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Effect of a marine bacterial biofilm on adhesion and retention of pseudo barnacle to silicone coating surface

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Abstract−The effect of a marine bacterial *Cytophaga lytica* (*C. lytica*) biofilm on the adhesion and retention of a pseudobarnacle (epoxy adhesive) to platinum-cured silicone coatings was investigated at varying coating thickness (100-800 μm), modulus (E=0.08-1.3 MPa), and shear rate (2-22 μm/s). The initial adhesion of *C. lytica* biofilm on the silicone coating surfaces was increased as the coating modulus was increased. Nonetheless, the adhesion strength of the pseudobarnacle was not significantly influenced by the attached biofilms, with its strength decreasing with increasing the coating modulus. Thus, these results suggest that the pseudobarnacle adhesion strength would be primarily determined by physico-mechanical properties of the silicone coatings. Also, the adhesion/detachment tests demonstrated that the retention of the pseudobarnacle after water jetting was minimal for the soft silicone coating (VP1), which showed better performance than the widely acceptable silicone resin of DC 3140.

Keywords: Biofilm, Biofouling, Silicone Coating, Pseudobarnacle, Adhesion

INTRODUCTION

Biofouling by marine organisms is widely found on ship hulls, resulting in an increase in maintenance cost and fuel consumption. One effective way to minimize fouling is to release toxic metal compounds in self-polishing antifouling marine coatings at a controlled rate, but accumulation of the toxic chemicals in a harbor simultaneously results in serious environmental problems [1,2]. In an effort to develop environmental friendly marine coatings to replace the conventional paints, release mechanisms of silicone-based materials have been extensively studied [3-9]. Low surface energy and modulus of typical silicone-based coatings have been found to play a crucial role in easy removal of barnacles and pseudobarnacles from a coating surface. Incorporation of a non-reactive fluid to a coating system is also known to decrease barnacle adhesion strength due to the migration of the fluid to the surface [10,11]. However, the adhesion and release characteristics of silicone-based coatings may be seriously altered upon immersion in sea water by rapid accumulation of organic molecules such as proteins, polysaccharides, and proteoglycans on the surface. This molecular fouling, so-called "conditioning film," makes the surface more favorable for attachment of bacteria by electrostatic attraction and physical forces [12-14].

As a result, the weakly adsorbed bacteria more strongly adhere to the surface with their extracellular polymeric substances, on which larvae of many marine invertebrates may selectively settle and metamorphose [15,16]. According to previous studies [17-19], the attachment of marine bacteria was significantly influenced by critical surface tension of substrates, exhibiting a minimal biological adhesion at below 25 mJ/m^2. However, such a correlation of bio-adhesion and critical surface tension was often contrary to each other [20].

The adhesion of marine bacteria *Cytophaga lytica* (*C. lytica*) has been carried out on various silicon coating substrates. *C. lytica* tends to more strongly adhere to a hydrophobic surface than a hydrophilic surface. Even though bacterial strains show a different correlation between bio-adhesion and critical surface tension, marine bacteria are well known to modify the surface properties of the marine coatings. As a result, the silicone-based coatings may lose their initial release properties and differently interact with macro-foulants. To address this issue, we studied the adhesion strength of pseudobarnacle on the silicone-based coating that was modified with *C. lytica* biofilm, to identify the effects of the biofilm on adhesion force at various experimental conditions. *C. lytica* is a marine bacterium used to evaluate fouling-release performance of potential marine coatings [21]. In addition, shear adhesion tests were also performed for the virgin silicone coatings, and the adhesion forces were compared with those obtained from the biofilm-covered silicone coatings.

EXPERIMENTAL

1. Materials

Two different vinyl-terminated polydimethylsiloxanes (V21: MW= 6 kg/mole, V35: MW=49.5 kg/mole), trimethylsiloxy terminated polydimethylsiloxane (DMS-T35: MW=49 kg/mole), a crosslinker tetrakis (dimethylsiloxy)-silane (SIT278.0), and a catalyst platinum-divinyltetramethyldisiloxane complex (SIP6830.3) were purchased from Gelest, and methylisobutylketone (MIBK) was obtained from Sigma-Aldrich.

