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REVIEW

Advances in the treatment of problematic industrial biofilms

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Abstract In nature, microorganisms tend to form biofilms that consist of extracellular polymeric substances with embedded sessile cells. Biofilms, especially mixed-culture synergistic biofilm consortia, are notoriously difficult to treat. They employ various defense mechanisms against attacks from antimicrobial agents. Problematic industrial biofilms cause biofouling as well as biocorrosion, also known as microbiologically influenced corrosion. Biocides are often used to treat biofilms together with scrubbing or pigging. Unfortunately, chemical treatments suppress vulnerable microbial species while allowing resistant species to take over. Repeated treatment cycles are typically needed in biofilm mitigation. This leads to biocide dosage escalation, causing environmental problems, higher costs and sometimes operational problems such as scale formation. New treatment methods are being developed such as enhanced biocide treatment and bacteriophage treatment. Special materials such as antibacterial stainless steels are also being created to combat biofilms. This review discussed some of the advances made in the fight against problematic industrial biofilms.

Keywords Biofilm · Biocide · D-Amino acid · Chelator · Norspermidine · Bacteriophage · Antibacterial stainless steel · Biocide resistance

Introduction

A community (also known as consortium) of microorganisms that adhere to abiotic surfaces is defined as a biofilm (Hall-Stoodley et al. 2004). Biofilms in nature typically consist of multiple synergistic microbial species, but pure-strain microbes can also form biofilms (O'Toole et al. 2000). The microbial cells embedded in a biofilm matrix are known as sessile cells while the cells suspended in the bulk fluid are called planktonic cells. Both types of cells have identical genotypes, but their phenotypes may differ (Donlan 2002). Most microorganisms in nature live in a biofilm (McDougald et al. 2012). Biofilms secreted extracellular polymeric substances (EPS) to embed sessile microbes. EPS provides a biofilm with protection to fend off harmful environmental conditions such as fluid shear stress, pH swings and antimicrobials (Hall-Stoodley et al. 2004; Zuo 2007).

Biofilms cause biocorrosion, biofouling (clogging) and reservoir souring. They decrease the quality of crude oil and they induce potential environmental problems and failures of infrastructures (Gittens et al. 2013; Kahrilas et al. 2015). Biocorrosion that is also known as microbiologically influenced corrosion (MIC) causes billions of dollars of economic losses in the USA each year. Many microorganisms involved in MIC and biofouling. They include sulfate reducing bacteria (SRB), acid producing bacteria, iron

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oxidizing bacteria. Archaea and fungi also get involved in the MIC process (Larsen et al. 2010). Physical scrubbing and chemical treatment methods are commonly used to mitigate problematic industrial biofilms (Carew et al. 2009). These two methods are often combined because physical scrubbing (or pigging in the pipeline industry) alone is often inadequate. Sessile cells close to a metal surface are directly responsible for MIC, because they can utilize extracellular electrons or they secrete corrosive metabolites at locally high concentrations underneath the biofilms (Xu et al. 2013). Planktonic cells in the bulk fluid usually are not directly involved in the MIC process. Thus, the control of problematic biofilms is the key to MIC mitigation.

Sessile cells are far more difficult to mitigate than planktonic cells. High dosages of biocides are typically required in field applications to eradicate sessile cells because of the various defense mechanisms employed by biofilms. They include a diffusional barrier to slow down the penetration of biocides, purposely lowered metabolic rate to reduce biocide intake, preservation of persister cells, upregulation of resistance genes that code for proteins to degrade antimicrobials, and efflux pumps to pump out harmful chemicals (Li et al. 2016c). It was reported that 10 times or higher concentrations of biocides are needed to mitigate sessile cells than those needed for planktonic cells (Videla 1996).

In many industrial systems, the complete eradication of biofilms is impractical due to various reasons. For example, the required biocide dosage or treatment time may be excessive. Sometimes, it is futile to do a complete job because flow will introduce microbes again. Unlike humans, industrial systems do not possess an immune system to combat residual microbes after biofilm treatment. This means biofilms will bounce back. Thus, repeated treatment cycles are likely needed as is the case of oil and gas operations. In any biocide treatment, the continued use of the same biocide will inevitably promote resistant microbes (Vance and Thrasher 2005). Over time, this leads to dosage escalation that causes environmental stress, cost increase and sometimes operational problems. For example, when the tetrakis hydroxymethyl phosphonium sulfate (THPS) dosage is sufficiently high, this biocide introduces a high concentration of sulfate that causes scale formation due to barium sulfate precipitation in the drilling fluid.

