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Surface coating on aluminum substrate with polymeric guanidine derivative to protect jet fuel tanks from microbial contamination

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ABSTRACT

Antimicrobial coatings can act as an effective barrier to microbial growth and colonization in jet fuel tanks, which can effectively avoid the safety problems and economic losses. In this study, we initially synthesized an antimicrobial and water-insoluble complex, poly (hexamethylene guanidine) hydrochloride-sodium stearate (PHMG-SS), and its minimum inhibitory concentrations (MICs) were measured as 0.25 g/L, 0.25 g/L, 0.25 g/Land 0.5 g/L against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Yarrowia lipolytica*, respectively; then, PHMG-SS was facilely coated onto the surface of aluminum substrate by the aid of a green linker, polyvinyl butyral (PVB). The PHMG-SS-coated aluminum exhibited a powerful microbicidal activity against the above four microorganisms, and provided a greater than 99.999% (5 log) reduction in viable counts of experimental microorganisms within 30 min through contact. In addition, the antimicrobial performance could still be observed after 7 d of ultraviolet radiation or soaking in jet fuel. Furthermore, the antimicrobial mechanism was investigated and concluded as damaging the microbicidal morphologies and suppressing the activity of respiratory chain dehydrogenase. Finally, PHMG-SS-coated aluminum was evaluated as interior surface of jet fuel tanks to protect jet fuel, and the experimental results showed that PHMG-SS-coated aluminum achieved a greater than 99.9999% (6 log) reduction in viable counts of a mixed suspension of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Yarrowia lipolytica* within 30 min. The outputs from this work represent a highly versatile approach to build an antimicrobial barrier to protect jet fuel from microbial contamination, and such approach is also worthy of wide application to treat other surfaces.

1. Introduction

Jet fuel, mainly composed of C_8-C_{16} hydrocarbons which are volatile and exhibit high saturated vapor pressure and hygroscopicity, is the crucial source to ensure the normal work of all the systems of aircraft and flying safety [1,2]. When free-water is present in tanks, the jet fuel-associated water environment is suitable for microorganism proliferation, particularity within biofilm communities of tank surfaces. Organic compounds which partition from the fuel to aqueous phase would serve as nutrients and facilitate jet fuel biodeterioration [3–5]. Furthermore, the moisture in the jet fuel provides a suitable environment for the growth of microorganisms, making it more susceptible to microbial contamination than gasoline [6]. Therefore, the microbial control of jet fuel is highly important and worthy of extensive study.

A great deal of publicity has been given in recent years on the problems caused by microbial attack on jet fuel, especially that microbial contamination of jet fuel has been widely aware in the aviation industry, and International Air Transport Association (IATA) originally published "Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks" in 2002 (currently in its 5th edition) which provided a detailed overview of the damage potentially caused by jet fuel-contaminating microorganisms and provided recommendations for minimizing biodeterioration risk in aircraft fuel systems [5,7,8]. In fact, microbial contamination of jet fuel has been reported as early as 1960s [9]. Jet fuel can pick up microbially contamination from each stage of the fuel transport infrastructure from refinery tank to airfield hydrant, and microorganisms can enter the inner fuel tank as contaminants due to the entry of airborne, waterborne microorganisms, or occasional errors in operational procedures, thus leading to the contamination of inner surface and jet fuel [10,11]. Studies have shown that diverse

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microorganisms, including various bacteria, yeasts and moulds, can all flourish in jet fuel. Of the organisms identified, *Hormoconis resinae* has been particularly prevalent in jet fuel tanks, which can utilize the hydrocarbons of jet fuel and results in the production of organic acids which are corrosive to metals [12,13]. Besides, the yeast of *Yarrowia lipolytica* (*Y. lipolytica*) and the bacterium of *Bacillus subtilis* (*B. subtilis*), have previously been isolated from jet fuel [8]. *Y. lipolytica* is well known for its ability to degrade hydrocarbon fuels by a specific pathway involving alkane monooxygenases, fatty-alcohol oxidases as well as dehydrogenases fatty-acyl-CoA synthetases, hence it can undoubtedly degrade jet fuel [14]. These microorganisms live in the water-bearing areas of jet fuel storage and transportation equipment [15]. Under normal circumstances, it is impossible to remove water thoroughly, and water accumulation can contribute to the biofilm accumulation on interior surface of jet fuel tanks. Consequently, the residual water provides habitats in which microbial communities can thrive, and then microorganisms can soon aggregate. Moreover, they are adept at attaching to surfaces to form wet slimes, and fouling on the interior surface to induce the biofilm accumulation. Finally, the fuel filters will be plugged by the microbial aggregates or biofilms [16]. Microbial growth and contamination in jet fuel storage tanks can cause metal corrosion, fuel filter block, and increased maintenance costs associated with these problems [7,8]. Moreover, once the jet fuel is contaminated by microorganism, the quality of fuel will be reduced [5]. In addition, the cleaning of jet fuel tank is a cumbersome and arduous work, which will require a huge cost and also pose a threat to the cleaners [17]. Therefore, it is of great importance to take effective measures to render the interior surface of jet fuel tanks with potent antimicrobial property and control the microbial contamination.

Surface coating is the most desirable and economic approach to obtain the antimicrobial properties owing to the simple operation and easy scalable production [18–20]. However, the existing common problem of surface coating is the persistence. The antimicrobial components will gradually leach out from the coated surface, thus resulting in a decrease in the durability and effectiveness of antimicrobial property and also possible danger to human health and environmental safety, especially when the coatings contain small molecules and inorganic particles [21–24]. Therefore, polymeric coatings with strong antimicrobial activity and sustainability without leaching should be highly developed but remains a critical challenge. Based on the long-term exploration of antimicrobial materials in our group [25–28], as well as considering the coating process and cost, poly (hexamethylene guanidine) (PHMG) was incorporated into the coating strategy, which is an ideal antimicrobial polymer due to its broad-spectrum and excellent antimicrobial properties, lower toxicity to human beings and environment [29,30]. Although PHMG has been extensively applied in various fields, and acted as a powerful agent to inactivate the microorganisms, the water-soluble characteristic easily causes PHMG leaching out from the coating systems. Therefore, modifying PHMG into water-insoluble derivatives would be a practicable method to avoid the attenuation of antimicrobial performance.

