Mitigation of MIC from the material and its surface

Dake Xu, Professor

Eurocorr 2017, Prague, Czech
Background

- BS and MS in Bioengineering Microbiology, Molecular biology
- Ph.D, Institute for Corrosion and Multiphase Technology, Ohio University, 2008-2013 Ph.D advisor: Tingyue Gu Corrosion, Bioelectrochemistry
- Associate Professor, Institute of Metal Research, Chinese Academy of Sciences, 2013-2017 Material, Electrochemistry
- Professor, Northeastern University (China), 2017

- Research interests: Biocorrosion, antibacterial material and biomaterial, biofilm and biocide. Mainly focused in the mechanism and mitigation of marine MIC and biofouling.
- Published more than 50 peer-review MIC-related journal papers.
- Editorial board member of NPJ Material degradation.
- 6 Ph.D and 12 master students in my group (3 international graduate students).
Extracellular electron transfer (EET) in microbial fuel cell (MFC)

1. Direct Electron Transfer (DET)
   Direct cell wall-metal surface contact
   Pili (conductive nanowires).

2. Mediated Electron Transfer (MET)
   FAD and riboflavin can act as electron shuttles. Some other chemicals such as H$_2$
   are also used as electron carriers.

✓ MIC is analogous to the biocathode process of MFC.
Biocatalytic cathodic sulfate reduction (BCSR) by sessile sulfate-reducing bacteria (SRB) on the iron surface:

Anodic: \[ 4\text{Fe} \rightarrow 4\text{Fe}^{2+} + 8\text{e}^- \]

Cathodic: \[ \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \]

Mechanism for MIC by SRB utilization of electrons from iron oxidation for sulfate reduction. BCSR can explain why and how MIC due to SRB happens.
1. EET explains how MIC occurs

The cathodic reactions take place in the cytoplasm of the corrosive bacteria, which is defined as “biocathode” in MFC.

✓ EET is the key to investigate the MIC mechanisms.
1. EET explains how MIC occurs

Electron mediators promote MIC caused by SRB

Bioelectrochemistry, 101:14-21, 2015
1. EET explains how MIC occurs

Electron mediators promote MIC caused by Nitrate-reducing bacteria (NRB)

Bioelectrochemistry, 118:38-46, 2017
Breakthrough (Proof of EET in MIC)

We found the genes that determined the expression of electron mediators. Then we overexpressed these genes using synthetic microbiology methods to figure out if MIC was accelerated.

Work in progress!!!
Breakthrough (Proof of EET in MIC)
Breakthrough (Proof of EET in MIC)

Another electroactive biofilm confirmed the important role of MET in MIC process.

✓ MET was proved in MIC from genetic level.
2. Bioenergetics explains why MIC occurs

<table>
<thead>
<tr>
<th>Redox couple</th>
<th>n</th>
<th>$E^{o'}$ (Mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\text{CO}_2 + 2\text{acetate} /\text{hexose}$</td>
<td>8</td>
<td>$-670$</td>
</tr>
<tr>
<td>$\text{Fe}^{2+}/\text{Fe}^{0}$</td>
<td>2</td>
<td>$-447$</td>
</tr>
<tr>
<td>$\text{CO}_2 + \text{acetate}/\text{lactate}$</td>
<td>4</td>
<td>$-430$</td>
</tr>
<tr>
<td>$\text{CO}_2 /\text{formate}$</td>
<td>2</td>
<td>$-432$</td>
</tr>
<tr>
<td>$2\text{H}^+ /\text{H}_2$</td>
<td>2</td>
<td>$-414$</td>
</tr>
<tr>
<td>Acetate/ethanol</td>
<td>4</td>
<td>$-390$</td>
</tr>
<tr>
<td>$\text{CO}_2/\text{methanol}$</td>
<td>6</td>
<td>$-370$</td>
</tr>
<tr>
<td>$2\text{Acetate}/\text{butyrate}$</td>
<td>4</td>
<td>$-290$</td>
</tr>
<tr>
<td>$2\text{CO}_2 /\text{acetate}$</td>
<td>8</td>
<td>$-290$</td>
</tr>
<tr>
<td>$\text{CO}_2/\text{CH}_4$</td>
<td>8</td>
<td>$-244$</td>
</tr>
<tr>
<td><strong>SRB</strong> $\text{SO}_4^{2-}/\text{HS}$</td>
<td>8</td>
<td>$-217$ (-200)</td>
</tr>
<tr>
<td>Fumarate/succinate</td>
<td>2</td>
<td>$+33$</td>
</tr>
<tr>
<td>$\text{NO}_2^-/\text{NH}_3$</td>
<td>6</td>
<td>$+330$</td>
</tr>
<tr>
<td>$\text{NO}_3^-/\text{NH}_3$</td>
<td>8</td>
<td>$+360$</td>
</tr>
<tr>
<td>$2\text{NO}_3^-/\text{N}_2$</td>
<td>10</td>
<td>$+760$</td>
</tr>
<tr>
<td>$\text{O}_2/2\text{H}_2\text{O}$</td>
<td>4</td>
<td>$+818$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2/2\text{H}_2\text{O}$</td>
<td>2</td>
<td>$+1350$</td>
</tr>
</tbody>
</table>

