak with a nonlinear least-squares fit program using the Gauss-Lorentzian equation, shows appearance of a smaller peak at around 530–531 eV which represents hydroxyl groups related to the adsorbed H₂O on the surface (Mohanty *et al.* 2012). Figure 2(c) shows the Ti2p main peak located at 458.4 eV (Ti2p_{3/2}) and another peak at 464.1 (Ti2p_{1/2}), which correspond to Ti⁴⁺ from TiO₂ (Liang *et al.* 2011). Silver photo deposition did not affect the TiO₂ as reported earlier (Wodka *et al.* 2010).

Figure 2(d) shows Ag3d scan. Peak fitting performed on AgTiO_{2-high} Ag suggested the existence of silver in three different states. The main peaks obtained with the AgTiO_{2-high} Ag sample at 367.3 and 373.3 eV can be assigned to 3d_{5/2} and 3d_{3/2} of Ag⁺, respectively. These peaks suggest the existence of Ag₂O (Liang *et al.* 2011; Li *et al.* 2015). Small peaks related to metallic silver (Ag⁰) and AgO (Ag²⁺) were also found at 368.16 and 366.74 eV, respectively (Liang *et al.* 2011; Li *et al.* 2015). Wodka *et al.* reported the formation of Ag⁰ (Wodka *et al.* 2010) as the main Ag state from photoreduction of CH₃COOAg on TiO₂ nanoparticles. They used a high-pressure xenon arc lamp of 250 W. On the contrary, Li *et al.* (2015) reported a formation of Ag₂O. They used AgNO₃ as a precursor of silver and performed photoreduction under 20-W UV light at a 10 cm distance for 10 min. In this study, photoreduction was performed using three 8W UVA blacklights from a 5.5 cm distance, which resulted in 3.3 mW/cm² light intensity. Weaker UV irradiation may cause the deposition of silver oxide instead of silver metal. Oxidation of metallic silver in the air (Viet *et al.* 2018) can be another possibility of the Ag₂O formation.

Silver dissolution study

Figure 3 shows the degree of silver leaching from AgTiO₂ membranes in water and in a NaCl solution. Results obtained with three AgTiO_{2-high} Ag membranes are shown in the figure with blue diamond-shaped markers. In case of AgTiO_{2-low} Ag membranes, results with two samples were shown with square markers. Smaller markers show the results of repeated experiments using the same membrane.