In the present work, we aim to develop an antimicrobial aluminum sheet with water-insoluble PHMG-SS as biocide through a facile surface modification method. PHMG-SS was synthesized *via* strong electrostatic interaction and embedded in a coating applied on aluminum sheets. The performances of the modified aluminum sheets were evaluated in terms of biocidal efficiency against microorganisms identified as contaminants in jet fuel tanks, the leaching of PHMG-SS, and the coating stability. Some aspects of the biocide action mechanism were presented and discussed (Scheme 1).

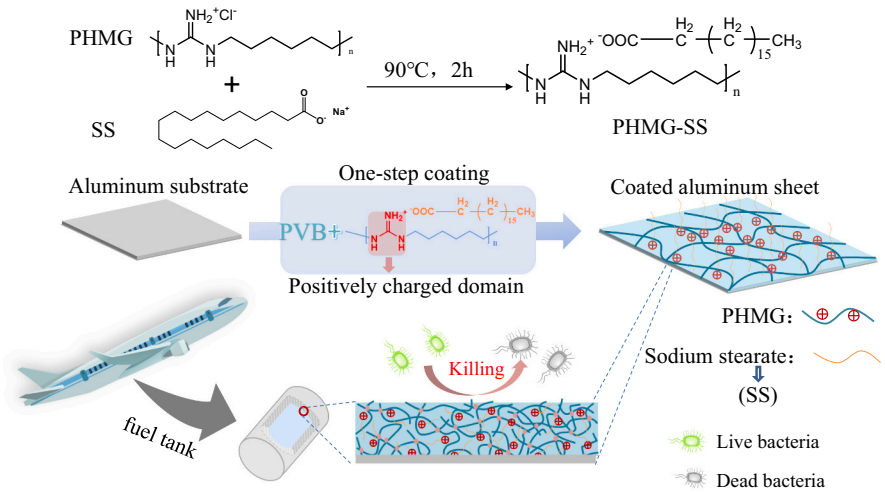
2. Experimental section

2.1. Materials

RP-3 jet fuel was provided by AVIC Chengdu Aircraft Industrial (Group) Co., Ltd., and the 1060-type aluminum sheet with thickness of 2 mm was purchased from Shenzhen Shengli Company; Polyhexamethylene guanidine hydrochloride (PHMG, 95%) as the antimicrobial agent was purchased from Shanghai High Poly Biotechnology Co., Ltd. China, and sodium stearate (SS, 96%), acetone and absolute ethanol were supplied by Chengdu Huaxia Chemical Reagent Co. Ltd. China; Polyvinyl butyral (PVB) as the linker was obtained from Chengdu Tianyuan Chemical Co. Ltd.; *Escherichia coli* (*E. coli*, ATCC8739), *Staphylococcus aureus* (*S. aureus*, ATCC6538), *Bacillus subtilis* (*B. subtilis*, CMCC (B)63501) and *Yarrowia lipolytica* (*Y. lipolytica*, AS2.1379) were supplied by R&D Lab of Functional Fibers of Sichuan University.

2.2. Synthesis of PHMG-SS complex

Both PHMG and sodium stearate were dissolved in deionized water, respectively. The sodium stearate solution was heated to 90 °C and thoroughly ionized by constant stirring. Subsequently, PHMG solution was slowly and dropwise added to the sodium stearate solution under continuously stirring. After reaction for 2 h, the complex is obtained by direct filtration, and the resulting product was washed with deionized water three times. Finally, the obtained complex were dried in a vacuum oven at 60 °C for 24 h.



Scheme 1. Synthetic route of PHMG-SS and the formation of antimicrobial aluminum sheet.

2.3. Pretreatment of aluminum sheets

The aluminum sheets (Als) were cut into pieces with the dimensional size of 50 mm * 50 mm * 2 mm, and then sanded with 1000 grit sandpaper. Thereafter, the sheets were ultrasonically washed with acetone, ethanol and distilled water for 10 min, respectively. Finally, the Als were dried and stored before use under normal condition.

2.4. Fabrication of PVB/PHMG-SS coatings

First, 1 wt% PVB solution was generated by adding 1 g PVB in 99 g anhydrous ethanol. PHMG-SS and PVB solution were mixed at different mass ratios and processed under stirring for 12 h. Subsequently, the pretreated Als were immersed in the above solution for 1 min and maintained at ambient temperature for drying. The PVB/PHMG-SS coated Als containing 0, 0.1 wt% and 0.5 wt% PHMG-SS was noted as PVB-0, PVB-0.1 and PVB-0.5, respectively. Lastly, dried Als were obtained and stored in a valve bag before test.

2.5. Evaluation of antimicrobial properties of PHMG-SS

The minimum inhibitory concentrations (MICs) of PHMG-SS complex against *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica* were tested with the agar dilution method according to the Ministry of Health of the PRC "Technical Standard For disinfection (2002 version)", and this method is suitable for insoluble antimicrobial products. The procedure was elucidated as below. The bacteria were cultured in Luria-Bertani (LB) medium or Malt Extract (ME) medium at 37 °C to logarithmic-phase, then diluted it to ~10^7 CFU/mL with PBS buffer by tenfold dilution series to obtain bacterial suspensions. Subsequently, we prepared a series of antimicrobial agent suspensions with PBS buffer to obtain different concentrations (0.05 g/L, 0.25 g/L, 0.5 g/L and 2.5 g/L), and placed them in a water bath at 50 °C for later use; Next, twice-concentrated LB and ME agar mediums were prepared, and then mixed with different concentrations of PHMG-SS to obtain antimicrobial agent mediums for inoculating the above prepared bacterial suspensions. Afterwards, 20 μL (concentration ~ 10^7 CFU/mL) of the bacterial suspensions were withdrawn and spotted on separate plate containing the antimicrobial agent medium, and the agar plates without PHMG-SS was set as a positive control. Finally, the inoculated LB medium plates and MEA medium plates were cultured in an incubator at 37 °C for 24 h or 28 °C for 72 h, respectively. The minimum concentration of antimicrobial suspension at which colony growth was completely inhibited was regarded as the MIC of PHMG-SS to the tested bacteria.