$\Delta G^{o'} = -nF E^{o'} < 0$

✓ Electrogenic bacteria corrode for maintenance energy.
✓ NRB is corrosive, and should be more aggressive than SRB.
2. Bioenergetics explains why MIC occurs

**Starvation of organic carbon accelerates MIC due to SRB (Field condition)**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Sessile Cell Count (cells/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full medium (ATCC 1249 Medium)</td>
<td>≥10⁶</td>
</tr>
<tr>
<td>Full medium minus 90% carbon source</td>
<td>≥10⁵</td>
</tr>
<tr>
<td>Full medium minus 99% carbon source</td>
<td>≥10⁴</td>
</tr>
<tr>
<td>Full medium minus 100% carbon source</td>
<td>≥10⁴</td>
</tr>
</tbody>
</table>

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

Int Biodeterior Biodegrad, 91:74-81, 2014
Starvation of organic carbon accelerates MIC due to NRB

2. Bioenergetics explains why MIC occurs

- With sufficient organic carbon in the medium:
  - Periplasm
  - Cytoplasm
  - Organic carbon oxidation
  - Nitrate reduction under biocatalysis
  - Energy release
  - *Pseudomonas aeruginosa* sessile cell

- Under organic carbon starvation:
  - Periplasm
  - Cytoplasm
  - Nitrate reduction under biocatalysis
  - Electron transfer chain
  - N$_2$ or NH$_4^+$ energy release
  - Ferric iron (Fe$^{III}$) reduction
  - Ferric iron (Fe$^{III}$) oxidation
  - *Pseudomonas aeruginosa* sessile cell

Graphs showing the relationship between time and various parameters under different conditions:

- Control (0% carbon source reduction)
- 90% carbon source reduction
- 100% carbon source reduction

- Voltage (V) vs. SCE
- Resistance ($R_p$) vs. Time (day)
- Logarithm of current density ($i$) vs. Voltage (V)
2. Bioenergetics explains why MIC occurs

In the absence of organic electron donors, the sulfate reducing Bacterium *D. vulgaris* could survive for a long period, up to 55 days.

Only when the methanogenic strains are deprived of energy sources such as organic carbon, they start to turn aggressive towards carbon steel, causing increased corrosion, indicating that the phenomenon is likely not strain specific.
2. Bioenergetics explains why MIC occurs

On-demand electron transfer

Starvation triggers pilus formation for better DET to harvest more electrons.


Currently DET is still disputable. Cogent and direct evidence is needed to prove the occurrence of DET.
Major drawbacks:

(1) We demonstrated that MET of EET played a key role in MIC due to SRB. We further confirmed MET in MIC from genetic level (data unpublished).

(2) $\text{H}_2\text{S}$ corrosion was not a sole chemical corrosion phenomenon.

3. MIC classification

**EET - MIC (electroactive or electrogenic biofilm)**

Biofilms rely on **anaerobic respiration** for energy. Oxidant (e.g., sulfate and nitrate) is reduced **inside cells under biocatalysis**. SRB, NRB, methanogens, etc. intentionally cause corrosion for energy.

**Metabolites - MIC**

**Fermentative** bacteria and fungi in biofilms secrete organic acids. The produced oxidants (organic acids, H\(^+\), etc.) in MIC are reduced **outside cells** without biocatalysis.