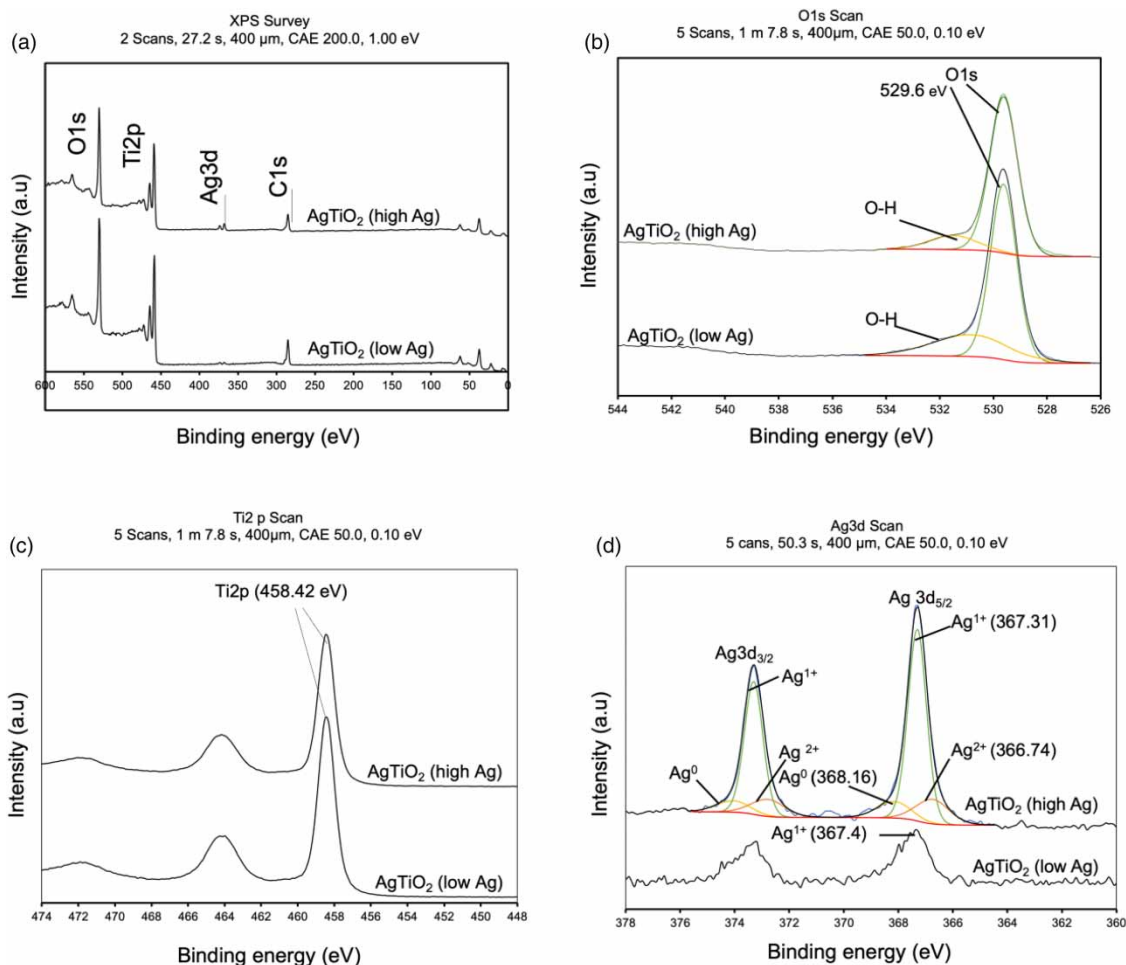


Figure 2 | XPS analysis of AgTiO₂ nanocomposites: (a) AgTiO_{2-high Ag} wide scan, (b) AgTiO_{2-high Ag} O1s peak, (c) AgTiO_{2-high Ag} Ti2p, and (d) Ag3d peaks of AgTiO_{2-high Ag} and AgTiO_{2-low Ag} with doublet fitting.

Without any salt, the amount of silver dissolved from AgTiO_{2-low Ag} membrane was less than 0.03 $\mu\text{mol/L}$. Less than 0.4% of silver on the membrane leached out in 36 h. The results showed a high stability of the membrane in water. This result agrees well with other studies reporting on the low silver dissolution in water. For example, half encapsulated silver nanoparticles in polyamide coated on ceramic membrane showed the total silver leaching stabilized at a concentration of 0.007 $\mu\text{mol/L}$ after 25 h (Peng *et al.* 2020).

Salinity of water is reported to induce the silver leaching (Levard *et al.* 2013). In this study also, the dissolution of silver was enhanced when the membranes were soaked in a 0.09 M NaCl solution. The concentration of silver ion dissolved from AgTiO_{2-low Ag} membrane increased almost linearly in the first 6 h as shown in Figure 3. Then the silver concentration in the solution reached a plateau of 1.5–2.1 $\mu\text{mol/L}$. Differently, membranes with higher silver deposition, AgTiO_{2-high Ag} in the figure, increased quickly within 60 min and reached a stable value of $1.7\text{--}2.3 \times 10^{-6}$ mol/L. The trends of concentration increase were reproducible with a few different membrane samples prepared under the same conditions as shown in the figure.

When the same AgTiO_{2-low Ag} membranes were used for the soaking study repeatedly, the silver dissolution became negligible when the total soaking time exceeded 70 h. This phenomenon occurred after the dissolution of about 21% of deposited silver. The membrane surface was slightly colored even though almost no silver leaching was observed by ICP. This may be explained by the dissolution of silver oxides present at the surface of the membrane as observed by XPS. Metallic silver has a lower dissolution rate (Goderecci *et al.* 2017) and remained on the membrane. Different from AgTiO_{2-low Ag} membranes, the amount of dissolved silver from AgTiO_{2-high Ag} membranes in a NaCl solution during a week was about 4% and the