2.6. Antibacterial mechanism of PHMG-SS

2.6.1. Observation of bacterial morphology

SEM was conducted to characterize bacterial morphology after the contact with PHMG-SS. In detail, 10 mL *S. aureus* suspensions (~10^8 CFU/mL) were centrifuged under 8000 rpm to obtain bacterial pellet, and then resuspend it in 10 mL PBS buffer. The resuspended bacterial suspensions (5 mL) and PHMG-SS complex (0.1 g) were co-cultured in a water bath shaker for 12 h, and the suspensions without PHMG-SS complex was used as control. Then, the above suspensions were centrifuged under 8000 rpm again. Subsequently, 2.5% glutaraldehyde aqueous solution was applied to fix the bacterial precipitates of experimental and control groups at 4 °C overnight. Next, the bacterial precipitates were dehydrated with gradient alcohol (*i.e.*, 30%, 50%, 70%, 90% and 100%) for 15 min, respectively. Afterwards, the sample was dispersed in tert-butanol and then freeze-dried. Lastly, dried bacterial precipitates were sputter coated with gold for SEM test. Likewise, the above procedures were repeated for *E. coli*.

2.6.2. Effect on the activity of respiratory chain dehydrogenase

The activity of cell respiratory chain dehydrogenase exerts a direct impact on the growth of bacteria, so it can act as a vital factor to depict the antibacterial mechanism [28,31]. For the testing, *E. coli* and *S. aureus* suspension were first mixed evenly with Tris HCl buffer (pH = 8.6), 0.1 mol/L glucose solution and 1.0 mg/mL of 2,3,5-triphenyltetrazolium chloride (TTC) solution; then, the PHMG-SS complex of different qualities (*i.e.*, 0.5 mg, 1 mg, 1.5 mg, 2 mg and 2.5 mg) were introduced to the mixed suspensions, and the mixtures without PHMG-SS were set as the control. All the testing mixtures underwent the standing culture in a constant temperature incubator at 37 °C for 5 h. Next, two drops of concentrated sulfuric acid (100 μL) were separately added to the mixtures to terminate the reaction. Afterwards, N-butanol was added to extract the metabolites, and the mixtures were then centrifuged under 8000 rpm for 3 min. Lastly, the activity of cell respiratory chain dehydrogenase was determined by determining the OD value of the supernatant at 490 nm, and both experimental and control groups were performed in triplicate.

2.7. Antimicrobial activity of the coating and the coated Als

Surface antimicrobial activity assays were performed by complying with JIS Z 2801 standards [32]. In brief, coated Als specimens and respective control specimens were cut into 1.5 × 1.5 cm^2 pieces. In addition, *E. coli*, *S. aureus B. subtilis* and *Y. lipolytica* suspension (25 μL) was added at the center region of the samples. Subsequently, polyethylene film (1 × 1 cm^2) was employed to cover the bacterial (fungi) suspension. The samples, after being cultivated at 37 °C and 90% humidity for 30 min, were immersed in 3 mL of phosphate-buffered saline (PBS) buffer, and then 100 μL eluent was withdrawn and uniformly spread on the LB (ME) agar plates for another 24 h (72 h) incubation at 37 °C (28 °C).

Moreover, the inhibition zone assay and UV–Vis analysis were adopted to prove that the PVB/PHMG-SS composite coatings were characterized as non-leaching. First, 100 μL *S. aureus* and *E. coli* suspensions (~10^6 CFU/mL) were separately to cover the full surface of agar plates. PVB-0, PVB-0.1, PVB-0.5 samples with the size of 1.5 × 1.5 cm^2 were then placed on agar plates and cultured at 37 °C for 24 h. In addition, PVB-0.5 coated Als (4 × 4 cm^2) were soaked in 20 g DI water for 3 d under room temperature. Then the absorption of solution after soaking was measured using an ultraviolet (UV) spectrophotometer (Unico, UV2900A, USA) in the range of 190–700 nm to determine whether PHMG was leaching out from the coating, and pristine PHMG solution was used as a control.

In addition, considering that majority of microbial growth occurs in jet fuel-associated water biofilms on internal surfaces in actual situations, thus, the aqueous microbial suspension mixed with jet fuel was applied to simulate the contaminated jet fuel. In order to verify the antimicrobial properties of coated Als in jet fuel environment, microbial contaminated jet fuel was prepared by adding 100 μL mixture microbial (*E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica*) into 3 mL jet fuel, and the final microbial concentration was around 10^6 CFU/mL. Subsequently, coated Als (1.5 × 1.5 cm^2) were co-cultured with jet fuel containing mixed microbial in a water bath shaker at a speed of 200 r/min for 30 min to make the Als can fully contact with the pre-added microorganisms in jet fuel, and six parallel samples were set for each group. After the contact interaction, the quantitative analysis of the alive microorganisms in jet fuel was carried out according to the part of "standard practice for enumeration of viable bacteria and fungi in liquid fuels-Filtration and culture procedures" from IP 385 and ASTM D5259. The specific steps included 1) shaking the contaminated fuel after co-cultivation on a fast vortex mixer, then taking out the Als and transferring the contaminated fuel to a sterile syringe; 2) a filter preconfigured with a filter membrane (0.45 μm) was connected to the syringe, and then the plunger of the syringe was gently pressed by hand to ensure the microorganisms depositing on the filter membrane; 3) using a sterile tweezer to transfer the filter membrane to a centrifuge tube containing PBS buffer solution, then it was shaken on a fast vortex mixer

to elute the microbial on the membrane surface. The eluant was diluted into serial concentrations of bacterial suspensions by a ten-fold dilution method for colony counting, and the determination assay was referred to: GB4789.2-2010 "Food Safety National Standard Food Microbiological Inspection Total Number of Colonies Determination". The specific methods were as follows: the tested bacteria suspensions were serially diluted in 10-fold increments with PBS buffer, and then 100 μL of each dilution was withdrawn and uniformly spread on the LB agar or MEA plates. After incubating it for a certain period, the number of colonies formed in each plate was recorded, then the total number of microbial colonies per mL of the original microbial suspension based on the dilution factor could be calculated.

2.8. Coating stability test

In order to demonstrate the stability of the coating, two factors were taken into account, including ultraviolet irradiation and immersion in jet fuel, which are common conditions for the coating materials. Particularly, for the former factor, the coated and uncoated samples were exposed to ultraviolet light (~15 W/m^2) at room temperature for seven days; Regarding the latter one, the samples were soaked in jet fuel for 7 d. Then cleaning the surface of the samples with deionized water and dry it for the further use. After the treatment by the factors, the antibacterial properties against *E. coli* and *S. aureus* of Als were examined by complying with JIS Z 2801 standard.

