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Desulfovibrio vulgaris Corroded X65 Carbon Steel and Copper with Two Different Types of MIC Mechanisms

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ABSTRACT

This study demonstrates that the mechanisms of microbiologically influenced corrosion (MIC) by *Desulfovibrio vulgaris*, a sulfate reducing bacterium (SRB), against X65 carbon steel and pure copper belong to two different types of MIC. Type I MIC involves extracellular electron transfer across cell walls of sessile cells in biofilms. This type of MIC is also called extracellular electron transfer MIC (EET-MIC). Type II MIC, also known as metabolite MIC (M-MIC), is caused by secreted corrosive metabolites that are more concentrated locally under a biofilm. The corrosive metabolites secreted by planktonic cells can also contribute to Type II MIC. The metabolites oxidize metals extracellularly without biocatalysis or EET. Experimental data in this work show that 20 ppm (w/w) riboflavin, a universal electron mediator, did not enhance sessile cell growth, but it accelerated EET-MIC by *D. vulgaris* in the ATCC† 1249 medium against X65 carbon steel with a 90% increase in weight loss and a 284% increase in the average maximum pit depth. However, 20 ppm riboflavin did not increase copper MIC, because copper MIC by SRB was due to secreted metabolites (i.e., M-MIC) rather than the direct result of sulfate reduction. This work also shows that copper MIC weight loss caused by the SRB was at least one order of magnitude higher than that of X65 carbon steel even though the SRB sessile cell count on copper was 10 times lower.

Key words: microbiologically influenced corrosion, mechanism, biofilm, *Desulfovibrio vulgaris,* carbon steel, copper

† Trade name.

INTRODUCTION

The awareness of MIC increased significantly in the past decade in many fields such as the oil and gas industry, water utilities, and the biomedical implant industry.[1-3] Sulfate is a ubiquitous oxidant (electron acceptor) in seawater, brackish water and even in human saliva. Thus, SRB are often found in anaerobic environments because they can utilize this readily available oxidant.[4,5] Most environments in the oil and gas industry such as reservoirs and oil transport pipelines are strictly anaerobic.[6] In systems that are open to the air, SRB may grow underneath aerobic biofilms such as an iron oxidizing bacterium biofilm.[7,8] Although SRB are often blamed for MIC in various settings, nitrate reducing bacteria and acid producing bacteria are shown to be corrosive as well.[9-11]

Carbon steel and copper are two widely used metals.[12] These two metals are prone to MIC by SRB. It is important to understand the mechanisms of their MIC caused by SRB.[13,14] Under anaerobic condition, anaerobic MIC mechanisms can be classified into two distinct categories.[15] EET-MIC is the corrosion caused by biofilms that harvest energy from energetic metals.[16] The biofilms are capable of cross-cell wall electron transfer, also known as extracellular electron transfer (EET).[17] SRB and NRB are two primary types of microbes that cause EET-MIC against carbon steel because they can transport electrons released by extracellular iron oxidation across cell walls to the cytoplasm for biocatalyzed sulfate reduction and nitrate reduction, respectively for energy production.[9,18]

The following reactions have been used to explain the bioenergetics of SRB MIC mechanisms for carbon steel and stainless steel corrosion,[19,20]

$$4Fe \rightarrow 4Fe^{2+} + 8e^{-} \qquad E^{\circ\prime} = -447\ mV \qquad (1)$$

$$SO_4^{2-} + 9H^{+} + 8e^{-} \rightarrow HS^{-} + 4H_2O \qquad E^{\circ\prime} = -217\ mV \qquad (2)$$

$E^{o\prime}$ is defined as the reduction potential at 25 °C, pH 7, and 1 M solutes (or 1 bar partial pressure for gases). The cell potential for the redox reaction combining Reaction (1) and Reaction (2) is +230 mV at these conditions. This positive potential means the redox reaction combining the two half reactions has a negative Gibbs free energy change at these conditions. Thus, EET-MIC by SRB against steel is thermodynamically favorable.[17] Because insoluble Fe is oxidized extracellularly, while sulfate reduction occurs intracellularly, EET is required to link these two reactions.