May or may not be intentional. These oxidants will corrode in conventional chemical corrosion (such as acetic acid corrosion) without biofilms! APB (and some SRB and NRB) that perform anaerobic fermentation cause this type of MIC.
4. D-amino acids as biocide enhancer


Advantages of D-amino acids as biocide enhancer
1. Broad-spectrum signal molecular
2. Low toxicity
3. Biodegradable
4. Low price
Microbiologically Influenced Corrosion (MIC) Resistance of a Novel Cu-bearing 2205 Duplex Stainless Steel in the Presence of a Marine Pseudomonas aeruginosa Biofilm

Work done@IMR
MIC background

- MIC corrodes carbon steel.
- MIC attacks 304/304L SS, 316/316L SS, 2205 DSS and 2707 HDSS.
- MIC even attacks Copper and Ti.

316L pitting corrosion due to SRB after coculture for 7 days
Biofilms are responsible for MIC

It is widely accepted that MIC related pitting corrosion is caused by the biofilm. So if the biofilm can be effectively inhibited or mitigated, the occurrence of pitting corrosion due to MIC can be considerably decreased.

Larson et al., NACE Paper 07507, 2007
Mitigation of MIC

Current Mitigation Methods

- Biocides/Biostats (THPS and glutaraldehyde, etc.)
  Problems with toxicity, resistance, high costs, strict environmental regulations.

- Physical scrubbing (pigging)
  Some pipelines cannot be pigged.

- Microbial competition
  NRB can be used to mitigate souring, but not necessarily MIC. Because they are corrosive bugs!

In USA, $1.2 billon was spent annually on biocide to fight MIC. Aim to eradicate biofilm (planktonic cells are much easier to be killed). Biofilms are far more difficult to eradicate than planktonic cells. 10X or higher doses. 1,000X reported.
Antibacterial Stainless Steels

Copper ions show strong antibacterial ability, and copper is a vital alloy element. Based on this, adding suitable quantity of copper is the technical approach to develop antibacterial stainless steel.

IMR developed various types of stainless steel, including austenitic antibacterial SS (304-Cu, 316L-Cu, 317L-Cu, 201-Cu), ferritic antibacterial SS (430-Cu) and martensitic antibacterial SS (420-Cu, 2Cr13Mo-Cu,17-4PH Cu).

A newly developed 2205-Cu duplex SS was aimed to mitigate the MIC due to corrosive microbes in marine environments.
Antibacterial stainless steel

Broad antibacterial spectrum

<table>
<thead>
<tr>
<th>Experimental bacteria</th>
<th>Ferritic antibacterial</th>
<th>Austenitic antibacterial</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stainless steel</td>
<td>stainless steel</td>
<td>steel</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>99.9</td>
<td>99.9</td>
<td>0</td>
</tr>
<tr>
<td><em>Shigella flexneri</em> ATCC12022</td>
<td>99.0</td>
<td>99.5</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC9721</td>
<td>99.9</td>
<td>99.9</td>
<td>0</td>
</tr>
</tbody>
</table>

Antibacterial spectrum of the antibacterial stainless steels against the gram-negative bacteria.
### Antibacterial stainless steel

**Broad antibacterial spectrum**

<table>
<thead>
<tr>
<th>Experimental bacteria</th>
<th>Antibacterial rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferritic antibacterial stainless steel</td>
</tr>
<tr>
<td><em>Staphylococcus epidermis</em> ATCC14990</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Sarcina lutea</em> ATCC9341-A</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> ATCC8100</td>
<td>98.2</td>
</tr>
<tr>
<td><em>Bacillus pumilus</em> ATCC14884</td>
<td>97.7</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC29212</td>
<td>91.0</td>
</tr>
</tbody>
</table>

**Antibacterial spectrum of the antibacterial stainless steels against the gram-positive bacteria.**
Antibacterial stainless steel

Tap water exposed in air after 24h, (A) 304 SS, (B) 304–Cu, 1-Live/Dead staining, 2-Live sessile cells, and 3-Dead sessile cells.
Antibacterial stainless steel