2.9. Other characterizations

Surface morphologies of antimicrobial coatings were observed by Scanning electron microscopy (SEM, Hitachi SU3500, Japan). ^{1}H NMR spectroscopy was conducted on a Bruker AV 400 MHz spectrometer with dimethyl sulfoxide-*d*(DMSO-*d*) as a solvent. The PHMG-SS complex was measured using FT-IR spectrometer with an attenuated total reflection mode. Thermal analysis were conducted by thermogravimetric analysis (TGA)/differential thermal analysis (METTLER TOLEDO) over a temperature range of 30–600 °C with a heating rate of 10 °C min^{-1} at an N_2 atmosphere. Water contact angle (WCA) of the coating surface was measured through a contact angle tester (Harke-SPCAX1, China) using a sessile drop method, and at least five individual values were collected and averaged.

2.10. Statistical analysis

All statistical analyses presented at least three parallel experiments. OriginPro9.1 software was applied for statistical analysis. Values were averaged and expressed as means ± standard deviation (SD).

3. Results and discussion

3.1. Characterization of PHMG-SS complex

The ^{1}H NMR spectra of PHMG and synthesized PHMG-SS are presented in Fig. 1(a). The peaks at 1.31, 1.46, and 3.15 ppm were attributed to the methylene protons e, d, c, respectively, and the broad

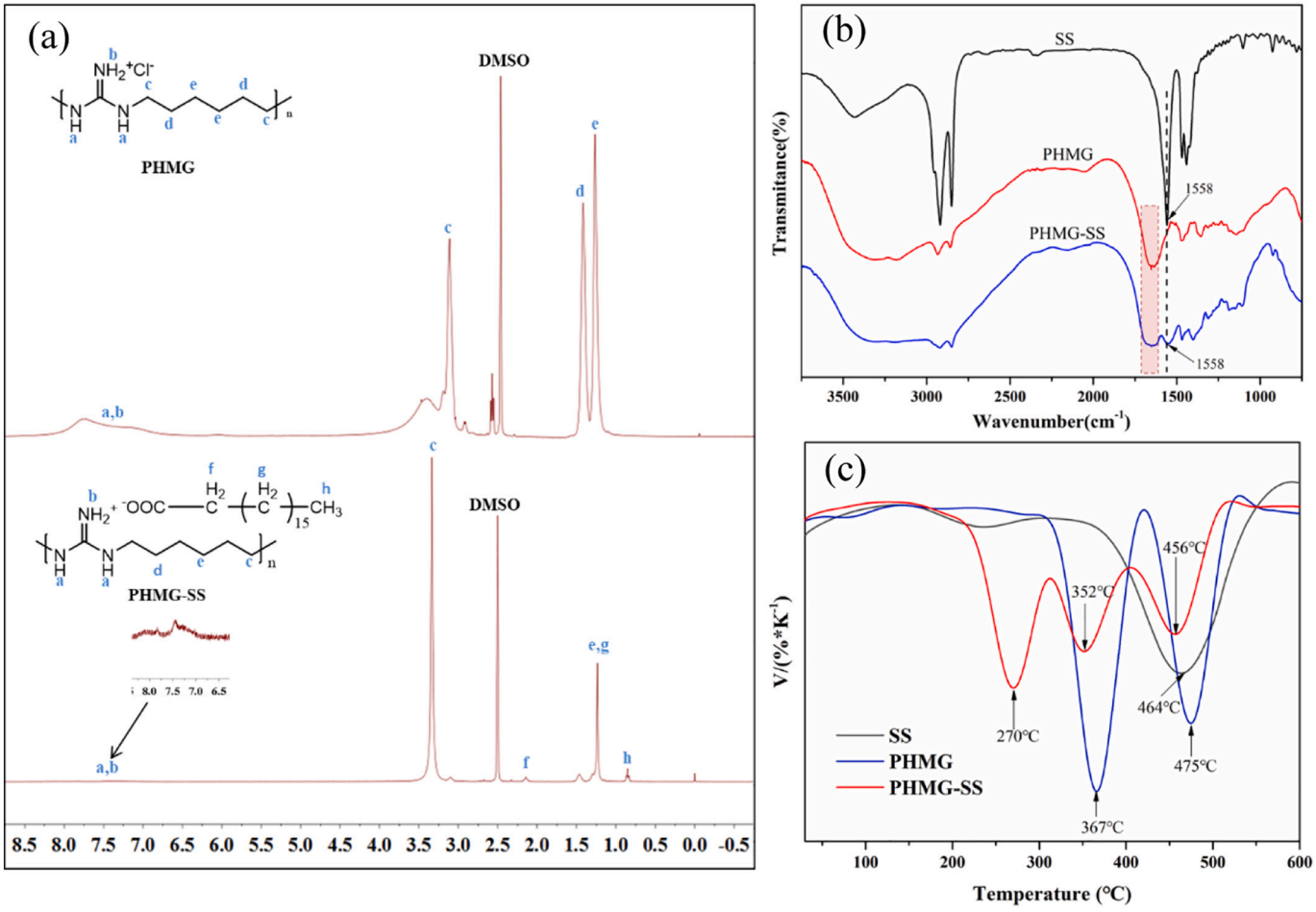


Fig. 1. (a) ^{1}H NMR spectra of PHMG and PHMG-SS complex in DMSO-*d*. (b) FT-IR spectra for SS, PHMG and PHMG-SS complex. (c) DTG curves for SS, PHMG and PHMG-SS complex.

chemical shift from 6.04 to 7.94 ppm belonged to the protons of guanidine groups (a, b, $-[NH]_2C{=}NH_2^+$). For PHMG-SS, the triplet peak around 0.91 ppm was assigned to the methyl groups of SS (h, $-CH_3$), the two sharp peaks at 1.20–1.55 ppm (d, e, g, $-CH_3$) appeared after compounding, which were ascribed to long-chain methylene protons on SS and PHMG. Besides, the peak located at $\delta = 2.14$ ppm was consigned to the methylene protons of SS structure (f, $OOC-CH_2-C$). Overall, the ^1H NMR spectra demonstrated that PHMG-SS complex was successfully synthesized.

In addition, FT-IR characterization was performed to confirm the successful synthesis of PHMG-SS complex. Fig. 1(b) shows the FT-IR spectra of SS, PHMG and PHMG-SS. The relatively broad peaks at 1621–1685 cm^{-1} belonged to integration of the stretching vibration (C=N) and bending vibration (N—H) in the PHMG structure, which were present in the complex [33]. Likewise, ν_{COO^-} at 1558 cm^{-1} was the characteristic peak of SS which was observed in PHMG-SS complex as well. The above evidence directly suggested the successful synthesis of PHMG-SS complex.