The bisulfide in Reaction (2) can gain or lose one proton to become H_2S or S^{2-},

$$HS^{-} + H^{+} \rightarrow H_2S \qquad (3)$$

$$HS^{-} \rightarrow S^{2-} + H^{+} \qquad (4)$$

H_2S can be corrosive, but it can also form an iron sulfate passivation film to inhibit corrosion. By using a special experimental design, Jia et al.[16] demonstrated that in anaerobic vials, higher dissolved [H_2S] led to partial iron sulfide passivation and decrease of the sessile cell count on C1018 carbon steel, resulting in less MIC. Their experimental data and H_2S corrosion model calculations showed that H_2S was not a

contributing factor in SRB MIC against carbon steel in anaerobic vials with culture medium pH not too far from 7. Their data also showed that M-MIC by SRB against C1018 carbon steel was negligible. Instead, EET-MIC with sulfate reduction was the primary factor in C1018 carbon steel MIC by *D. vulgaris*.[16]

In EET-MIC by SRB, sessile SRB cells must be able to perform EET. There are two electron transfer methods. One is direct electron transfer (DET) and the other mediated electron transfer (MET).[20] It has been shown that electron transfer is a bottleneck in SRB MIC of C1018 carbon steel and 304 stainless steel.[20,21] It was demonstrated that 10 ppm of either flavin adenine dinucleotide (FAD) or riboflavin (vitamin B_2) accelerated pitting and weight loss. These two chemicals are known as universal electron mediators which are utilized by many microorganisms in their electron transfer chains.[22,23] Similarly, it was also demonstrated that these two electron mediators accelerated the MIC of C1018 carbon steel by nitrate reducing *Pseudomonas aeruginosa*.[24] This is not surprising because NRB MIC against carbon steel is analogous to SRB MIC against carbon steel, both belonging to EET-MIC.[9]

Copper is much less energetic compared with steel because Cu^+ and Cu^{2+} reduction potentials are highly positive values as shown below,

$$Cu \rightarrow Cu^+ + e^- \qquad E^{\circ\prime} = +520 \text{ mV} \qquad (5)$$

$$Cu \rightarrow Cu^{2+} + 2e^- \qquad E^{\circ\prime} = +340 \text{ mV} \qquad (6)$$

Unlike iron oxidation coupled with sulfate reduction, copper oxidation coupled with sulfate reduction is not thermodynamically favorable. Thus, EET-MIC due to direct sulfate reduction by SRB against copper cannot happen spontaneously. However, M-MIC by SRB secreted HS^- can happen at neutral pH with the following reaction which has a negative Gibbs free energy change,

$$2Cu_{(crystal)} + HS^- + H^+ \rightarrow Cu_2S_{(crystal)} + H_{2(g)} \qquad \Delta G^{\circ\prime} = -62 \text{ kJ/mol} \qquad (7)$$

Thus, it is interesting to see that for steel and copper, SRB MIC has two very different mechanisms. One mechanism is EET-MIC (against steel), and the other is M-MIC (against copper) that does not rely on EET. This work was designed to prove the hypothesis that unlike EET-MIC by SRB against steel, M-MIC by SRB against copper cannot be accelerated by an electron mediator.

EXPERIMENTAL PROCEDURE

Modified Baar's medium for sulfate reducers (ATCC 1249) was used to culture *D. vulgaris* (ATCC 7757). The culture medium was adjusted to pH 7 before inoculation. The 200 ml anaerobic vials and other liquid solutions were autoclaved at 121 ^oC for 20 minutes. Liquid solutions were sparged with N_2 to remove dissolved oxygen. X65 carbon steel (composition in Table 1) and copper (99.9% mass purity) were used. Square coupons of the metals had an exposed 1 cm × 1 cm top surface. All other surfaces were protected by polytetrafluoroethylene paint. The coupon polishing and cleaning methods are reported elsewhere.[18] Riboflavin (Fisher Scientific, Pittsburgh, PA, USA) was used as the electron mediator.