Cultured in 0.9% NaCl solution for 24h
Cultured in 0.9% NaCl solution for 24h in presence of *E. coli*
Electrochemical parameters for stainless steel specimens exposed to LB medium with and without *E.coli*.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Solutions</th>
<th>Time (day)</th>
<th>$E_{\text{corr}}$ (mV)</th>
<th>$E_{\text{pit}}$ (mV)</th>
<th>$I_{\text{corr}}$ (μA/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304 SS</td>
<td>LB</td>
<td>2</td>
<td>-131</td>
<td>332</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>-131</td>
<td>325</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>LB+bacteria</td>
<td>2</td>
<td>-286</td>
<td>221</td>
<td><strong>1.42</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>-519</td>
<td>-369</td>
<td><strong>4.98</strong></td>
</tr>
<tr>
<td>304-Cu SS</td>
<td>LB</td>
<td>2</td>
<td>-186</td>
<td>161</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>-187</td>
<td>168</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>LB+bacteria</td>
<td>2</td>
<td>-192</td>
<td>264</td>
<td><strong>0.08</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>-324</td>
<td>-174</td>
<td><strong>1.24</strong></td>
</tr>
</tbody>
</table>
Antibacterial stainless steel

Corrosion characteristics of stainless steels exposed to LB medium with *E. coli* for 21 days.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Maximum pit depth (μm)</th>
<th>Pitting rate* (mm/year)</th>
<th>R&lt;sub&gt;a&lt;/sub&gt; (μm)</th>
<th>Weight loss (mg/cm&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>304 SS</td>
<td>13.4</td>
<td>0.23</td>
<td>1.17 ± 0.03</td>
<td>0.6 ± 0.05</td>
</tr>
<tr>
<td>304-Cu SS</td>
<td>8.3</td>
<td>0.14</td>
<td>0.65 ± 0.02</td>
<td>0.2 ± 0.03</td>
</tr>
</tbody>
</table>

![Graph showing pit depth and distance from pit source for 304 SS and 304-Cu SS](image-url)
Antibacterial stainless steel

Summary:
Unlike traditional mitigation methods, the innovative idea of this research is to utilize the antibacterial stainless steel surface (the Cu-rich phase and the Cu ions released from the matrix) where the biofilm attached to directly mitigate the corrosive biofilm.
Pseudomonas aeruginosa is a Gram-negative motile rod bacterium widely distributed in nature.

P. aeruginosa is an aerobic marine corrosive microbe, which have caused many MIC cases.

It has been recognized as the pioneer colonizer in the process of biofilm formation.

Biofilm formation + Corrosive
2205 DSS has been widely used in the marine environments, such as ships, offshore platform, subsea equipment, coastal facility and the use of seawater cooling equipment.
Antibacterial Stainless Steel used in marine environment

2205 duplex SS is widely used in marine environments. In recent, the failures of 2205 DSS due to MIC were reported. The MIC resistance of a novel Cu-bearing 2205 Duplex Stainless Steel (2205 Cu-DSS) against an aerobic marine *P. aeruginosa* biofilm was investigated.

Microstructure of 2205 Cu-DSS after
(a)  solution at 1050°C.
(b)  solution at 1050°C, and aging at 540°C for 4 h.
2205-Cu DSS mitigated MIC

Mechanical properties

<table>
<thead>
<tr>
<th>Samples</th>
<th>$\delta_s$ (MPa)</th>
<th>$\delta_b$ (MPa)</th>
<th>$\sigma$ (%)</th>
<th>$\psi$ (%)</th>
<th>H (Hv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>540</td>
<td>770</td>
<td>76</td>
<td>38</td>
<td>380</td>
</tr>
<tr>
<td>b</td>
<td>636</td>
<td>886</td>
<td>74</td>
<td>32</td>
<td>471</td>
</tr>
<tr>
<td>c</td>
<td>571</td>
<td>810</td>
<td>77</td>
<td>30</td>
<td>369</td>
</tr>
</tbody>
</table>

Where $\delta_s$ is the yield strength, $\delta_b$ tensile strength, $\sigma$ elongation, $\psi$ cross sectional area and hardness H.

a. commercial 2205 DSS
b. 2205 Cu-DSS after solution and aging treatment
c. 2205 Cu-DSS after solution treatment

The mechanical properties of 2205-Cu were slightly better than those of the commercial 2205.
2205-Cu DSS mitigated MIC

MIC resistance test – Linear polarization resistance

The variations of $R_p$ with exposure time for 2205 DSS and 2205 Cu-DSS coupons in the presence of *P. aeruginosa* at 30°C.