2. Silicon Coating Preparation

Silicone coating solutions were prepared by thoroughly mixing

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Table 1. Mixing ratio, modulus, water contact angle and surface energy of various virgin silicone coatings used in the experiments. All experiments results were performed at least 10 times (n>10)

Samples	V35 (MW=49 kg/mole)	V21 (MW=6 kg/mole)	Modulus (MPa)	Water (degree)	Surface energy (mJ/m^2)
VP1	1	0	0.08	117	17
VP2	0.75	0.25	0.2	117	16
VP3	0.5	0.5	0.7	118	18
VP4	0.25	0.75	1.0	116	18
VP5	0	1	1.3	117	19

vinyl-terminated polydimethylsiloxanes (silicone resins), tetrakis (dimethylsiloxy)-silane (crosslinker), platinum-divinyltetramethyl-disiloxane complex (catalyst) and MIBK solvent. The two different silicone resins, V21 and V35, were mixed at five different weight ratios to vary coating modulus. Five V35-V21 formulations with 0 (V1), 0.25 (V2), 0.5 (V3), 0.75 (V4) and 1 (V5) wt% V21 monomer were prepared, as illustrated in Table 1. Coating modulus was also varied by changing the crosslink density, which was obtained by measure the deformation of the specimen swollen in toluene [22]. Coating thickness was controlled by gently pouring different amounts of the homogeneous solution onto silane-treated glass microscope slide. The coatings were cured at ambient condition for at least one week before tests and the thickness was measured with a digital caliper. The modulus and thickness of the coating were in the range of 0.08-1.3 MPa and 100-800 μm, respectively.

3. Coating Deposition in a Multi-well Plate

A 400 μl aliquot of a coating solution was dispensed in each well of a 24-well polystyrene plate using a micropipette for biofilm retention and water-jet adhesion tests. A coating formulation was deposited in two columns of the plate. A commercial silicone resin DC 3140 (Dow Corning Corp.) was used as a control. After the silicones in the plate were cured at ambient condition, all the plates were pre-leached in a re-circulating deionized water tank for at least one week.

4. Marin Bacterial Biofilm Treatment

C. lytica was generously provided by Dr. Park of the Coastal Engineering & Ocean Energy Research Department, Korea Institute of Ocean Science and Technology [15]. The 40 ml, artificial sea water nutrient medium (ASWNM) was inoculated with 0.5 ml of an overnight culture of the marine bacterial *C. lytica* and incubated at 28 °C for 24 h. After the *C. lytica* culture was centrifuged, the supernatant was discarded. The collected bacterials were resuspended in 35 ml ASWNM and centrifuged again. The 35 ml bacterial cell suspension was added to 800 ml of ASWNM and mixed well and the suspended solution was slowly poured into a sterilized dish and 24-well plate, which were pre-leached in distilled water for one week. The dish was placed in an incubator at 28 °C for 24 h. Each glass slide was individually lifted out of the culture media with forceps and dipped in distilled water three times to remove any non-attached bacteria. The biofilm-treated glass slide was then allowed to dry at ambient conditions for one week before pseudobarnacle adhesion tests.

5. Water-jet Test

The 24-well plates were then transferred to the water tank of an automated water-jet apparatus for biofilm adhesion tests. The first column was not treated with the water-jet and used to determine the initial amount of biofilm retained on each coating as control. The second column of each coating was treated with a jet pressure of 20 and 30 psi for 5 seconds. After water-jet treatments, each coating surface was rinsed with distilled water three times and dried at room temperature for 1 h. Then, the coating surfaces were stained with crystal violet (CV) as biomass indicator. To elute CV dye solution, each plate was extracted with 33% glacial acetic acid. Each eluate was transferred to ELISA plate and measured for absorbance at 600 nm using multi-well plate reader [23].