Although there are many biocides that are marketed, only a few of them are suitable for large-scale applications such as oilfield applications. So far, THPS and glutaraldehyde are the two dominant biocide choices in oil and gas operations because of their excellent broad-spectrum efficacy, cost, biodegradability and safety profile. They are also used in water treatment and other operations. While the pursuit for new antimicrobials is ongoing, the chances for a new blockbuster biocide on the market any time soon are not optimistic based on the track record in the past four decades. Thus, researchers have been searching for other effective ways for biofilm mitigation. This work is a review of these new approaches.

D-Amino acids as biocide enhancers

Using biocide enhancers is one way to lower biocide dosages. Surfactants have long been used in biocide blends to help distribute biocides (Simões et al. 2006). Biocide enhancers do not need to be biostatic or biocidal themselves. For example, if a chemical agent disperses sessile cells and converts them into planktonic cells, it can be used as a biocide enhancer. This is because planktonic cells are far more susceptible to antimicrobial agents. Biofilm dispersal occurs owing to the presence of dispersal factors or signals, physiological cues and changes in nutrients, etc. (McDougald et al. 2012). One recently discovered biofilm dispersal agent is D-amino acids (Kolodkin-Gal et al. 2010).

D-Amino acids were once considered rare in nature decades ago, unlike L-amino acids that are used in protein synthesis. Nowadays, with advanced analytical tools and due to increased interests in their biological functions, D-amino acids are found to be ubiquitous. They are widely distributed in microorganisms, plants, animals, and even in humans (Konno 2007). A significant percentage of D-amino acids among all amino acids can be found in various foods (Man and Bada 1987). The biological function of D-amino acids in biofilm regulation is still not clear. It is suspected that D-amino acids serve as a signal molecular. Cava et al. (2011) suggested D-amino acids were necessary for the remodeling of cell wall structures. Lam et al. (2009) stated that D-amino acid synthesis might be a common method of self-adjustment of bacterial cells to adapt to their changing local environment. Some D-amino acids are hypothesized to modify the synthesis of peptidoglycan molecules that exist in all bacterial cell walls (Lam et al. 2009).

Kolodkin-Gal et al. (2010) found that D-methionine (D-met), D-tyrosine (D-tyr), D-leucine (D-leu), and D-tryptophan (D-trp) triggered the *Bacillus subtilis* biofilm disassembly. Hochbaum et al. (2011) confirmed that D-amino acids signaled the *B. subtilis* biofilm disassembly. A glycopeptide dendrimer containing D-amino acids was found to inhibit the biofilm formation of *Pseudomonas aeruginosa* (Johansson et al. 2011). Xu and Liu (2011) demonstrated that D-tyr dispersed biomass build-up on microfiltration membranes. They suggested that the mixture of four aforementioned D-amino acids at a relatively small overall concentration (10 nM) dispersed the *B. subtilis, Staphylococcus aureus, P. aeruginosa* biofilms (Kolodkin-Gal et al. 2010).

D-Alanine exists in the peptidoglycan molecules in all bacterial cell walls (Cava et al. 2011). Figure 1 shows that the D-alanine terminus in both types of peptidoglycan molecules (Royet and Dziarski 2007). One hypothesis is that

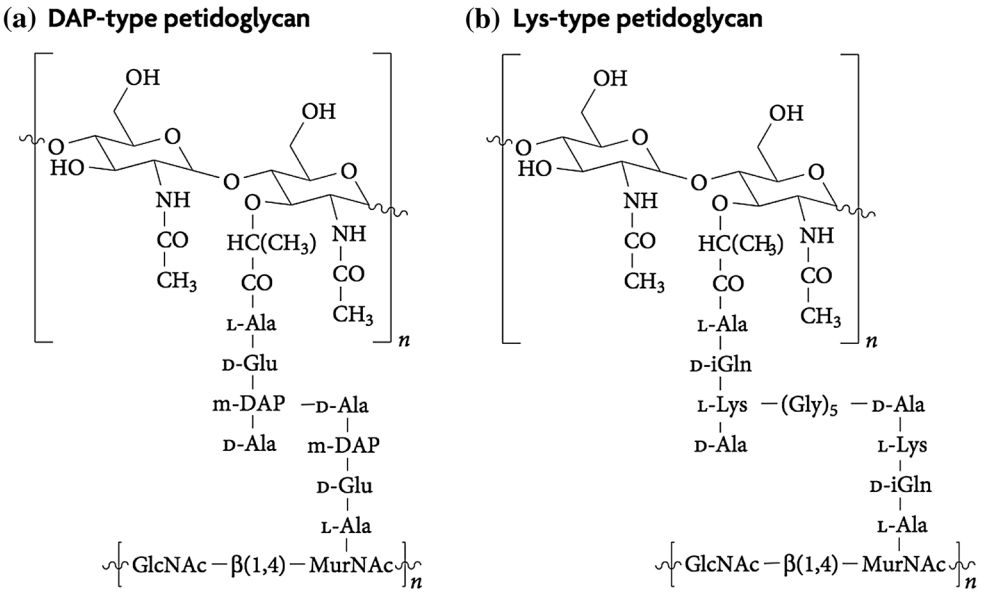

Fig. 1 Two types of peptidoglycan molecules in bacterial cell walls (Royet and Dziarski 2007) (figure reproduced with permission from Nature Publishing Group)