Fig. 1(c) plots the DTG curves of SS, PHMG and PHMG-SS which were adopted to assess the thermal stability of the PHMG-SS complex. As indicated from the DTG curves, the weight loss process of PHMG-SS exhibited three degradation stages which were located at around 270 °C (T_{max1}), 456 °C(T_{max2}) and 352 °C(T_{max2}), respectively, and such thermal degradation behavior was also consistent with previous study [34]. Though the point of weight loss for PHMG-SS complex was lower than that of the pure PHMG and SS, the mass of the complex only degraded by 1% with the temperature rising to 200 °C. As revealed from the mentioned results, PHMG-SS exhibited high thermal stability at 200 °C and could satisfy majority of processing requirements.

3.2. Evaluation of antimicrobial properties of the PHMG-SS complex

We measured the MIC values of PHMG-SS against *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica*. According to Fig. 2, the MICs for *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica* were measured as 0.25 g/L, 0.25 g/L, 0.25 g/L and 0.5 g/L, respectively. As fungi are eukaryotes while bacteria are prokaryotes, this may be the reason that PHMG-SS showed stronger inhibitory effect on bacteria. Briefly, PHMG-SS complex exhibited an ideal antimicrobial activity against *E. coli*, *S. aureus B. subtilis* and *Y. lipolytica*.

3.3. Surface characterization of coated Als

3.3.1. Surface morphology

Fig. 3 presents the scanning electron microscope (SEM) images of uncoated Al surface and other three coated surfaces. Obviously, pristine Al surface mainly exhibits the rough topography and fracture sections caused by the sandpaper treatment but without deposited covers (Fig. 3a). In contrast, after the introduction of PVB, an obvious network fabricated by relatively uniform distributed "streamlets" can be observed (Fig. 3b). However, due to the applied amount of PVB and the interfacial tension between PVB and Al, PVB could not fully cover Al surface [35]. Also, the unsteady and uneven evaporation of solvent (anhydrous ethanol) was another possible reason [36]. Furthermore, compared to the surface network of PVB-0 coated Als, the introduction of PHMG-SS caused the surface to become more densely covered (Fig. 3d). Apart from that, comparing PVB-0 coated Als with PVB-0.5 coated Als, there was no obvious microscopic phase separation, demonstrating the good compatibility between PVB and PHMG-SS, and the possible reason could be attributed to the formation of hydrogen bonding and hydrophobic interaction between the two polymers.

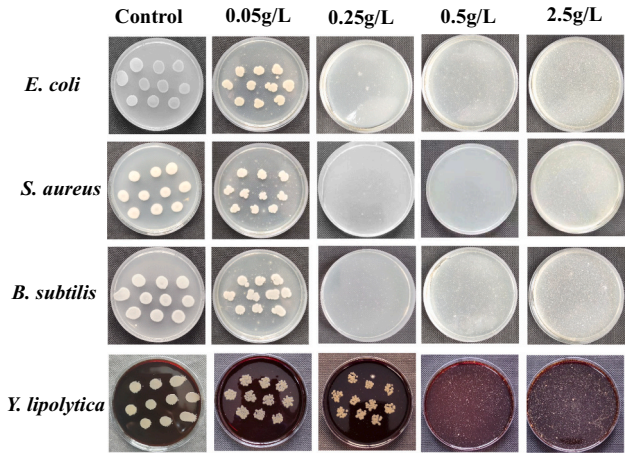


Fig. 2. Photographs of incubated plates showing the biocidal effect of the PHMG-SS complex on *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica*, respectively.

3.3.2. Surface hydrophilicity

The surface hydrophilicity of coated samples was assessed by performing contact angle measurements. According to Fig. 4, the uncoated aluminum surface displayed a hydrophobicity with WCA value at 101 ± 1.19°. However, the hydrophilicity increased slightly after coating owing to the presence of the hydroxyl group in PVB. Furthermore, the WCA value continued to descend after the introduction of hydrophilic PHMG-SS complex, the more incorporation amount of PHMG-SS, the lower WCA could be recorded, and the WCA of PVB-0.5 was 53.7 ± 2.8°.

3.4. Evaluation of antimicrobial activity of coating

Normally, untreated surface may be easily colonized by diverse microorganisms, and thus the microorganisms on the interior surface of jet fuel tanks may negatively influence the quality of fuel and plug the fuel filter. Fig. 5(a) shows the microbicidal activity of different samples against *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica*. Neither pristine Al sheet nor Als coated by only PVB exhibited antimicrobial activity, while strong antimicrobial efficiency was observed from PVB-0.1 and PVB-0.5 groups in which few microbial colonies were observed. Strikingly, the sample incorporated with only 0.1% antimicrobial complex (PVB-0.1) could kill 99.999% of *E. coli*, *S. aureus* and *B. subtilis* within only 30 min, while the kill of PVB-0.1 to *Y. lipolytica* was 77.29%. Moreover, the PVB-0.5 killed all the microorganisms mentioned above to below the detection level of the method within 30 min, which demonstrated that PVB-0.5 had good potential to control microbial growth.

In addition, both inhibition zone assay and UV–Vis analysis (Fig. 5b) verified the non-leaching characteristic of PHMG-SS in the coatings. Once the antimicrobial performance was completed by the leaching of PHMG-SS, an obvious inhibition zone would appear. Notably, no inhibition zones were observed in the sample groups of PVB-0, PVB-0.1 and PVB-0.5, the explanation for the former one was due to the lack of antimicrobial activity of PVB, and that for the latter two was because there was no leaching of antimicrobial substances, even the concentration of PHMG-SS reached to 0.5 wt%. Additionally, through three days of immersion, the leaching of PHMG from PVB-0.5 coated Als was below the detection limit [37]. Therefore, the obtained antimicrobial coatings could be regarded as stable without PHMG leakage.