6. Contact Angle Measurement and Surface Energy

Static advancing contact angles of water on five different virgin silicone coatings and the biofilm-treated coatings were measured by using a home-built contact angle apparatus. The image of the drop was recorded with DVD and analyzed with data acquisition software after 1 min. Then, static advancing contact angles were measured by drawing a line at the edge of the drop using image analysis software. The surface energies of the specimens were calculated from the Owens-Wendt equation. Contact angle measurements can be related to surface tensions or energies via Young's equation:

$$\gamma sv=\gamma sl+\gamma lv\cos\Theta \quad (1)$$

where θ is the measured contact angle and γ is the surface energy of the solid-vapor (sv), solid–liquid (sl) and liquid-vapor (lv) interface. Depending on the model used, an expression for ãsl can be combined with Eq. (1) and results in a relation between the contact angle and the liquid's surface tension from which the solid's surface energy can be extracted. Water contact angle and surface energy of the five different virgin silicone samples are shown in Table 1.

7. AFM Analysis

Imaging of the marine bacteria biofilm onto various silicone coatings was obtained using the Dimension 3100 equipped with a Nanoscope IIIa controller (Veeco Instruments, CA, USA). The AFM measurements were at ambient conditions (25 °C) using tapping mode probes with constant amplitude. A silicon cantilever with a spring constant of 1-5 N/m and an oscillation frequency of 75 kHz (FESP, Veeco instruments, CA, USA) was used to scan the surface of the biofilm-covered silicone coatings. The scan rate was 1.0 Hz and the resolution was 512 samples per line. Scanning was performed at three different positions of each sample and representative images are shown here. The adhesion force was determined from force-distance curve obtained from contact mode AFM that was conducted at room temperature in dry air to avoid the influence of capillary condensation of water. The silicon nitride probe (NP20, Veeco instruments, CA, USA), having a square pyramidal shape with a spring constant 0.12 N/m was used for this analysis. For a given sample the images and force data obtained in air were stable and reproducible with repeated AFM operation.

8. Adhesion Tests

Shear adhesion tests were conducted at four different shear rates

(2, 7, 12, 22 mm/s). Three aluminum studs (Diameter: 7.2 mm) were glued to the biofilm-covered/virgin silicone coatings with a commercial epoxy adhesive (Loctite, 1C-LV, Hysol) after 1 week. A maximum adhesion force was measured using data acquisition software (Labview 7) during the detachment of the pseudobarnacle from the silicone coatings at the constant shear rates. After the adhesion test, the real contact area of the stud was calculated by image analysis (Labview Vision Assistant 7). A combined design method provided by Stat-Ease Inc. (Design Expert 7.0) was used to statistically analyze the effect of modulus, thickness, and shear rate on pseudobarnacle adhesion strength of the virgin silicone coatings and the biofilm-treated silicone coatings. The adhesion apparatus is described in detail elsewhere [24].

RESULTS AND DISCUSSION

1. Surface Energy

The surface energy of the virgin silicone coatings was calculated using the Owens-Wendt equation [25], yielding 18±2 mJ/m^2. Fig. 1 shows the water contact angle images of VP1 and VP5 silicon coating without and with *C. lytica* marin bacteria biofilm. The water drops onto VP1 and VP5 virgin silicon coatings (Fig. 1(a) and 1(d)) barely changed the shape within a time, whereas the water drops onto the *C. lytica* biofilm treated coatings (Fig. 1(b) and 1(e)) were rapidly spreading on the surface after 1 min. This suggests that the biofilm significantly modifies the wetting properties of the silicone coatings. As shown in Fig. 1(b) and 1(e), the water contact angles of VP1 and VP5 (covered with a biofilm) were smaller than those for the virgin silicon coatings, resulting in higher surface energies (38-48 mJ/m^2) than the virgin coatings (18 mJ/m^2). This is probably because the biofilm produces the polysaccharides by marine bacteria.