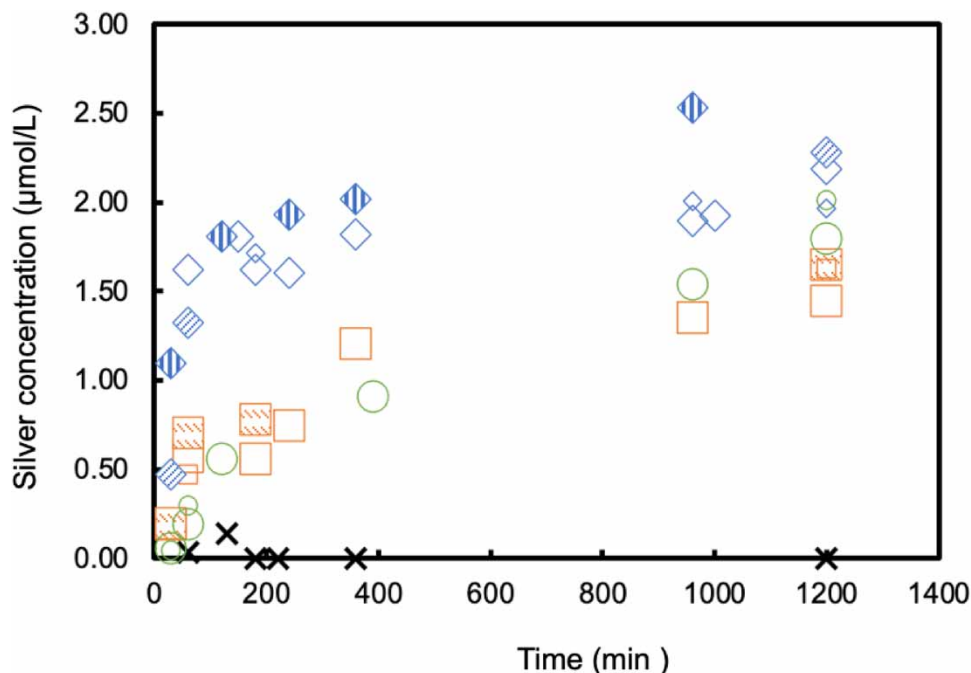


Figure 3 | Silver dissolution study. (+AgTiO₂-low Ag in water, ◇ for AgTiO₂-high Ag, □ for AgTiO₂-low Ag, and ○ for Support-Ag in 0.09 M NaCl. Different patterns show different sets of membranes. Smaller markers show repeated results using the same membrane.) Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wst.2023.003>.

dissolution kinetics did not change after a week of immersion. These results suggest that the mass of Ag₂O increases with increasing the amount of photochemically deposited silver.

In the figure, silver releasing results obtained with the Support-Ag samples prepared by immersing porous supports to the silver acetate solution are also included. The change of silver concentration in the solution was comparable to the AgTiO₂-low Ag membrane within 1,200 min. With these results, silver concentrations in the 0.09 M NaCl solution after soaking the membranes for 1,200 min were almost the same among the membranes prepared under different conditions.

Antibacterial study with membranes

The medium used for the bacterial culture contained 0.09 M NaCl, which concentration is the same as in the dissolution study. Figure 4 shows average results of at least five cultures for Bare support, Support-Ag (AB), TiO₂, AgTiO₂-low Ag (AB), and AgTiO₂-high Ag (AB) and Treated AgTiO₂-low Ag (AB). For Vitamin C studies, the result shows the average of two cultures.

As shown in Figure 4, supports ('Bare support' in the figure), supports coated with silver salt (Support-Ag (AB)), and supports coated with TiO₂ powder (TiO₂) showed negligible effect on the *E. coli* growth. On the contrary, deposition of silver on TiO₂ membranes hindered the growth: about 20% inhibition was observed compared to blank with AgTiO₂-low Ag (AB) samples. A membrane sample with higher silver deposition (AgTiO₂-high Ag (AB)) showed larger inhibition of about 80%.

Silver ions are reported to have antibacterial activity (Durán *et al.* 2016). The Support-Ag (AB) samples released silver ion as shown in Figure 3, therefore these samples were expected to show some growth inhibition. However, no inhibition on the *E. coli* growth was observed. Another interesting observation is the difference between AgTiO₂-low Ag (AB) and AgTiO₂-high Ag (AB) samples. While the amount of silver leaching from these samples approached a similar concentration as shown in Figure 3, higher silver deposition showed much stronger inhibition on the *E. coli* growth. These results suggest that the silver ion released from the membranes into the solution was not the major cause of the *E. coli* growth inhibition under the conditions used in this study.

AgO_x is reported to show stronger antibacterial activity than metallic silver (Rebello *et al.* 2016). To study the influence of AgO_x, fresh AgTiO₂-low Ag (AB) was soaked in 0.09 M NaCl for more than 50 h to remove the silver oxides from the membrane by dissolution. After this treatment, about 26% of the deposited silver on the membranes was dissolved into the solution as measured by ICP. This dissolved fraction of the amount of silver on the membrane was comparable to the soluble