The $R_p$ of 2205-Cu was larger than that of the commercial 2205, indicating its better MIC resistance against *P. aeruginosa.*
2205-Cu DSS mitigated MIC

(a) 2205 DSS in the uninoculated medium
(b) 2205 DSS in the medium inoculated with *P. aeruginosa*
(c) 2205 Cu-DSS in the uninoculated medium
(d) 2205 Cu-DSS in the medium inoculated with *P. aeruginosa* after 14 days of incubation

<table>
<thead>
<tr>
<th></th>
<th>Sterile medium</th>
<th>After inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2205 DSS</td>
<td>2205 Cu-DSS</td>
</tr>
<tr>
<td>$E_{corr}$ / mV (Vs. SCE)</td>
<td>-308.2</td>
<td>-478.5</td>
</tr>
<tr>
<td>$i_{corr}$/ ($\mu$A cm$^2$)</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>$\beta_a$ (V/dec)</td>
<td>0.18</td>
<td>0.99</td>
</tr>
<tr>
<td>$\beta_c$ (V/dec)</td>
<td>-0.09</td>
<td>-0.08</td>
</tr>
</tbody>
</table>
The Bode plots of 2205 DSS and 2205 Cu-DSS coupons with or without exposure to *P. aeruginosa* biofilm: (a) 2205 Cu-DSS in the medium inoculated with *P. aeruginosa*, (b) 2205 DSS in the medium inoculated with *P. aeruginosa*, (c) 2205 DSS in the uninoculated medium, and (d) 2205 Cu-DSS in the uninoculated medium.
MIC resistance test – The equivalent physical models and the corresponding circuit models

(a) a single, and (b) a double layer model with a biofilm.
2205-Cu DSS mitigated MIC

MIC resistance test – The efficiency of MIC resistance

<table>
<thead>
<tr>
<th>Day</th>
<th>11</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \eta_p )%</td>
<td>n/a</td>
<td>n/a</td>
<td>89</td>
</tr>
<tr>
<td>( \eta_R )%</td>
<td>57</td>
<td>81</td>
<td>88</td>
</tr>
</tbody>
</table>

The inhibition efficiency of 2205 Cu-DSS in the presence of *P. aeruginosa*. 
\( \eta_p \) was calculated by the \( i_{corr} \) of 2205-Cu DSS vs. the \( i_{corr} \) of 2205 DSS in the presence of *P. aeruginosa*.

\( \eta_R \) was calculated by the \( R_{ct} \) of 2205-Cu DSS vs. the \( R_{ct} \) of 2205 DSS in the presence of *P. aeruginosa*.

\[
\eta_p = \frac{i_{corr(uninh)} - i_{corr(inh)}}{i_{corr(uninh)}} \times 100\%
\]

\[
\eta_R = \frac{R_{ct(inh)} - R_{ct(uninh)}}{R_{ct(inh)}} \times 100\%
\]

The \( \eta_p \) and \( \eta_R \) demonstrate that the 2205-Cu DSS showed its MIC resistance against *P. aeruginosa* after 11 days.
CLSM to investigate the growth of the *P. aeruginosa* biofilm on the surface of: (a) 2205 DSS after 1 day, (b) 2205 Cu-DSS after 1 day, (c) 2205 DSS after 7 days, and (d) 2205 Cu-DSS after 7 days.
2205-Cu DSS mitigated MIC

(a) 2205 DSS in the presence of *P. aeruginosa* after 7 days, (b) 2205-Cu DSS in the presence of *P. aeruginosa* after 7 days, (c) 2205 DSS in the presence of *P. aeruginosa* after 14 days, and (d) 2205-Cu DSS in the presence of *P. aeruginosa* after 14 days.

The Live/Dead staining and SEM images confirmed the strong biofilm removal efficacy of 2205-Cu DSS compared with 2205 DSS.
MIC resistance test – Surface morphology observation

The CLSM 3-D images of (a) 2205 Cu-DSS and (b) 2205 DSS incubated in the medium inoculated with *P. aeruginosa* for 14 days.