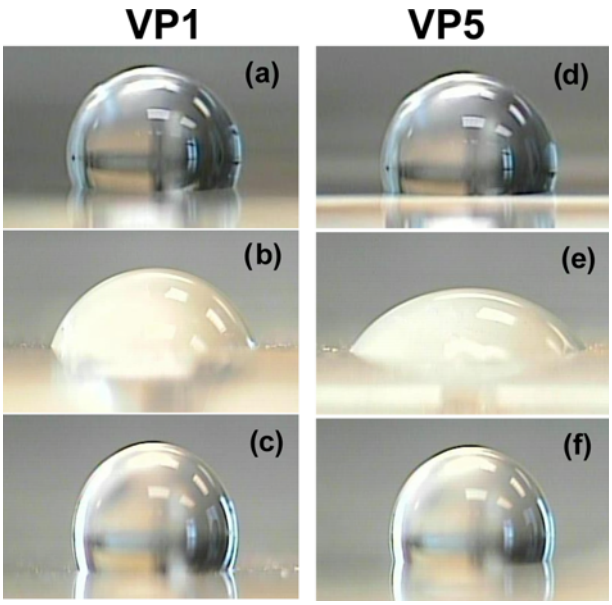

Fig. 1. Measurements of water contact angle of two different silicone coating surfaces: (a) Virgin silicon VP1, (b) biofilm-covered VP1, (c) after pseudobarnacle detachment from biofilm-covered VP1, (d) virgin silicon VP5, (e) biofilm-covered VP5, and (f) after pseudobarnacle detachment from biofilm-covered VP5.

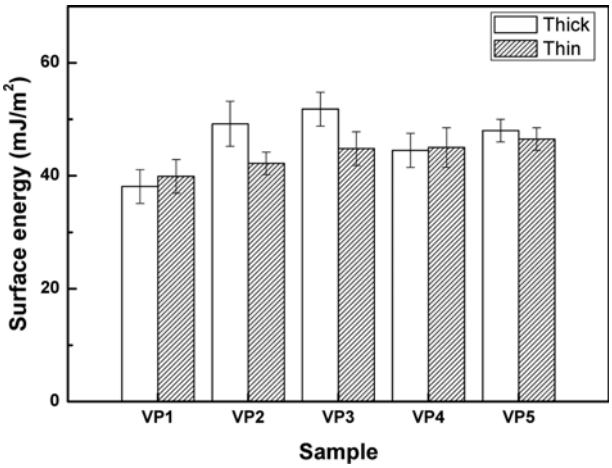

Fig. 2. Surface energy of various silicon coatings covered with *C. lytica* biofilm with different coating thickness. The thickness of thick and thin coatings was 750 and 200 μm, respectively.

Interestingly, the water contact angles onto the detached spots of the coating showed slightly higher contact angles compared with the virgin coatings (Fig. 1(c) and 1(f)). The water contact angles of various coatings with *C. lytica* biofilm ranged from 50 to 65°, which was in agreement with the data reported by Grasland et al. [26].

In Fig. 2, the surface energies of various silicone coatings covered with *C. lytica* biofilm are shown as a function of coating modulus at different thickness. As shown, the surface energy of *C. lytica* biofilm-covered silicone coatings appears to increase from 38 to 48 mJ/m^2 with increasing the coating modulus, except for some deviation of VP2 and VP3 coatings with the higher thickness. In the case of a thick coating with 750 μm thickness, the surface energy (38 mJ/m^2) of the bacterial-covered soft VP1 coating (E=0.08 MPa) was smallest of the samples tested. The surface energy of a thin coating with 200 μm showed a monotonous increase with increasing the modulus from VP1 to VP5. Detailed statistical analysis demonstrated that the least significant difference (LSD) bars of thin and thick coatings were not overlapping with each other, suggesting that the effect of the coating thickness was not significant.

2. Pseudobarnacle Adhesion onto Biofilm

Shear adhesion measurements were conducted to understand the effect of the physic-mechanical properties of silicon coatings on barnacle release force using a barnacle reattachment technique. This technique can be utilized as an alternative way to rapidly measure adhesion strength of the adult barnacles compared to field tests. The mean shear stress of the barnacles required to detach from the platinum cured silicones tested was in the range 0.032-0.13 MPa, depending on coating thickness and modulus. For the pseudobarnacle tests, the bacterial film did not seriously alter adhesion characteristics of the silicone coatings, showing a similar thickness and modulus dependence to the virgin silicone coatings.