some D-amino acids trigger biofilm disassembly by replacing the D-alanine terminus in bacterial cell walls' peptidoglycan molecules (Kolodkin-Gal et al. 2010). Another mechanism was proposed to explain why the *S. aureus* biofilm could be dispersed by D-amino acids. It hypothesized that D-amino acids prevented the second step in biofilm growth, that is the development of small foci into larger assemblies of cells (Hochbaum et al. 2011). More recently, a new explanation of biofilm dispersal triggered by D-amino acids was proposed by Leiman et al. (2013). It suggested that D-amino acid interfered with protein synthesis.

When D-amino acids were used to treat the biofilm of *Desulfovibrio vulgaris*, a corrosive SRB species, on carbon steel surfaces, Xu et al. (2012, 2014) and Jia et al. (2017) noticed that D-amino acids were inadequate in achieving logarithmic reductions of sessile cells. They suggested that for recalcitrant industrial biofilms, a biocide stress is required. They used D-amino acids as biocide enhancers for THPS and alkyldimethylbenzylammonium chloride. Glutaraldehyde was not chosen because it deactivates D-amino acids due to its function as an amino acid cross-linker. Table 1 shows a synergistic effect between THPS and D-tyr. The combination of 50 ppm (w/w) THPS + 1 ppm D-tyr was far more effective against the *D. vulgaris* biofilm on carbon steel in ATCC 1249 medium than 100 ppm THPS and 1 ppm D-tyr used individually in a 7-day biofilm prevention test, in which treatment chemical(s) were added to the culture medium before inoculation.

Biofilm consortia can be much more recalcitrant than pure-strain biofilms due to biofilm synergy. A biofilm consortium can even deliberately recruit new microbial species in the bulk fluid, not for short-term advantages, but for long-term advantages, such as antimicrobial defense

Table 1 Sessile cell counts on carbon steel coupons after a 7-day biofilm prevention test against *D. vulgaris* biofilm in ATCC 1249 medium mixed with different treatment chemicals (Xu et al. 2012)

Treatment	Sessile cell count (cells/cm^2)
No treatment (control)	$\geq 10^7$
100 ppm D-tyr	$\geq 10^6$
50 ppm THPS	$\geq 10^4$
100 ppm THPS	$\geq 10^2$
50 ppm THPS + 1 ppm D-tyr	<10

in the future (Costerton 2007). Li et al. (2016c) found that 50 ppm THPS + 1 ppm D-tyr reduced the SRB sessile cell count by 2 logs in the biofilm prevention test using a corrosive oilfield biofilm consortium in the ATCC 1249 medium (Table 2). This efficacy is much less than that reflected in Table 1 for the pure-strain *D. vulgaris* biofilm. When 50 ppm THPS was enhanced by 50 ppm of a D-amino acid mixture (equimolar mixture of D-tyr, D-met, D-leu, and D-trp), 4 log reduction of sessile SRB cell count was achieved. Sanchez et al. (2013a) reported that an equimolar mixture of D-met, D-phenylalanine (D-phe), D-proline (D-pro), and D-trp enhanced the dispersal of *S. aureus* compared with using individual D-amino acids. It is likely that different species of sessile cells in a biofilm consortium are susceptible to different D-amino acids. The corresponding SEM and CLSM images in Fig. 2 are consistent with the sessile cell counts in Table 2 from the most probable number assays using an SRB test kit. Figure 2d implies that most observed sessile cells in Fig. 2b were dead cells (red

Table 2 SRB sessile cell counts of an oilfield biofilm consortium on carbon steel coupons after a 7-day biofilm prevention test in ATCC 1249 medium using D-tyr and an equimolar mixture of D-tyr, D-met, D-leu, and D-trp (Li et al. 2016c)

Treatment	Sessile cell count (cells/cm^2)
No treatment (control)	$\geq 10^7$
50 ppm THPS	$\geq 10^7$
50 ppm THPS + 1 ppm D-tyr	$\geq 10^5$
50 ppm THPS + 10 ppm D-tyr	$\geq 10^5$
50 ppm D-amino acid mixture	$\geq 10^5$
500 ppm D-amino acid mixture	$\geq 10^5$
50 ppm THPS + 50 ppm D-amino acid mixture	$\geq 10^3$

dots) and very few cells were live cells (green dots). The comparison of SEM and CLSM images suggests that SEM may be used accurately only if dead sessile cells are already dislodged from the biofilm before coupon preparation for SEM analysis. CLSM images present more accurate pictures. However, they do not see cell morphology.