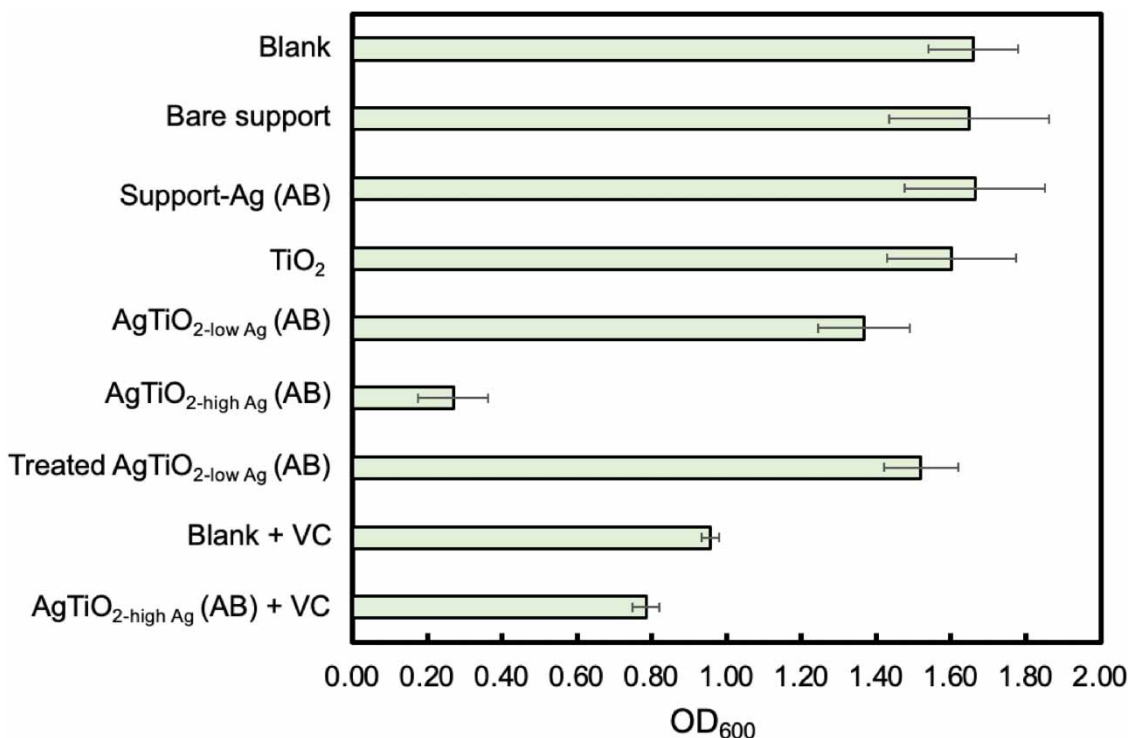


Figure 4 | OD₆₀₀ of *E. coli* by different membranes after overnight incubation.

fraction of silver estimated in the dissolution study, suggesting most of the soluble silver was removed from the membrane. The results obtained with these treated membranes were labeled as ‘Treated AgTiO_{2-low Ag} (AB)’ in Figure 4. The Treated AgTiO_{2-low Ag} (AB) membrane showed less inhibition compared to untreated samples (‘AgTiO_{2-low Ag} (AB)’). The reduced influence on the growth inhibition can be explained by the lower amount of silver oxides on the treated samples.

The influence of adding Vitamin C to the LB media was also studied. The addition reduced pH of the media from 6.1 to 5.1. The growth of *E. coli* (‘Blank + VC’ in the figure) became about half compared to the blank. When AgTiO_{2-high Ag} (AB) membrane samples were added to the media with Vitamin C, the growth was further inhibited (‘AgTiO_{2-high Ag} (AB) + VC’ in the figure). The OD₆₀₀ value was about 40% to the OD₆₀₀ value obtained with the blank test. On the other hand, the inhibition effect decreased significantly: the OD₆₀₀ value obtained with AgTiO_{2-high Ag} (AB) membranes was about 20% to that with blank when no VC was added. These changes may be explained by the decrease of reactive oxygen species formed by Ag that inhibit the growth of *E. coli* as Vitamin C scavenges these reactive oxygen species (Rebello *et al.* 2016). Another possible explanation is the adsorption of negatively charged Vitamin C ion to the TiO₂ surface, which is positively charged in acidic conditions (Verhovšek *et al.* 2012). The Vitamin C ions adsorbed on the TiO₂ surface may have prevented the direct contact between *E. coli* and silver oxide, and resulted in the reduced antibacterial activity.