Fig. 3 shows the maximum pseudobarnacle shear stresses of the virgin and biofilm-treated silicone coatings as a function of coating thickness at two different shear rates. The results demonstrated that the coating thickness and modulus were two significant factors influencing the adhesion force for the virgin coatings as well as the biofilm-treated coatings. In both shear rates, the maximum shear adhe-

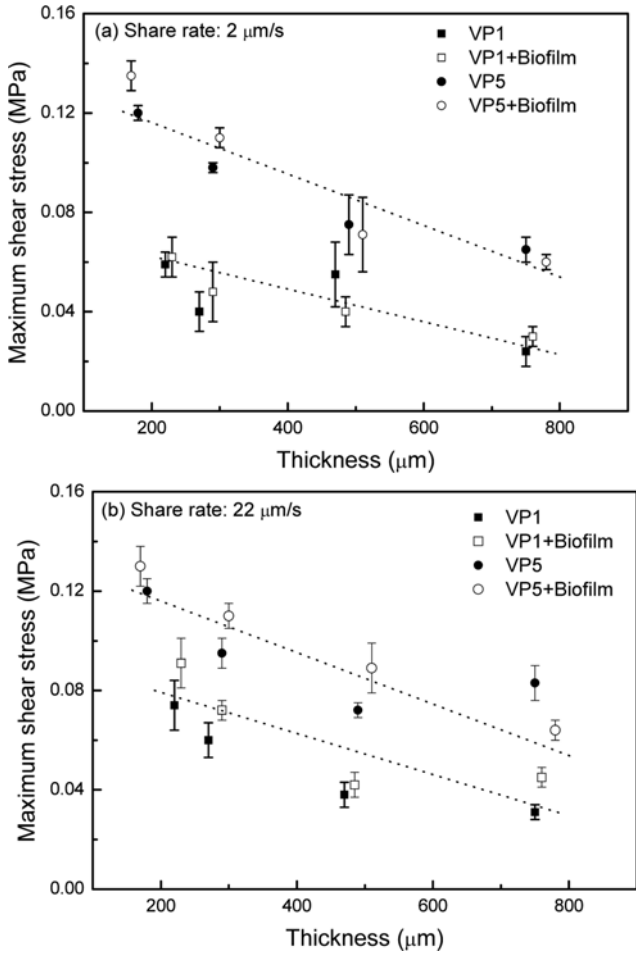

Fig. 3. Maximum shear stress for the detachment of pseudobarnacle of the platinum cured silicon coatings and marine biofilm-covered ones at two different shear rates: (a) 2 μm/s and (b) 22 μm/s. The square and circle symbols represent the VP1 (E=0.08 MPa) and VP5 coatings (E=1.3 MPa), respectively.

sion for detachment was decreasing with increasing the coating thickness. For the lowest modulus coating (VP1 and VP1+biofilm), mean pseudobarnacle adhesion forces ranged from 0.032 to 0.083 MPa. The values ranged from 0.074 to 0.13 MPa for the highest modulus coatings (VP5 and VP5+biofilm), suggesting that the adhesion force becomes higher with a thicker coating. Shear rate is believed to be an important factor especially for the soft VP1 coatings, but it turned out that the coating thickness and modulus were more dominant than the shear rate. Based on the results above, the detachment force of the pseudobarnacle from the biofilm-covered silicone coatings may be determined mainly by physico-mechanical properties of the coatings such as modulus and thickness as compared to chemical properties like surface energy.

3. AFM Images and Adhesion Force Measurement

When the silicone coatings are immersed in *C. lytica* marine bacterium solution, the micro-organism rapidly adsorbs on the coating surface. Fig. 4 shows topographic and phase images of VP1 and VP5 biofilm-covered coatings without non-reactive silicone oil, in which the average root mean square (RMS) surface roughness was 40.9 and 36.5 nm for a scan area of 20 μm×20 μm, respectively.