In summary, D-amino acids can enhance biocides in the mitigation of problematic biofilms. D-amino acids are promising anti-biofilm agents for biofilm dispersal (Romero et al. 2011; Vlamakis et al. 2013; Wood et al. 2011). Limited information on the effects of D-amino acids on the biofilm matrix structures has been reported in the literature (Sanchez et al. 2013b). It was noticed that D-amino acids did not enhance the biocide mitigation of planktonic cells (Sanchez et al. 2014), but rather it had the dispersal effect on sessile cells. This is actually advantageous because the same biofilms are unlikely to develop resistance, making it attractive for long-term applications.

Chelators as biocide enhancers

Another reported class of chemicals that can be used as biocide enhancers are chelators. Ethylenediaminetetraacetic acid (EDTA) has been identified as an enhancer for antibiotics in lock solutions to treat biofilms on catheters, especially those used by cancer patients with a weakened immune system due to chemotherapy (Alakomi et al. 2006; Raad et al. 2003a, b, 2007). The use of chelators as biocide enhancers for biofilm treatment in industrial applications was patented by Raad et al. (2000, 2003). Since EDTA is slowly biodegradable, it accumulates in aqueous systems in the environment. It has been gradually replaced by ethylenediaminedisuccinate (EDDS), which is a readily biodegradable chelator (Schowanek et al. 1997). It was reported that 2000 ppm EDDS enhanced 30 ppm THPS and also 30 ppm

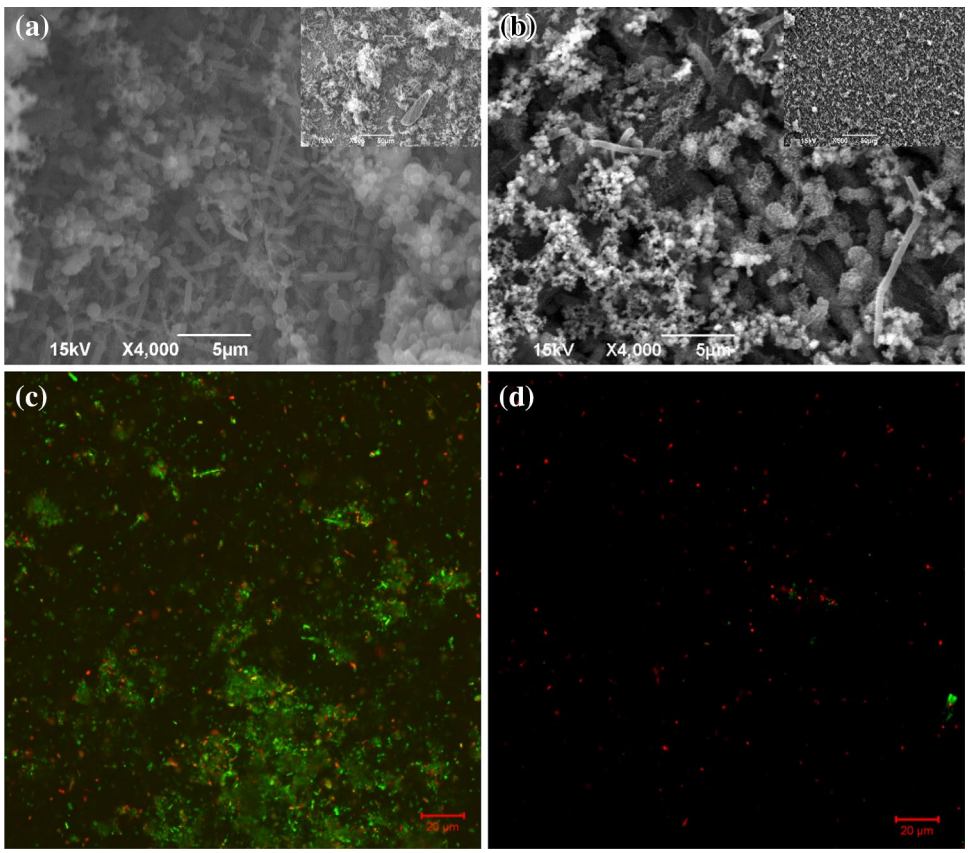

Fig. 2 SEM (*top* row) and CLSM (*bottom* row) images of biofilms after 7-day incubation in the biofilm prevention test against an oilfield biofilm consortium on carbon steel in ATCC 1249 medium with: **a** 50 ppm THPS, **b** 50 ppm THPS + 50 ppm D-amino acid mixture, **c** 50 ppm THPS, and **d** 50 ppm THPS + 50 ppm D-amino acid mixture (image reproduced from Li et al. (2016c) with no permission requirement). (Color figure online)