The MIC resistance performance of 2205-Cu was supported by the pit depth data.
2205-Cu DSS mitigated corrosive biofilm

2205-Cu DSS possessed strong MIC pitting resistance.
2205 Cu-DSS showed considerably larger CPT values, indicating its strong pitting resistance.
(a) The wide XPS spectra of the surface of the 2205 DSS and 2205 Cu-DSS in the medium with and without *P. aeruginosa* after 14 days of incubation, (b) the high resolution XPS spectra of Cl 2p for 2205 Cu-DSS with exposure to *P. aeruginosa* after 14 days of incubation, and (c) the high resolution XPS spectra of Cl 2p for 2205 DSS with exposure to *P. aeruginosa* after 14 days of incubation.

A protective Cu$_2$(OH)$_3$Cl layer was formed on the 2205 Cu-DSS surface.
Copper was “evenly” distributed in the 2205-Cu DSS.
2205-Cu DSS mitigated corrosive biofilm

Initial inoculation of $10^5$ cells/ml *P. aeruginosa* in artificial seawater for 1, 3, 5 days.
2205-Cu DSS mitigated corrosive biofilm

Initial inoculation of $10^3$ cells/ml *P. aeruginosa* in artificial seawater for 1, 3, 5 days.
2205-Cu DSS mitigated corrosive biofilm

(a) 10^3 cells/ml inoculation

(b) 10^5 cells/ml inoculation
2205-Cu DSS mitigated corrosive biofilm

Quantitative PCR further confirmed the strong antibacterial ability of 2205 Cu-DSS.

Forward primer:
5′-AGACACCGTCCAGACTCCTAC-3′

Reverse primer:
5′-CCAACTTTGCTGAACCACCTAC-3′
Environmental toxicity of 2205-Cu DSS

No death and abnormality

2205 Cu-DSS was environmentally safe.
2205-Cu DSS mitigated corrosive biofilm

Biofilm and pit morphology of 2205 DSS (A, C) and 2205-Cu DSS (B, D) after the 7-day incubation with nitrate reducing *Pseudomonas aeruginosa* PAO1 in anaerobic condition. Data provided by Prof Tingyue Gu, Ohio University.

2205 Cu-DSS effectively mitigated anaerobic biofilm.
2205-Cu DSS mitigated MIC

2205 Cu-DSS effectively mitigated anaerobic MIC.
The possible MIC resistance mechanism

The Cu$^{2+}$ ions released from the Cu-rich phases and the direct contact killing by the ε-Cu phases synergistically mitigated the corrosion biofilm and MIC.
Conclusion:

➢ The newly designed 2205-Cu DSS was found to be a potential anti-MIC material used in marine environments.

➢ The novelty is that a new method is developed to mitigate MIC from the material aspect.

➢ The investigation of 2nd generation 2205-Cu DSS is ongoing to pursue a better antibacterial ability with broad spectrum.
Mussel-inspired superhydrophobic surfaces with enhanced corrosion resistance and dual-action antibacterial and anti-MIC properties

Cooperated with Prof. Dawei Zhang
Improving the hydrophobic (hydrophilic) of the material surface: (1) reducing (improving) the surface energy of the material, (2) changing the surface microstructure and increasing the surface roughness.
Mimicing the natural nanostructure (superhydrophobic) is not enough to prevent the biofilm formation and attachment.
Preparation of superhydrophobic surface

Dopamine → Polydopamine

\[ \text{Dopamine} \xrightarrow{\text{pH}=8.5, 25^\circ\text{C}, 24\text{h}} \text{Polydopamine} \]

\[ \text{AgNO}_3 \xrightarrow{25^\circ\text{C}, 5\text{h}} \text{AgNP} \]

\[ \text{PFDH} \xrightarrow{25^\circ\text{C}, 12\text{h}} \text{AgNP} \]

\[ \text{thiol groups} \xrightarrow{\text{OH hydroxyl group}} \]

316L → PDA-deposited surface (PDS) → Ag-deposited surface (ADS) → Superhydrophobic surface (SS)

Su et al. 2016

Material Science and Engineering C, 80:566-577, 2017
### Surface micro-topography

<table>
<thead>
<tr>
<th></th>
<th>PDS</th>
<th>ADS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a1)</td>
<td><img src="a1.png" alt="Image" /></td>
<td><img src="b1.png" alt="Image" /></td>
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</table>

- **PDS**: $S_a = 124$ nm
- **ADS**: $S_a = 197$ nm
- **SS**: $S_a = 164$ nm
Surface analysis

PDS film: C 1s
ADS film: C 1s, Ag 3d
SS film: C 1s, Ag 3d, S 2p, F 1s
Surface wettability

CA = 80°

CA = 26°

CA = 30°

CA = 153°

SA < 3°
The chelation of polydopamine (PDA) film with Fe showed a protective effect on stainless steel substrate.