Influence of silver ions on the *E. coli* growth

The influence of silver ions on the *E. coli* growth was studied by adding CH₃COOAg solution to the media. In order to examine the influence of acetate ions, sodium acetate (CH₃COONa) solution was also used. The inhibition rate on the *E. coli* growth in Figure 5 was calculated using the equation below.

$$\text{Inhibition rate \%} = \left(1 - \frac{OD_{600}}{OD_{600\text{blank}}}\right) \times 100$$

As shown in the figure, increasing the silver acetate concentration increased the inhibition of *E. coli* growth. This concentration range is similar to what was reported as a minimal inhibition concentration of silver acetate against *E. coli* (Peetsch *et al.* 2013). On the contrary, addition of CH₃COONa did not increase the inhibition rate, showing that acetate ions are inert

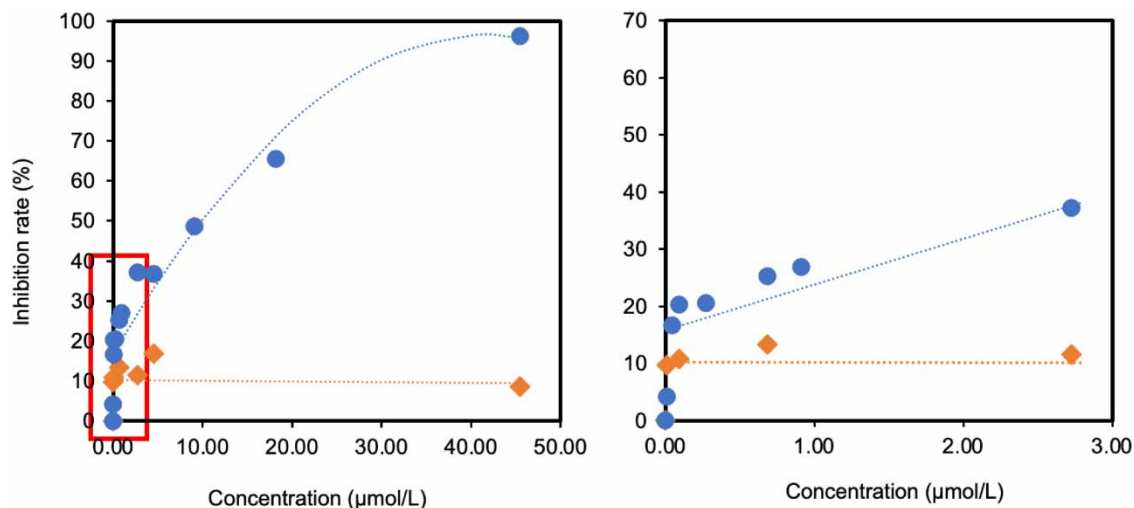


Figure 5 | Effect of CH₃COOAg (○) and CH₃COONa (◇) concentrations on *E. coli* growth: (a) whole result and (b) magnified for the red box area. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wst.2023.003>.

in terms of microbial activity. Silver ions show antibacterial activity as reported (Marambio-Jones & Hoek 2010). Almost 70% inhibition was observed with 20 μmol/L CH₃COOAg while increasing the concentration higher than 40 μmol/L inhibits the bacteria more than 90%.

AgTiO₂-high Ag (AB) membranes showed around 80% *E. coli* inhibition as shown in Figure 4. To have this inhibition, the silver concentration needs to be higher than 20 μmol/L as shown in Figure 5. However, the silver concentration released from the membrane was one order smaller as shown in Figure 3. These results also suggest that the antibacterial property did not depend solely on the released silver ion.

Direct observation of antibacterial activity

AgTiO₂ may prevent the growth of *E. coli* or may kill the bacteria. To observe the influence directly by a microscope, AgTiO₂ were deposited on glass plates instead of porous ceramics and *E. coli* cells dispersed in water were applied. The *E. coli* cells expressing red fluorescent proteins were used to count the number of *E. coli* cells, because they were difficult to be distinguished on AgTiO₂ (Figure 6(d)). The dead *E. coli* cells were detected by green fluorescent of Sytox Green. Figure 6 shows an example of the observations. The number of red and green spots corresponds to the number of *E. coli* cells and dead cells, respectively. Table 1 summarizes the counts of red and green cells with and without AgTiO₂ treatment. The number of green spots was larger with AgTiO₂. The clear difference between the blank plates and AgTiO₂ plates suggest that the AgTiO₂ contributed the death of *E. coli* cells. In these tests no salinity existed. Accordingly, the silver ion formed by the dissolution from AgTiO₂ is negligible, as described earlier. These results support the antibacterial activity of AgTiO₂.