3.5. Coating stability test

The test with respect to the resistance of jet fuel was performed to assess the antibacterial stability of the prepared coating. As shown in Fig. 6, as expected, the PVB-0 coatings exhibited no antibacterial activity toward both *S. aureus* and *E. coli* after soaking. By contrast, after being immersed in jet fuel for 7 days, both PVB-0.1 and PVB-0.5 Als could still exhibit desirable disinfection efficiency. Besides, the durability of coating against UV light was also investigated as a vital index of stability, and the results showed that all the coating containing

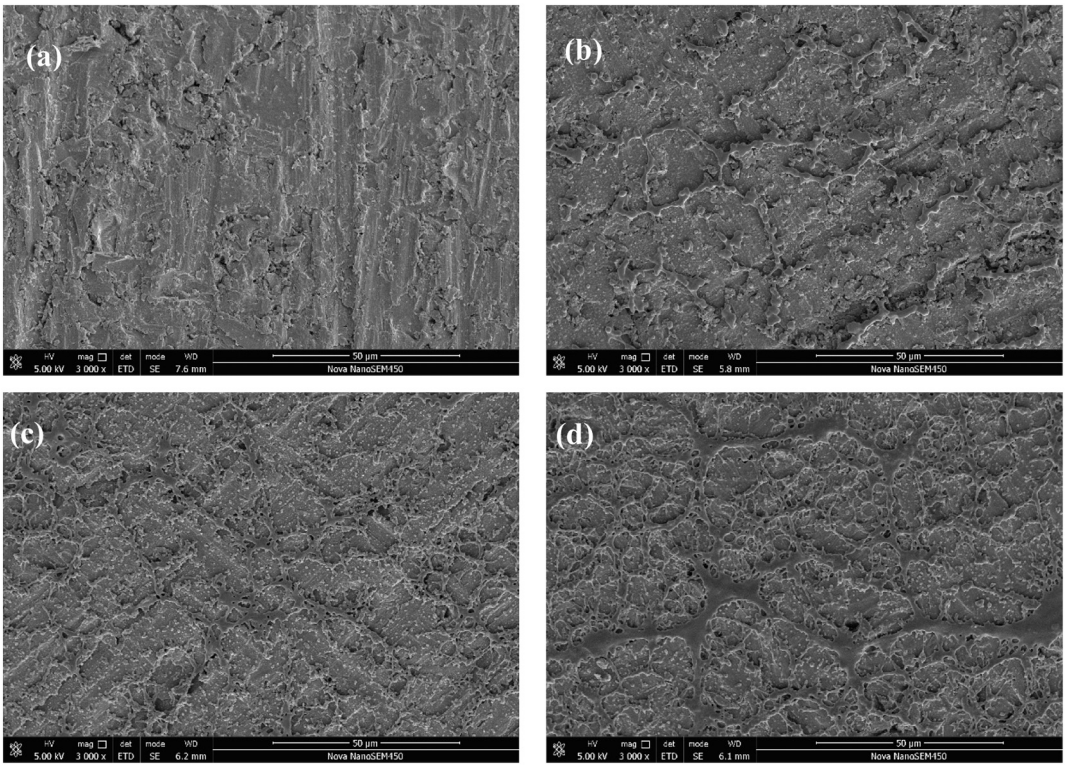


Fig. 3. SEM image of (a) Al, (b) PVB-0 coated Als, (c) PVB-0.1 coated Als, and (d) PVB-0.5 coated Als.

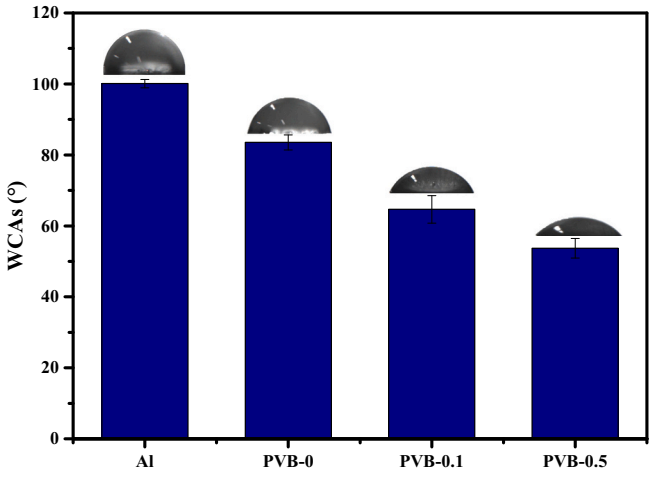


Fig. 4. Water contact angle of Al and different coated samples.

antibacterial complex (PVB-0.1, PVB-0.5) exerted antibacterial effect on both bacteria without decline, supporting the fact that the coatings could maintain stable under both conditions of UV irradiation and soaking in jet fuel for a certain period (Fig. 7).

3.6. Investigation of antibacterial mechanism

3.6.1. Bacterial morphology

The morphological variations of the bacteria treated by PHMG-SS complex were observed by SEM (Fig. 8). The normal morphologies of living *E. coli* and *S. aureus* were rod-shaped and spherical, respectively. After contact with PHMG-SS complex, apparent deformation or disruption of bacterial morphology was found, and even that the intracellular cytoplasm leaked out from the debris of dead bacteria (Fig. 8b and d). This was caused by the biocidal action of PHMG containing positively charged domain and hydrophobic alkyl groups, which acted on the negatively charged bacterial membrane and thus led to cell lysis [38,39].

3.6.2. The effect of PHMG-SS on bacterial respiratory chain dehydrogenase

Given the negatively-charged nature of bacterial surface, bacteria could adhere to and encased in the positively-charged coatings due to the electrostatic attraction, causing the block of respiratory channels and inhibition of cell metabolism. In order to verify the hypothesis, the activity of respiratory chain dehydrogenase was determined. Normally, if TCC enters a healthy cell, it will be reduced to red 1,3,5-Triphenyl tetrazolium formazan (TTF) by respiratory chain dehydrogenase. Once the activity of respiratory chain dehydrogenase is suppressed, TCC can be only partially reduced and the color changes from dark red to light red accompanied by the descend of bacterial OD valve. As obviously shown in Fig. 9, when the content of the PHMG-SS was 2.5 mg, the corresponding OD value of *E. coli* and *S. aureus* suspensions were 96.94% and 98.79% lower than that of control group. Besides, the color of the solution of PHMG-SS treated group was significantly lighter than that of control group, which indicated the bacterial metabolic activity was considerably inhibited. According to the above results, it was concluded that PHMG-SS complex could kill bacteria by at least two mechanisms including membrane disrupt mechanism and inhibiting the metabolism. More importantly, the synergetic effect of multiple antibacterial modes will further reduce the possibility of bacterial natural selection and evolution.