glutaraldehyde against the planktonic cells of two corrosive SRB (*D. vulgaris* and *Desulfovibrio desulfuricans*), while 2000 ppm EDDS alone did not inhibit the SRB growth (Wen et al. 2010). EDDS at concentration of 2000 ppm enhanced the efficacy of 30 ppm glutaraldehyde against the *D. desulfuricans* biofilm on carbon steel coupons (Wen et al. 2009). Methanol is a winterizing agent in reservoir operations. It was found that 1000 ppm EDDS + 10% (v/v) methanol considerably enhanced 30 ppm glutaraldehyde in the inhibition of SRB planktonic cells, prevention of biofilm formation, and mitigation of souring caused by *D. vulgaris* on carbon steel coupons (Wen et al. 2012; Xu et al. 2012a). The results showed that EDDS can cut down the biocide (e.g., THPS and glutaraldehyde) dosages considerably for the prevention of SRB biofilms and the eradication of established SRB biofilms (Wen et al. 2009). EDDS is considered non-hazardous and readily biodegradable (Schowanek et al. 1997). Although relatively high concentrations of EDDS are needed because of the abundance of ions in the fluid, EDDS is inexpensive.

Norspermidine for biofilm dispersal

Norspermidine is a polyamine (molecular weight 131) produced by some bacteria, algae and plants. It was recently found to inhibit biofilm formation. Hobley et al. (2014) showed that norspermidine was not produced by the wild-type *B. subtilis* NCBI 3610, but 250 μM norspermidine inhibited the *B. subtilis* biofilm formation. Ramón-Peréz et al. (2015) demonstrated that the norspermidine can be produced by *Staphylococcus epidermidis* after a 40-h incubation. Exogenous 25 μM norspermidine addition was found to have the highest inhibitory effect on the *S. epidermidis* biofilm formation. Fourteen of 31 different *S. epidermidis* strains isolated from healthy skins, healthy conjunctivas, and ocular infections were inhibited by 52.2–83.1% with 25 μM norspermidine. Furthermore, 100 μM norspermidine was found to disassemble the mature *S. epidermidis* biofilm. Disassembly took place in 10 strains out of the total 31 strains. The suggested mechanism is due to the attachment of norspermidine to negatively charged sugar residues, or to neutral sugars with polar groups causing a collapse of the exopolysaccharides and then the disassembly of the biofilm (Ramón-Peréz et al. 2015). It was also reported that a combination of 500 μM D-tyr and 500 μM norspermidine was capable of achieving the highest relatively disassembled biomass against a thick (900 μm) 6-month old biofilm consortium in wastewater treatment systems (Si et al. 2014). The combination of D-tyr and norspermidine was thought to reduce the EPS content and alter the protein and polysaccharide matrix structure in the microbial aggregates, promoting sessile cells to return to planktonic cells. Wu et al. (2016) reported that norspermidine acted as a biocide enhancer to treat a biofilm consortium from a wastewater treatment system. Their results showed that 1 ppm biocide alone treatment (silver ion) failed to remove biofilms. Norspermidine alone treatment at 500–1000 μM achieved a biofilm reduction of 21–34% after a 24-h exposure. However, the combination of 500 μM norspermidine and 0.01 ppm silver ion disrupted the biofilm with a higher biofilm reduction rate of 48% after the 24-h exposure.

The results above showed that norspermidine had a potential application against industrial biofilms. The combination of 500 μM (65 ppm) norspermidine and 0.01 ppm silver ion achieved better biofilm removal than the 1 ppm silver ion alone treatment (Wu et al. 2016). Two orders of biocide magnitude dosage reduction was achieved. The norspermidine is not expensive. Therefore, it is interesting to evaluate the combination of norspermidine and common industrial biocides (e.g., THPS, glutaraldehyde, quaternary ammonium compounds and chlorine based biocides) against industrial biofilms, especially those in the oil and gas industry. There is a possibility that the amine group in norspermidine may react with glutaraldehyde or chlorine based biocides. Therefore a chemical compatibility test is necessary if these biocides are used together with norspermidine.