The mechanism of antibacterial activity by silver is still not fully understood (Durán *et al.* 2016; Singh & Mijakovic 2022). Several mechanisms have been proposed and investigated. For example, silver ions react with thiol groups of protein and causing inactivation of the bacteria (Durán *et al.* 2016). Silver ions can also disturb ATP production and DNA replication (Marambio-Jones & Hoek 2010). Formation of reactive oxygen species by the reaction between Ti³⁺ and oxygen vacancy in AgTiO₂ is also proposed to be the cause of bacteria elimination in dark conditions (Viet *et al.* 2018). However, the results obtained in this study showed that the concentration of silver ion was too low to explain the inhibition degree. In addition, Ti³⁺ phase was not observed by XPS (Figure 2). Another potential cause of the inhibition is the direct contact between the silver and *E. coli* that can damage the cell walls of the bacteria (Marambio-Jones & Hoek 2010; Durán *et al.* 2016; Peng *et al.* 2020). The antibacterial property depends also on the silver state; for example, Ag₂O is reported to have higher activity than metallic silver (Rebelo *et al.* 2016). The antibacterial property obtained in this study is possibly attributed to the silver oxides at the surface of the membrane, but further study is needed to evaluate the mechanism. For practical applications, a longer lifetime of AgTiO₂ coating can be expected in solutions not containing chloride ion, as Ag₂O dissolution is negligible in water

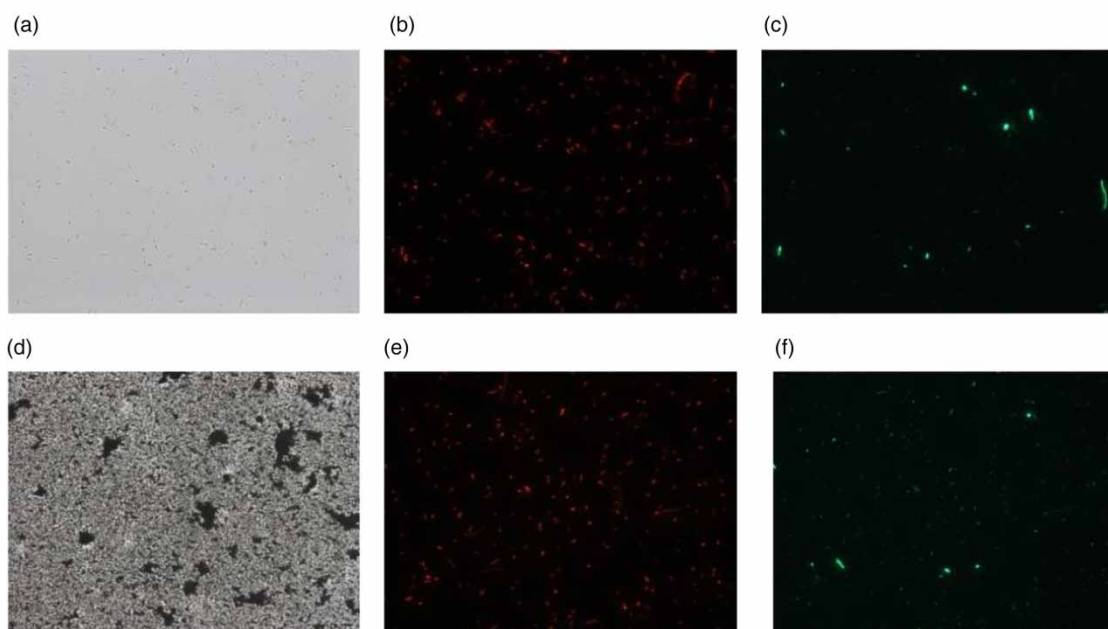


Figure 6 | Direct antibacterial study on nanoparticles: (a) bright field, (b) *E. coli* cells (red); (c) dead cells (green) without AgTiO₂, (d) bright field, (e) *E. coli* cells (red), and (f) dead cells (green) with AgTiO₂. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wst.2023.003>.

Table 1 | Summary of the cell counts on a glass without (blank) and with AgTiO₂

Sample no.	Blank		AgTiO ₂	
	Red	Green	Red	Green
1	170	37	231	187
2	220	51	196	160
3	225	27	196	121
4	173	25	172	132
5	193	25	246	164
6	224	33	–	–

but accelerates in the presence of chloride ion. Applying a periodical photochemical reduction has a potential to regenerate the bactericidal property of the coating.

CONCLUSIONS

AgTiO₂ membranes were prepared by a photoreduction method. The amount of silver deposition can be controlled by the concentration of initial silver acetate solution. Ag₂O was found at the surface of TiO₂ analyzed by XPS. Silver leaching from the AgTiO₂ membranes into water was negligible but was enhanced in 0.09 M NaCl solution.