Table 2 shows the test matrix. Three coupons, 100 ml medium and 1 ml *D. vulgaris* seed were added to each anaerobic vial. For each test condition, two replicate vials were used. The initial planktonic cell count was 10^6 cells/ml following inoculation. To prevent accidental oxygen ingress, 100 ppm L-cysteine

was used in the medium as an oxygen scavenger. The vials were incubated at 37 °C for 7 days before the coupons were taken out for analysis. Sessile cells were enumerated in modified Postgate's B liquid medium[25] using most probable number (MPN) methodology.[26] They were first removed from a coupon surface using a tiny sterile brush. The brush and the dislodged sessile cells were placed in 10 ml pH 7.4 phosphate buffered saline solution in a test tube and then the test tube was vortexed to distribute the cells evenly in the solution before MPN enumeration.[18] Pit images and pit depths were obtained using a scanning electron microscope (SEM) and an infinite focus microscope (IFM) as reported previously.[24]

Table 1
Chemical Composition of X65 Carbon Steel (wt %)

C	Si	Mn	P	S	V	Nb	Ti	Fe
0.16	0.45	1.65	0.020	0.010	0.09	0.05	0.06	Balance

Table 2
Electron Mediator Test Matrix

Microbe	*D. vulgaris*
Medium	ATCC 1249
Metal	X65 carbon steel, copper
Test combination	0 ppm riboflavin, 20 ppm riboflavin
Liquid volume	100 ml in 200 ml anaerobic vials
Temperature	37 °C
Incubation time	7 days
Assay	SRB sessile cell count, biofilm image, pit image, coupon weight loss, pit depth

RESULTS AND DISCUSSION

After the 7-day incubation, the culture medium pH with X65 coupons was 7.1 ± 0.03 with and without riboflavin, whereas the culture medium pH with copper coupons was 7.3 ± 0.04 with and without riboflavin. On X65 carbon steel, after the 7-day incubation, the MPN sessile cell counts were both 10^6 cells/cm^2 with and without riboflavin. On copper, the MPN sessile cell counts were both 10^5 cells/cm^2 with and without riboflavin. The results suggest that 20 ppm riboflavin did not impact sessile cell growth. The results here also indicate that in the same culture medium, sessile SRB grew better on X65 than on copper. In a previous study on stainless steel, Zhang et al.[20] demonstrated that riboflavin had no impact on *D. vulgaris* planktonic growth in the ATCC 1249 medium.

SEM images in Figure 1 show that there were far more sessile cells on the X65 surfaces than on the copper surfaces. More importantly, the addition of 20 ppm riboflavin to the culture medium did not visibly change the sessile cell density in both X65 and copper cases. Thus, SEM images in Figure 1 support the sessile cell count data.

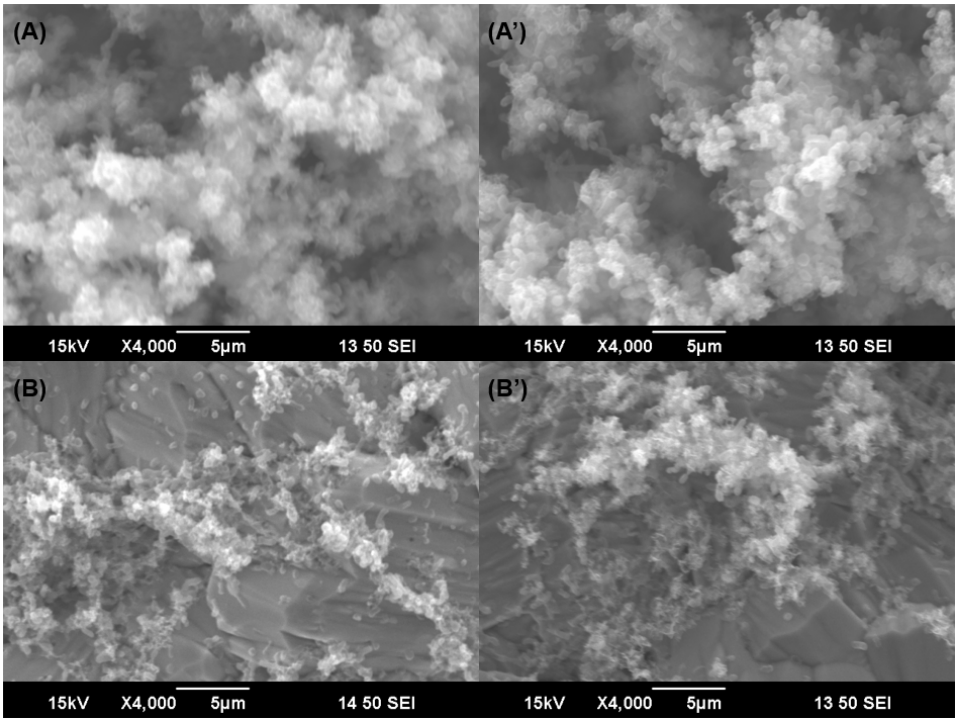

Figure 1. Biofilm images after the 7-day incubation: (A) carbon steel with 0 ppm riboflavin, (A') carbon steel with 20 ppm riboflavin, (B) copper with 0 ppm riboflavin, and (B') copper with 20 ppm riboflavin.