3.7. Application on protecting jet fuel from microbial contamination

As analyzed above, it was proved that the antimicrobial composite coating was chemically stable for at least one-week, non-leaching and highly efficient to inactivate microorganisms. The test on protecting real jet fuel from microbial contamination was conducted. According to Fig. 10, when the jet fuel contaminated with microorganisms was

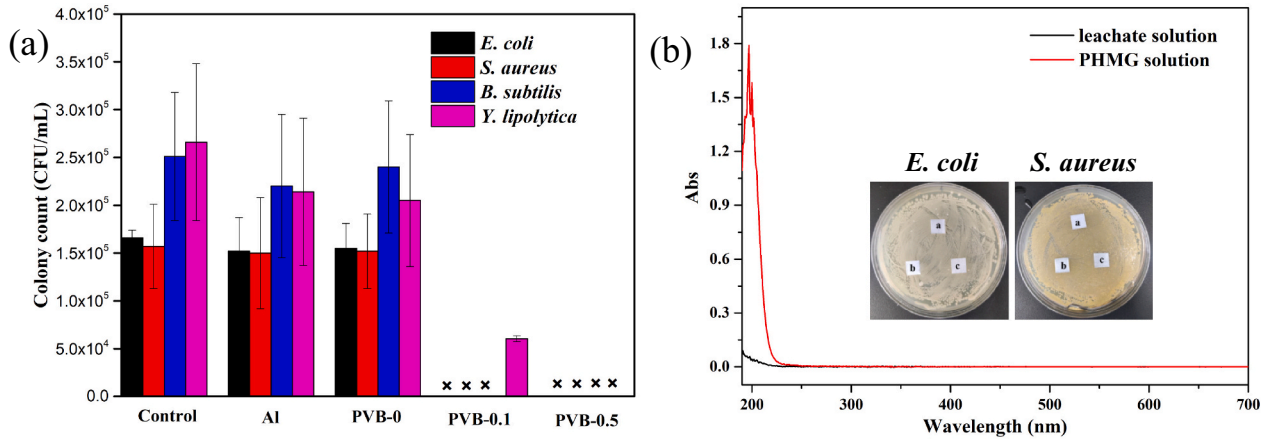


Fig. 5. (a) Microbicidal activity of different Als against *E. coli* and *S. aureus*, *B. subtilis* and *Y. lipolytica* microbial colonies, (b) UV absorption spectra of leachate solution of PVB-0.5 in DI water for 3 d and inhibition zone (a: PVB-0, b: PVB-0.1, c: PVB-0.5).

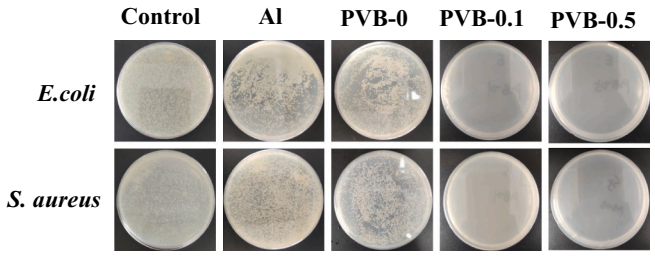


Fig. 6. Antimicrobial photographs against *E coli* (~10^5 CFU/mL) and *S. aureus* (~10^5 CFU/mL) of Al, PVB-0, PVB-0.1, PVB-0.5 after soaking in jet fuel for 7 days.

treated with pristine Al sheet or PVB-0 coated Als, the microorganisms still grew vigorously that could be attributed to the jet fuel containing possible essentials for microbial proliferation. However, compared to the jet fuel treated by the pristine Al sheet, the number of mixed microorganisms (~10^6 CFU/mL) in the jet fuel cultured with PVB-0.1 coated Als after a period of only 30 min decreased dramatically by 57.48%, and >99.999% reduction was recorded in that treated by PVB-0.5 coated Als. On the whole, PVB/PHMG-SS composite coating on aluminum substrate was highly effective on killing microorganisms in jet fuel, which was critical to protect the quality of fuel products and avoid microbial corrosion to fuel tank.

4. Conclusion

In brief, a highly antimicrobial surface was developed based on a water-insoluble polymeric guanidine derivative and polyvinyl butyral on aluminum substrate. Polyelectrolyte-surfactant complex (PHMG-SS) characterized by water-insoluble and prominent antimicrobial activities was synthesized, and the complex was facilely coated on aluminum substrate through a simple and scalable method. The produced antimicrobial coating exhibited hydrophilic characteristic, excellent UV resistance and demonstrated short term compatibility with jet fuel. Additionally, good disinfection was demonstrated by kills of 99.999% of *E. coli*, *S. aureus*, *B. subtilis* and *Y. lipolytica* within 30 min for PVB-0.5 Als. Moreover, the composite coating exhibited the advantage of non-leaching, thereby reducing the threat to environmental safety and human health. In addition, the coated aluminum substrate, such as PVB-0.5, could strongly inactivate mixed microorganisms (*E. coli*, *S. aureus*,

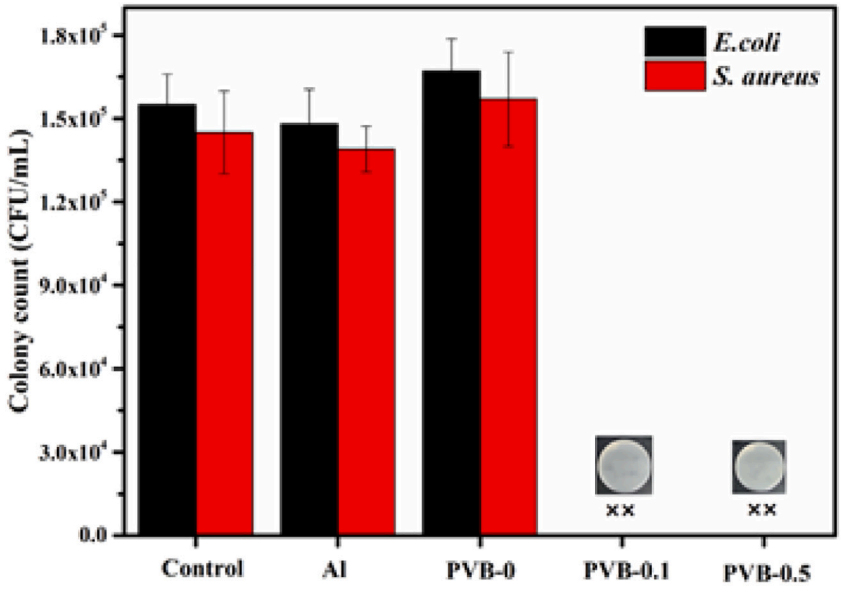


Fig. 7. The result of antibacterial test after irradiating blank substrate and coating sample under UV light for 7 days.