Prepared AgTiO₂ membranes inhibited the *E. coli* growth under dark conditions. The hindered growth cannot be explained only by the silver ion concentration leached out from the membranes. The inhibition degree was reduced when AgTiO₂ membranes were washed with NaCl solution before the *E. coli* cultivation, which may be due to the reduced amount of silver oxides on the membranes. Microscopic observation of *E. coli* on glass plates covered with AgTiO₂ showed that a direct contact with AgTiO₂ causes the death of *E. coli*.

The potential to apply photoreduction of silver to enhance the antibacterial activity of the membrane is demonstrated in this study. The antibacterial activity can be associated with silver oxides, but further study is required to understand the mechanism.

ACKNOWLEDGEMENT

This work was supported by JST SICORP Grant Number JPMJSC18C5, Japan.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Bogler, A., Lin, S. & Bar-Zeev, E. 2017 Biofouling of membrane distillation, forward osmosis and pressure retarded osmosis: principles, impacts and future directions. *Journal of Membrane Science* **542**, 378–398.
- Che Abdul Rahim, A. N., Yamada, S., Bonkohara, H., Mestre, S., Imai, T., Hung, Y.-T. & Kumakiri, I. 2022 Influence of salts on the photocatalytic degradation of formic acid in wastewater. *International Journal of Environmental Research and Public Health* **19** (23), 15736–15744.
- Durán, N., Durán, M., De Jesus, M. B., Seabra, A. B., Fávoro, W. J. & Nakazato, G. 2016 Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine* **12** (3), 789–799.
- Fan, X., Liu, Y., Wang, X., Quan, X. & Chen, S. 2019 Improvement of antifouling and antimicrobial abilities on silver-carbon nanotube based membranes under electrochemical assistance. *Environmental Science & Technology* **53** (9), 5292–5300.
- Goderecci, S. S., Kaiser, E., Yanakas, M., Norris, Z., Scaturro, J., Oszust, R., Medina, C. D., Waechter, F., Heon, M., Krchnavek, R. R., Yu, L., Lofland, S. E., Demarest, R. M., Caputo, G. A. & Hettlinger, J. D. 2017 Silver oxide coatings with high silver-ion elution rates and characterization of bactericidal activity. *Molecules* **22** (9), 1487–1501.
- Guin, D., Manorama, S. V., Latha, J. N. L. & Singh, S. 2007 Photoreduction of silver on bare and colloidal TiO₂ nanoparticles/nanotubes: synthesis, characterization, and tested for antibacterial outcome. *The Journal of Physical Chemistry C* **111** (36), 13393–13397.
- Iravani, S., Korbekandi, H., Mirmohammadi, S. V. & Zolfaghari, B. 2014 Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences* **9** (6), 385–406.
- Jeon, S. & Lee, J.-H. 2020 Rationally designed in-situ fabrication of thin film nanocomposite membranes with enhanced desalination and anti-biofouling performance. *Journal of Membrane Science* **615**, 118542–118552.
- Kumakiri, I., Diplas, S., Simon, C. & Nowak, P. 2011 Photocatalytic membrane contactors for water treatment. *Industrial & Engineering Chemistry Research* **50** (10), 6000–6008.
- Kumakiri, I., Murasaki, K., Yamada, S., Rahim, A. N. b. C. A. & Ishii, H. 2022 A greener procedure to prepare TiO₂ membranes for photocatalytic water treatment applications. *Journal of Membrane Science and Research* **8**, 549416–549422.
- Le, L., Xu, J., Zhou, Z., Wang, H., Xiong, R. & Shi, J. 2018 Effect of oxygen vacancies and Ag deposition on the magnetic properties of Ag/N co-doped TiO₂ single-crystal films. *Materials Research Bulletin* **102**, 337–341.
- Levard, C., Mitra, S., Yang, T., Jew, A. D., Badireddy, A. R., Lowry, G. V. & Brown, G. E. 2013 Effect of chloride on the dissolution rate of silver nanoparticles and toxicity to *E. coli*. *Environmental Science & Technology* **47** (11), 5738–5745.
- Li, J.-H., Yan, B.-F., Shao, X.-S., Wang, S.-S., Tian, H.-Y. & Zhang, Q.-Q. 2015 Influence of Ag/TiO₂ nanoparticle on the surface hydrophilicity and visible-light response activity of polyvinylidene fluoride membrane. *Applied Surface Science* **324**, 82–89.
- Li, S., Zhu, Q., Sun, Y., Wang, L., Lu, J., Nie, Q., Ma, Y. & Jing, W. 2020 Fabrication of Ag nanosheet@TiO₂ antibacterial membranes for inulin purification. *Industrial & Engineering Chemistry Research* **59** (16), 7797–7804.
- Liang, Y. Q., Cui, Z. D., Zhu, S. L., Liu, Y. & Yang, X. J. 2011 Silver nanoparticles supported on TiO₂ nanotubes as active catalysts for ethanol oxidation. *Journal of Catalysis* **278** (2), 276–287.
- Marambio-Jones, C. & Hoek, E. 2010 A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research* **12** (5), 1531–1551.
- Mohanty, P., Mishra, N. C., Choudhary, R. J., Banerjee, A., Shripathi, T., Lalla, N. P., Annapoorni, S. & Rath, C. 2012 Oxygen vacancy induced phase formation and room temperature ferromagnetism in undoped and Co-doped TiO₂ thin films. *Journal of Physics D: Applied Physics* **45**, 325301–325308.
- Nakamura, M., Aihara, J., Hoshida, H. & Akada, R. 2018 Identification and mutational analysis of escherichia coli sorbitol-enhanced glucose-repressed srlA promoter expressed in LB medium by using homologous recombination and one-round PCR products. *Molecular Biotechnology* **60** (12), 912–923.