The gas film formed by the superhydrophobic surface improved the corrosion resistance.
Biofilm prevention of *E. coli*

1 day

(a1) Bare surface (BS)  (b1) Ag-deposited surface (ADS)  (c1) Superhydrophobic surface (SS)

3 day

(a2) Bare surface (BS)  (b2) Ag-deposited surface (ADS)  (c2) Superhydrophobic surface (SS)

Truong et al. 2012
Biofilm prevention of *S. aureus*
Biofilm mitigation (E. coli)

1 day

Live staining

Dead staining

(a1)

BT = 128.8 ± 2.9 μm
BC = 31.4 ± 3.1%

50μm

BS

(a2)

BT = 195.2 ± 10.6 μm
BC = 58.3 ± 6.1%

50μm

3 day

Live staining

Dead staining

(c1)

BT = 47.4 ± 2.7 μm
BC = 9.3 ± 0.9%

50μm

ADS

(c2)

BT = 106.1 ± 4.3 μm
BC = 37.5 ± 4.2%

50μm

(d1)

BT = 17.9 ± 0.8 μm
BC = 1.1 ± 0.2%

50μm

SS

(d2)

BT = 38.1 ± 7.1 μm
BC = 12.4 ± 1.1%

50μm
Biofilm mitigation ( *S. aureus* )

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>3 day</th>
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<tbody>
<tr>
<td></td>
<td>Live staining</td>
<td>Dead staining</td>
</tr>
<tr>
<td>BS</td>
<td><img src="a1" alt="Image" /></td>
<td><img src="a2" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>BT = 111.5 ± 7.2 μm</td>
<td>BC = 33.7 ± 4.0%</td>
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<td><img src="d1" alt="Image" /></td>
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<tr>
<td></td>
<td>BT = 54.0 ± 6.7 μm</td>
<td>BC = 15.4 ± 2.1%</td>
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<td></td>
<td><img src="d1" alt="Image" /></td>
<td><img src="d2" alt="Image" /></td>
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<tr>
<td></td>
<td>BT = 33.7 ± 2.2 μm</td>
<td>BC = 0.5 ± 0.1%</td>
</tr>
</tbody>
</table>
Biofilm thickness and coverage

(a1) Biofilm thickness (µm)

1 day
3 day

US
ATS
SS

(b1) Biofilm thickness (µm)

1 day
3 day

US
ATS
SS

(a2) Bacteria coverage

1 day
3 day

US
ATS
SS

(b2) Bacteria coverage

1 day
3 day

US
ATS
SS
Planktonic cell inhibition (OD$_{600nm}$)

1 day: SS > BS > ADS

3 day: BS > SS > ADS

1 day: the superhydrophobic surface inhibited the attachment of the biofilm, most of the bacteria were in planktonic status, leading to a high OD value of the SS.

3 day: with the release of Ag$^+$ from SS, the growing of bacteria was inhibited, so the increase rate of the OD value significantly decreased.
The fast release of Ag$^+$ from nanosilver surface at the beginning of immersing was not good for long-term antibacterial effect.

The superhydrophobic surface gradually released Ag$^+$. 
The nanosilver surface showed the highest cytotoxicity, while the cytotoxicity was decreased for superhydrophobic surface because of the release inhibition of Ag$^+$. 
The superhydrophobic surface separated the surface and the medium in initial immersing stage, reduced the adhesion of the biofilm, and inhibited the release of Ag⁺.

The superhydrophobic surface and released Ag⁺ ions synergistically mitigated the biofilm.
Antibacterial superhydrophilic surface

1 day

3 day

nano-silver particles surface

Superhydrophobic surface

Superhydrophilic

S. aureus
Conclusion:

➢ A superhydrophobic (hydrophilic) surface was constructed on the surface of 316L stainless steel by self-assembly of polydopamine, nano-silver particles and PFDT.

➢ Biofilm was synergistically inhibited by the superhydrophobic surface and the released silver ions.

➢ The superhydrophilic surface also exhibited strong anti-biofilm ability.
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