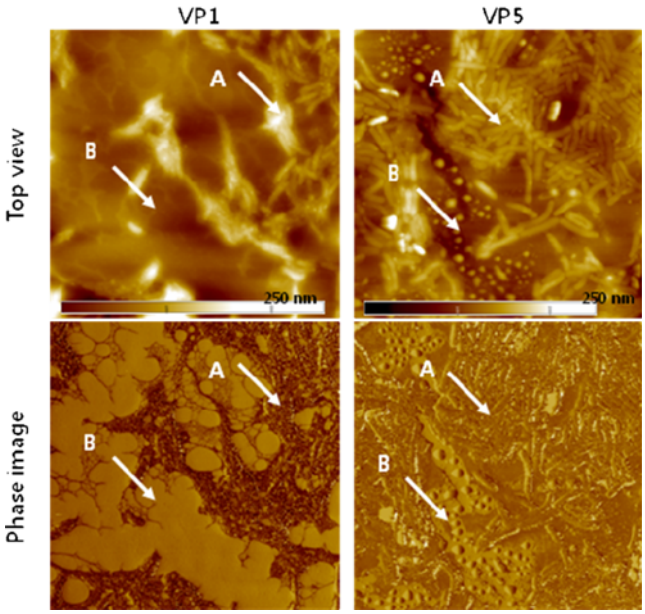

Fig. 4. Topographic (top) and phase (bottom) images for VP1 and VP5 coating surfaces that are covered with marine bacterial biofilm: (a) VP1 coating with roughness of 40.9 nm and (b) VP 5 coating with roughness of 36.5 nm. Scan size is 20× 20 μm. The coating thickness is 750 μm. The white arrows indicate the marine bacteria (A) and the bare silicone surface (B).

For the hard coating (VP5), the surface appears to be almost fully covered with *C. lytica* compared with that of the soft coating (VP1). The difference between the silicone and marine bacteria is well represented by the phase images in Fig. 4 as indicated by the white arrows of A and B.

To calculate the contact and adhesion forces of the bacteria, force-distance plots were obtained from contact mode AFM. Pembrey et al. [27] reported that air drying affected some cell properties, such as affinity to Sepharose columns, attachment to solid substrate, and electrophoretic mobility of cells, but had little or no effect on hydrophobicity and viability. Thus, dehydration effects, if any, would probably occur immediately for a short time after samples treatments. Adhesion force was calculated from the pull-off portion of the retracting curve for the VP1 and VP5 samples covered with biofilm. Similarly, the contact force was calculated from the horizontal distance curve traversed by piezo in the Z-direction. In the case of VP1 biofilm, the pull-off adhesion force of the marine bacteria was lower (190.0±2 nN) than that that of VP5 (377.2±2 nN). These results are in good agreement with those obtained using the pseudobarnacle adhesion tests.

4. Biofilm Settlement and Retention

Fig. 5 shows the relative UV absorbance of the crystal violet bound to the bacterial film on five different silicone coatings deposited in the 24 well plate before and after water jetting. The degrees of absorbance of crystal violet were taken at 600 nm. Here, the UV absorbance is proportional to the biofilm mass remaining on the surface. The relative absorbance of the soft VP1 coating with respect to the control commercial DC 3140 was 0.85±0.06, indicating that the biofilm formed less on the surface than the control. Furthermore, the biomass retained on the soft coating was lower than the other

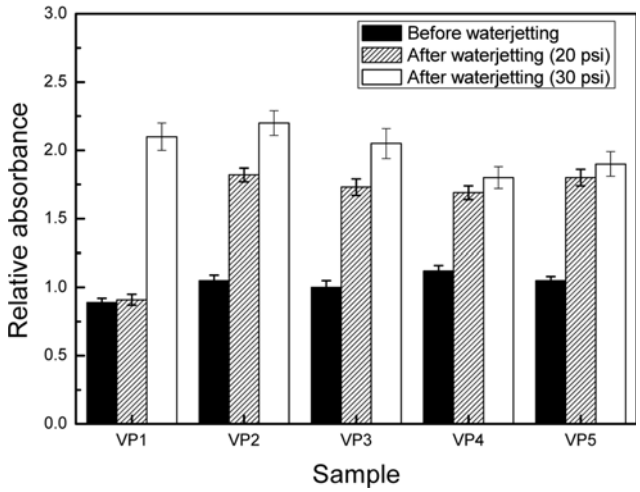

Fig. 5. Relative UV absorbance of the five different silicone coating samples with respect to DC 3140 before and after water jetting for two different jetting pressures (20 psi and 30 psi).

harder silicone coatings. Note that the biofilm retention of the virgin silicone coatings is well correlated with the modulus of the bacterium-covered coatings. After water jetting at 20 psi pressure for 5 sec, there was a significant difference in the relative absorbance between the soft VP1 coating and the other coatings. The soft coating showed the best performance in terms of bacterial retention, yielding a relative absorbance of 0.93±0.07. However, as the water jetting pressure increased to 30 psi, the biomass retained on the five different silicone coatings was almost the same without any appreciable changes among the samples tested.