- Naoi, K., Ohko, Y. & Tatsuma, T. 2004 TiO₂ films loaded with silver nanoparticles: control of multicolor photochromic behavior. *Journal of the American Chemical Society* **126** (11), 3664–3668.
- Nguyen, T., Roddick, F. A. & Fan, L. 2012 Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures. *Membranes (Basel)* **2** (4), 804–840.
- Obotey Ezugbe, E. & Rathilal, S. 2020 Membrane technologies in wastewater treatment: a review. *Membranes* **10** (5), 89–116.
- Pandian, A. M. K., Rajamehala, M., Singh, M. V. P., Sarojini, G. & Rajamohan, N. 2022 Potential risks and approaches to reduce the toxicity of disinfection by-product – A review. *Science of the Total Environment* **822**, 153323–153336.
- Peetsch, A., Greulich, C., Braun, D., Stroetges, C., Rehage, H., Siebers, B., Koller, M. & Epple, M. 2013 Silver-doped calcium phosphate nanoparticles: synthesis, characterization, and toxic effects toward mammalian and prokaryotic cells. *Colloids Surf B Biointerfaces* **102**, 724–729.
- Peng, S., Chen, Y., Jin, X., Lu, W., Gou, M., Wei, X. & Xie, J. 2020 Polyimide with half encapsulated silver nanoparticles grafted ceramic composite membrane: enhanced silver stability and lasting anti-biofouling performance. *Journal of Membrane Science* **611**, 118340–118351.
- Rebelo, R., Calderon, S. V., Fanguero, R., Henriques, M. & Carvalho, S. 2016 Influence of oxygen content on the antibacterial effect of Ag-O coatings deposited by magnetron sputtering. *Surface and Coatings Technology* **305**, 1–10.
- Singh, P. & Mijakovic, I. 2022 Antibacterial effect of silver nanoparticles is stronger if the production host and the targeted pathogen are closely related. *Biomedicines* **10** (3), 628–643.
- Tan, Z.-K., Gong, J.-L., Fang, S.-Y., Li, J., Cao, W.-C. & Chen, Z.-P. 2022 Outstanding anti-bacterial thin-film composite membrane prepared by incorporating silver-based metal–organic framework (Ag-MOF) for water treatment. *Applied Surface Science* **590**, 153059–153070.
- Verhovšek, D., Veronovski, N., Lavrenčič Štangar, U., Kete, M., Žagar, K. & Čeh, M. 2012 The synthesis of anatase nanoparticles and the preparation of photocatalytically active coatings based on wet chemical methods for self-cleaning applications. *International Journal of Photoenergy* **2012**, 1–10.
- Viet, P. V., Phan, B. T., Mott, D., Maenosono, S., Sang, T. T., Thi, C. M. & Hieu, L. V. 2018 Silver nanoparticle loaded TiO₂ nanotubes with high photocatalytic and antibacterial activity synthesized by photoreduction method. *Journal of Photochemistry and Photobiology A: Chemistry* **352**, 106–112.
- Wodka, D., Bielanska, E., Socha, R. P., Elzbieciak-Wodka, M., Gurgul, J., Nowak, P., Warszynski, P. & Kumakiri, I. 2010 Photocatalytic activity of titanium dioxide modified by silver nanoparticles. *ACS Applied Materials & Interfaces* **2** (7), 1945–1953.
- Zodrow, K., Brunet, L., Mahendra, S., Li, D., Zhang, A., Li, Q. & Alvarez, P. J. 2009 Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. *Water Research* **43** (3), 715–723.

First received 18 September 2022; accepted in revised form 22 December 2022. Available online 10 January 2023