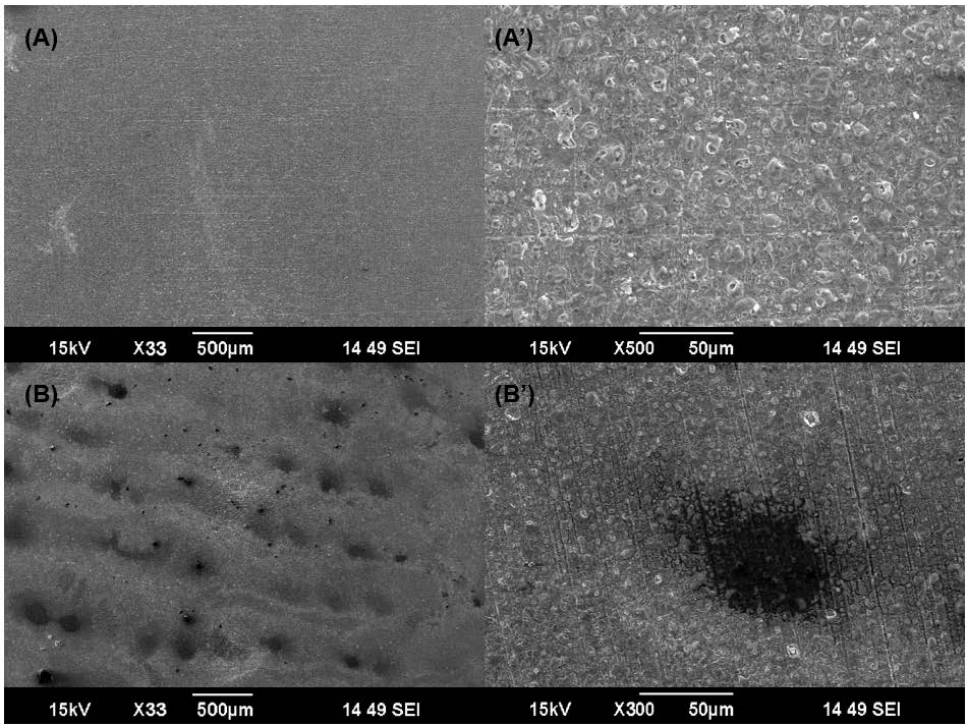

Figure 2. SEM pit images after the 7-day incubation on X65 carbon steel without (A and A') and with (B and B') 20 ppm riboflavin under different magnifications.