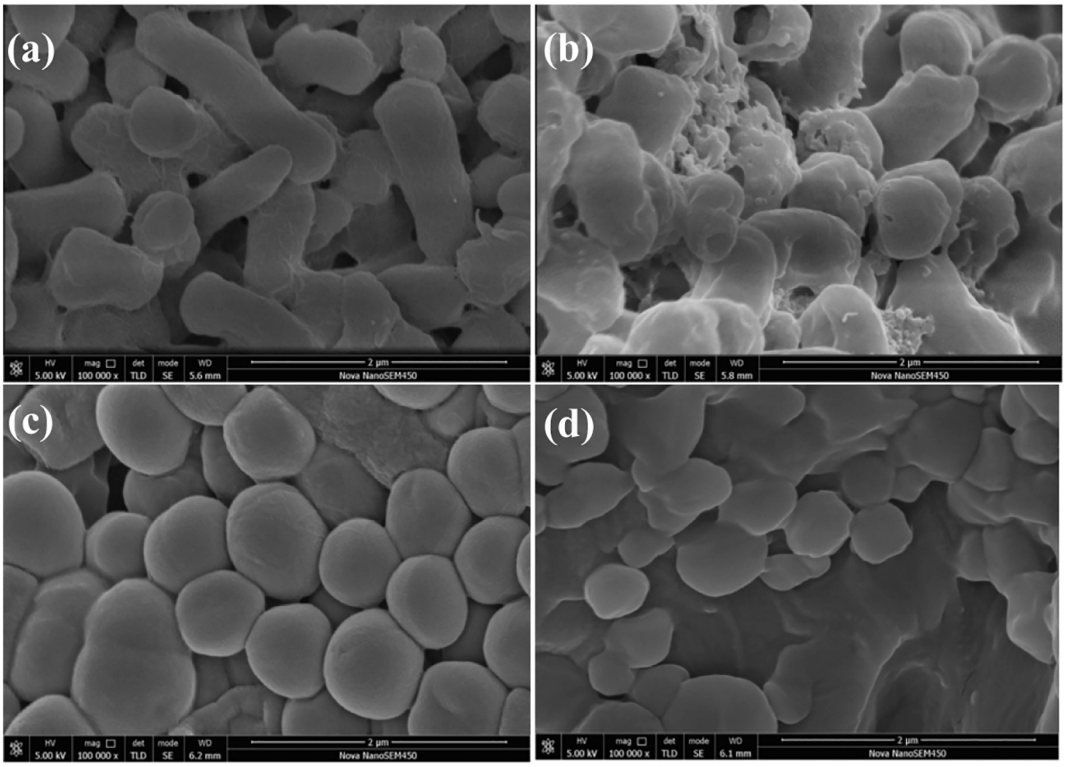


Fig. 8. SEM images of *E. coli* (a), *S. aureus* (c) in control groups and *E. coli* (b), *S. aureus* (d) in PHMG-SS treated groups.

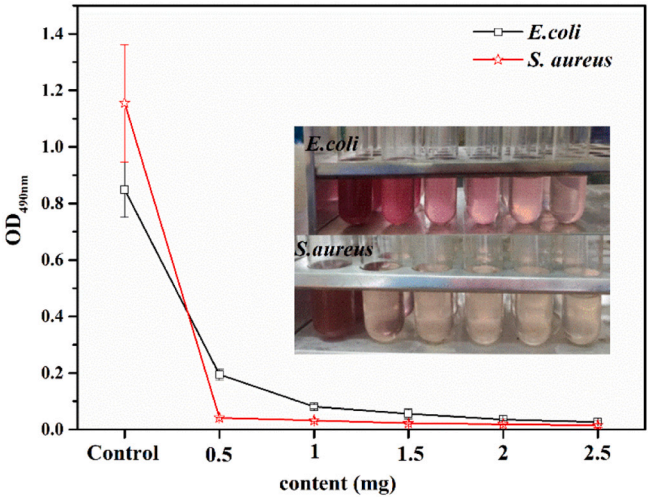


Fig. 9. The photograph and the corresponding OD values of *E. coli* and *S. aureus* suspensions mixed with TCC of control group and PHMG-SS treated group.

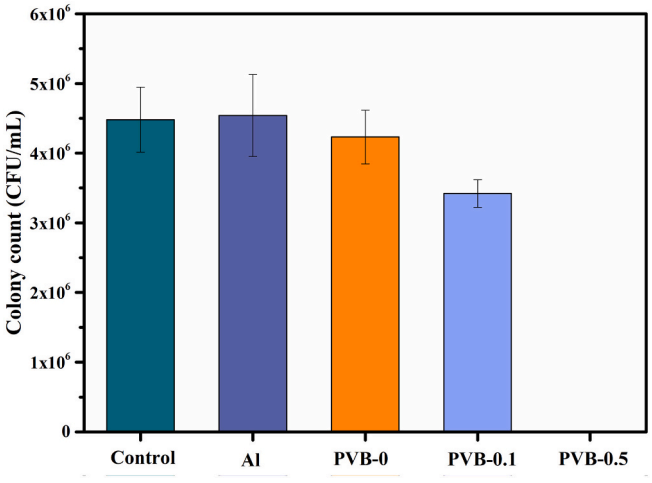


Fig. 10. Inactivating efficiency of coated Als on mixed microorganisms in jet fuel.

B. subtilis and *Y. lipolytica*) with the concentration of ~10^6 CFU/mL through breaking the bacterial integrity of bacterial structure and suppressing the enzymatic activity. This coated aluminum substrate exhibited the potential application as the interior surface of jet fuel tanks to protect jet fuel from microbial contamination. Furthermore, the application of the developed antimicrobial coating and the facile coating technology on aluminum sheet could be informative during aircraft manufacture or retro-coating process, and have the potential to substantially reduce the risk of microorganism proliferation and wing tank biodeterioration.

CRediT authorship contribution statement

1) Miss. Jie Zhang completed the majority of the experiments and characterizations and partially wrote the first draft;
2) Mr. Hao Luo completed partial experiments, completed the first draft and revisions;
3) Mr. Xueqian Yin provided some assistance on data analysis;
4) Prof. Yidong Shi provided many suggestions on the design of this work, and also helped to revise the manuscript;
5) Dr. Yong Zhang provided some assistance on improving the manuscript;

6) Dr. Lin Tan proposed the idea, provided the research platform, guidance on data analysis, helped to write and finalize the manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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