Bacteriophages for biofilm treatment

There have been some recent reports using bacteriophages as anti-biofilm agents (Ashraf et al. 2014; Gutiérrez et al. 2016; Motlagh et al. 2016). Bacteriophages can prevent biofilm formation and achieve biofilm eradication. Bacteriophages can permeate into biofilms and lyse the cells with degradation of its EPS by phage depolymerases (Parasion et al. 2014). Even the persister cells in biofilms could be infected and removed by phages (Gutiérrez et al. 2014; Pearl et al. 2008). Several studies confirmed that biofilms were removed by phages. The infection of three bacteriophages (LiMN4L, LiMN4p and LiMN17) individually or as a cocktail at 10^9 PFU/ml of 7-day *Listeria monocytogenes* biofilms on stainless steel coupons were investigated. Each individual phage reduced the sessile cell counts on stainless steel coupons by 3–4.5 logs, while the cocktail of phages reduced the sessile cells to an undetectable level (Arachchi et al. 2013). The phage P100 was active against a wide range of *L. monocytogenes* biofilms on stainless steel (Montañez-Izquierdo et al. 2012; Soni and Nannapaneni 2010). On a stainless steel coupon surface, 10^9 PFU/ml phage P100 reduced *L. monocytogenes* sessile cell counts by 3.5–5.4 log (Soni and Nannapaneni 2010).

In order to achieve better efficacies, phages were sometimes combined with biocides. It was reported that a 1-day old *S. aureus* biofilm was treated with a cocktail of 10^9 PFU/ml phage SAP-26 and 0.6 ppm rifampicin

for 24 h. The 10^9 PFU/ml phage SAP-26 alone treatment achieved 3 log sessile cell reduction and the 0.6 ppm rifampicin alone treatment achieved 4 log reduction. However, the cocktail of 10^9 PFU/ml phage SAP-26 + 0.6 ppm rifampicin achieved 5 log sessile cell reduction (Rahman et al. 2011). Bacteriophages were also used to reduce microbial attachment to membrane in wastewater treatment systems against biofouling (Goldman et al. 2009; Motlagh et al. 2016). Bhattacharjee et al. (2015) showed that a bacteriophage (10^5–10^6 PFU/mL) isolated from a full-scale wastewater treatment plant could remove the *Delftia tsuruhatensis* ARB-1 biofilm on a glass slide. The bacteriophage at 10^{12} PFU/mL was also found to eliminate the *D. tsuruhatensis* ARB-1 biofilm on membrane filters. Results showed that the water flux through the membrane that previously decreased due to biofilm formation increased after the phage application.

In the field, it is a major challenge to apply bacteriophages because of the extreme host specificities (Calendar 2006). Phages that infect *D. vulgaris* or *Desulfovibrio aespoeensis* may not infect other *Desulfovibrio* species (Eydal et al. 2009; Seyedirashti et al. 1991; Summer et al. 2011). Therefore, a cocktail of phages will be necessary for a field biofilm consortium. However, the large-scale use of phage mixtures is expensive. Thus, phages may be combined with biocides and biocide enhancers in the same batch application, since some specific phages may infect persister cells in biofilms. More research is needed before phages are used in practical applications.

Antibacterial stainless steel

Instead of protecting existing materials against problematic biofilms using chemical and microbiological agents, new materials are being developed to possess antimicrobial properties. Stainless steels have been widely used in many industries. However, they are not immune to biofilm attachment and MIC pitting corrosion. An antibacterial stainless steel was first invented by Nisshin Steel (Chiyoda-ku, Japan) in 1990s (Sun et al. 2015). Yang's group subsequently developed various antibacterial stainless steels for applications in different environments with broad antibacterial spectra (Lin et al. 2007; Yang et al. 2005, 2006). Nan et al. (2015) reported that 304L-Cu antibacterial stainless steel exhibited a strong MIC resistance against *Escherichia coli* in the Luria–Bertani culture medium in a test lasting 21 days. The 304L-Cu antibacterial stainless steel considerably reduced the corrosion pit depth and weight loss. The corrosion resistance was confirmed by the reduced corrosion current density in their potentiodynamic polarization test.