Together with the shear adhesion measurements, the biomass retention on various silicone coatings demonstrated that the formation of a bacterial film is highly limited on the soft VP1 silicone coating (E=0.08 MPa). This implies that the physico-mechanical property of the coatings affects the bacterium settlement and retention in addition to their chemical and biological properties. This is in accordance with a recent work by Gray et al. [28] in which they reported that the settlement density of some larvae increased as the elastic modulus of the silicone coating (0.01-0.1 MPa) increased. In a separate study of the physico-mechanical properties of silicone coatings on the settlement of green alga Ulva linza [5], no appreciable effect of the elastic modulus (0.2-9.4 MPa) and thickness (16-430 μm) was found. In the current study concerning the modulus effect on *C. lytica* settlement, the bacterium did not show any settlement preference on coating thickness, as can seen from our shear adhesion measurements. The marine foulants would respond differently to the physico-mechanical properties of the coatings of different thickness. These interactions collectively lead to minimal biofouling and result in a better release performance due to weak interactions between the biofilm and the silicone coatings [5,29].

Fig. 6 shows the optical microscopy images of the biofilm-treated silicone coatings and the detached pseudobarnacles after being stained with crystal violet. When the pseudobarnacle (epoxy) was applied to the surface of the biofilm-covered coatings, the epoxy was more strongly bound to the biofilm than the silicone coatings, which eventually resulted in a complete biofilm transfer to the epoxy side. Thus, no trace was observed on the detached spots of the coatings and

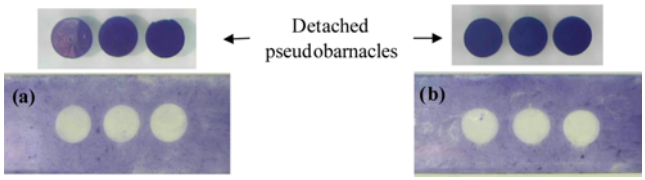

Fig. 6. Optical microscopic images of the biofilm-covered silicone coatings and the detached pseudobarnacles (epoxy) after being stained with crystal violet: (a) VP1 coating and (b) VP5 coating. The coating thickness is 750 μm. As shown, the biofilm was completely transferred to the detached epoxy side.

the contact angles on the spots recovered to the value of the virgin silicone coatings. As illustrated in the crystal violet test and the surface energies on the pseudobarnacle-detached spots of coating, adhesion failure occurred at the interface of the biofilm and the silicone coating. Therefore, the adhesion strength is in the order of epoxy-biofilm>coating-biofilm≈coating-epoxy. This also suggests that the micro-organism weakly adhered to the silicone surface possibly by van der Waals force rather than hydrogen or covalent bonds.

CONCLUSIONS

We performed adhesion and retention experiments of pseudobarnacle on silicone coatings with different coating modulus and thickness to evaluate fouling performance of potential marine coatings and to understand their release mechanisms. Shear adhesion tests as well as relative absorbance measurements demonstrated that the adhesion strength of pseudobarnacle was primarily determined by physico-mechanical properties of the silicone coatings such as modulus and thickness as compared to chemical and biological properties. The contact angle measurements indicated that the biofilm modified the surface properties from hydrophobic to hydrophilic and tended to strongly adhere to the hard coatings. Nonetheless, no significant effect of the biofilm on the pseudobarnacle adhesion strength was observed compared with the biofilm-free silicone coatings. Of the five silicone coatings with different modulus ranging from 0.08-1.3 MPa, the softest coating named VP1 showed the best performance in terms of the adhesion and retention of the pseudobarnacle, which would be useful to gain an understanding on adhesion and releasing mechanism of the marine coatings and to assist the widely accepted silicone resin of DC 3140.

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