The corrosive marine *P. aeruginosa* biofilm caused severe MIC attack against various types of materials including carbon steels, stainless steels, duplex stain steels and hyper duplex stainless steels (Li et al. 2016a, b; Xu et al. 2017). Copper containing 2205 duplex stainless steels (2205-Cu DSS) was created and tested by Xia et al. (2015). This new antibacterial DSS material possessed a strong antibacterial ability against the aerobic *P. aeruginosa* biofilm with an antibacterial efficiency of 97% in a test lasting 7 days of incubation. The biofilm thickness formed on the 2205-Cu DSS surface was substantially reduced due to the presence of Cu ions released from the copper-rich phases on the steel matrix in the initial corrosion process. The maximum pit depth caused by *P. aeruginosa* biofilm was reduced from 9.5 to 1.4 μm compared with the conventional 2205 DSS. Their electrochemical corrosion test also confirmed the corrosion resistance of 2205-Cu DSS in the presence of *P. aeruginosa*. Compared with 2205 DSS, the 2205-Cu DSS also possessed a much stronger pitting corrosion resistance against the *P. aeruginosa* biofilm. The critical pitting temperature (CPT) values of 2205-Cu DSS (54 °C) was considerably larger than that of 2205 DSS (45 °C) in the presence of the *P. aeruginosa* biofilm after 14 days (Li et al. 2016d). It should be noted copper ions are biocidal against many microorganisms, but SRB such as *D. vulgaris* are not affected. They can form biofilms even on pure copper pipes, such as those used in fire sprinkler systems (Fu et al. 2014).

As shown in Fig. 3, with an initial planktonic *P. aeruginosa* of 10^3 cells ml^{-1}, 2205-Cu DSS exhibited strong antibacterial performance in the artificial seawater. The live/dead staining of biofilm indicated that less live and more dead sessile cells appeared on the 2205-Cu DSS surface after 3 days compared with the 2205 DSS control. After 5 days, the antibacterial efficacy reached 79%, demonstrating a strong inhibition of *P. aeruginosa*. The biofilm thickness is sometimes used to evaluate the biocidal effect of antimicrobial agents. In this case, the thickness of *P. aeruginosa* was reduced substantially on the 2205-Cu surface as shown in Fig. 3. Figure 4 depicts the proposed mechanism of how 2205-Cu DSS kills bacteria. The direct contact kill by copper-rich phases and the Cu^{2+} ions released from the steel matrix synergistically endow 2205-Cu DSS an excellent antibacterial ability. Unlike protective coatings, antibacterial steels do not face the problems of coating crack and disbondment. The drawback of this novel antibacterial DSS is that the general corrosion rate is increased due to the addition of copper. Because MIC pitting resistance is much more vital than its general corrosion resistance, which is negligibly small, the antibacterial DSS has the potential to be used where MIC pitting is a real threat.

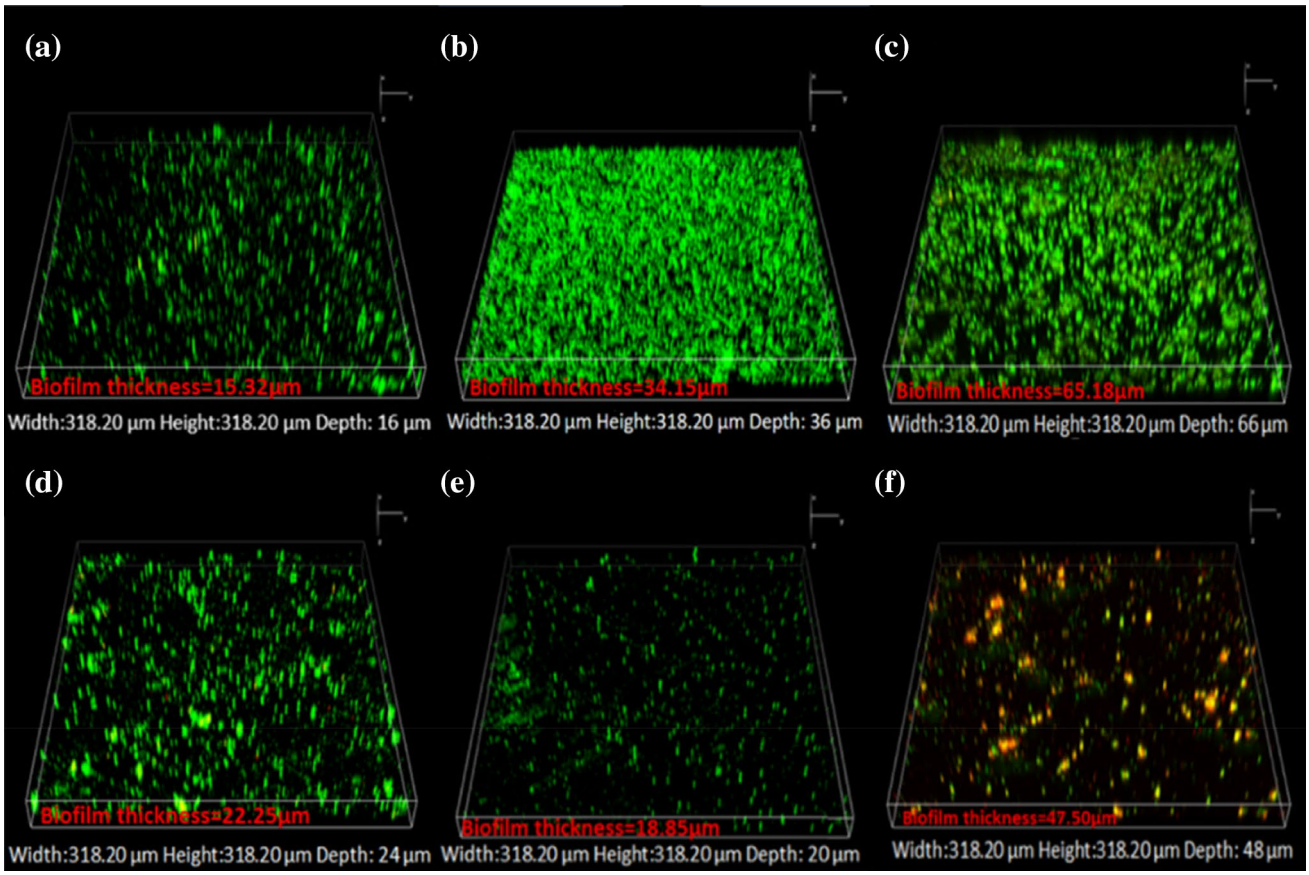

Fig. 3 The 2205 DSS and 2205-Cu DSS surfaces after live/dead staining under CLSM with initial planktonic cell concentrations of 10^3 cells ml^{-1}: **a** 2205 DSS after 1 day, **b** 2205 DSS after 3 days, **c** 2205 DSS after 5 days, **d** 2205-Cu DSS after 1 day, **e** 2205-Cu DSS after 3 days, and **f** 2205-Cu DSS after 5 days (image reproduced from Lou et al. (2016) with permission from Elsevier)

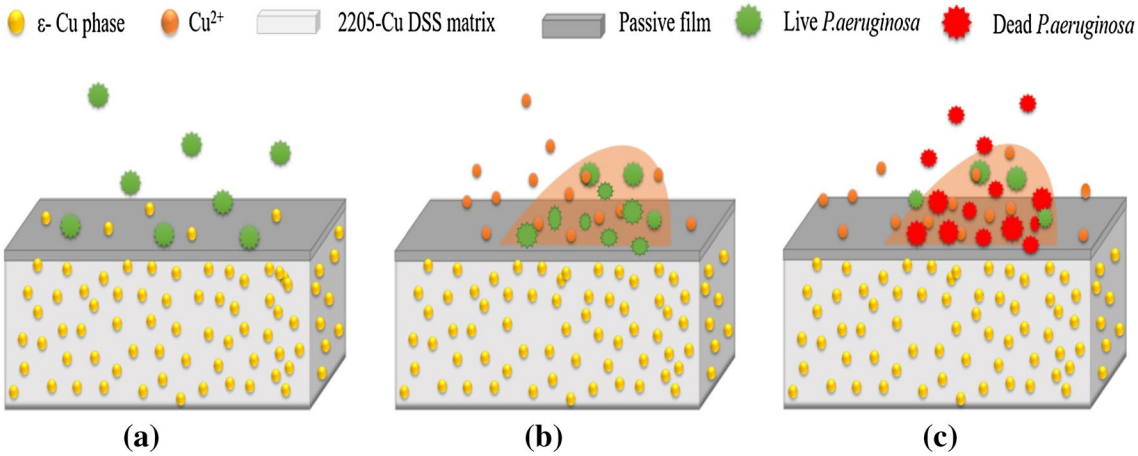

Fig. 4 Schematic illustration of the antibacterial mechanism of 2205-Cu DSS (figure reproduced from Lou et al. (2016) with permission from Elsevier)

Conclusion

Problematic industrial biofilms cause biofouling and MIC, resulting in tremendous economic losses in many industries. They are very resilient against treatment efforts that tend to suppress the vulnerable microbial species while unwillingly allowing resistant species to take over in the long run. This review discussed some recent advances in treating biofilms. They included chemical and microbiological treatments as well as new antibacterial stainless steels. Due to biocide resistance, the battle against problematic industrial biofilms will be everlasting. Researchers will continue to get inspirations from nature for new chemical and microbiological agents to control biofilms. For example, researchers have been testing synthetic peptides as biocide enhancers at ppb levels. This class of biocide enhancers is inspired by the anti-biofilm peptides secreted by sea anemones to keep their surfaces clean. It is foreseeable that some of chemical agents will likely be incorporated into special coatings in addition to liquid dosing for biofilm prevention in the future.

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