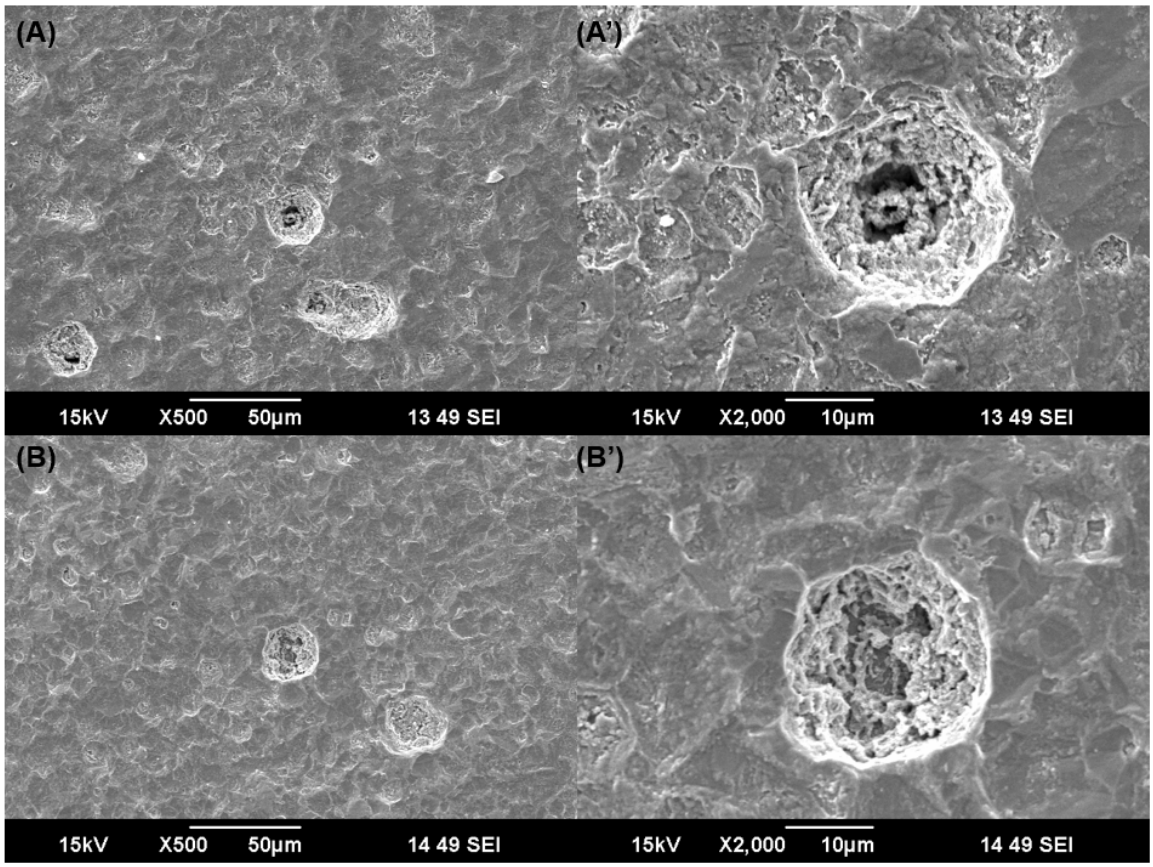

Figure 3. Pit images of copper MIC after the 7-day incubation without (A and A') and with (B and B') 20 ppm riboflavin under different magnifications.

The SEM images in Figure 2 show that there were more numerous and larger pits on X65 carbon steel with 20 ppm riboflavin compared with those without 20 ppm riboflavin. These results clearly show that the electron mediator promoted the pitting corrosion by *D. vulgaris* against X65 carbon steel. On the contrary, the SEM images in Figure 3 show that that the pitting corrosion on copper surface was not increased by 20 ppm riboflavin. It demonstrates that the electron mediator was not effective in promoting MIC of copper because electron transfer is not a bottleneck in M-MIC.

The weight losses of X65 carbon steel and copper with and without 20 ppm riboflavin after the 7-day incubation are shown in Figure 4A. Each data point was the average from three coupons in the same vial. Figure 4 demonstrates that 20 ppm riboflavin increased X65 weight loss from 1.0 ± 0.1 mg/cm^2 to 1.9 ± 0.4 mg/cm^2, reflecting a 90% increase. This clearly indicates that the electron mediator promoted the EET-MIC by *D. vulgaris* against the X65 carbon steel. This also suggests that electron transfer is a bottleneck in this EET-MIC. In comparison, Figure 4B shows that 20 ppm riboflavin resulted in a statistically insignificant lower weight loss for copper (27.0 ± 1.6 mg/cm^2 vs. 25.2 ± 1.5 mg/cm^2, indicating that 20 ppm riboflavin did not enhance copper corrosion by *D. vulgaris* and that in this M-MIC, electron transfer was not a bottleneck. It is interesting to see that in this system, the copper weight loss was at least one order of magnitude higher than X65 weight loss while the sessile cell count on copper was 10 times lower. The large weight loss in Figure 4 and the absence of polishing lines in Figure 2 point to considerable weight loss accompanied by pitting. Thus, SRB MIC could be detrimental to copper systems such fire sprinkler and heat exchanger systems that use copper or copper alloys.

Xu et al. noted that in EET-MIC, sessile cells harvest electrons from extracellular iron oxidation for energy production.[9] This process requires EET that uses an elaborate electron transport chain[27] to move the extracellular electrons to the cytoplasm inside SRB sessile cells for sulfate reduction. SRB sessile cells will only harvest enough electrons for energy needed for growth or maintenance. Thus, the extent of EET-MIC is limited by a biofilm's need for energy. In comparison, the extent of M-MIC does not have this limit. In this work, copper weight loss was one order of magnitude greater because it was the result of M-MIC caused by hydrogen sulfide secreted by sessile cells as well as planktonic cells.

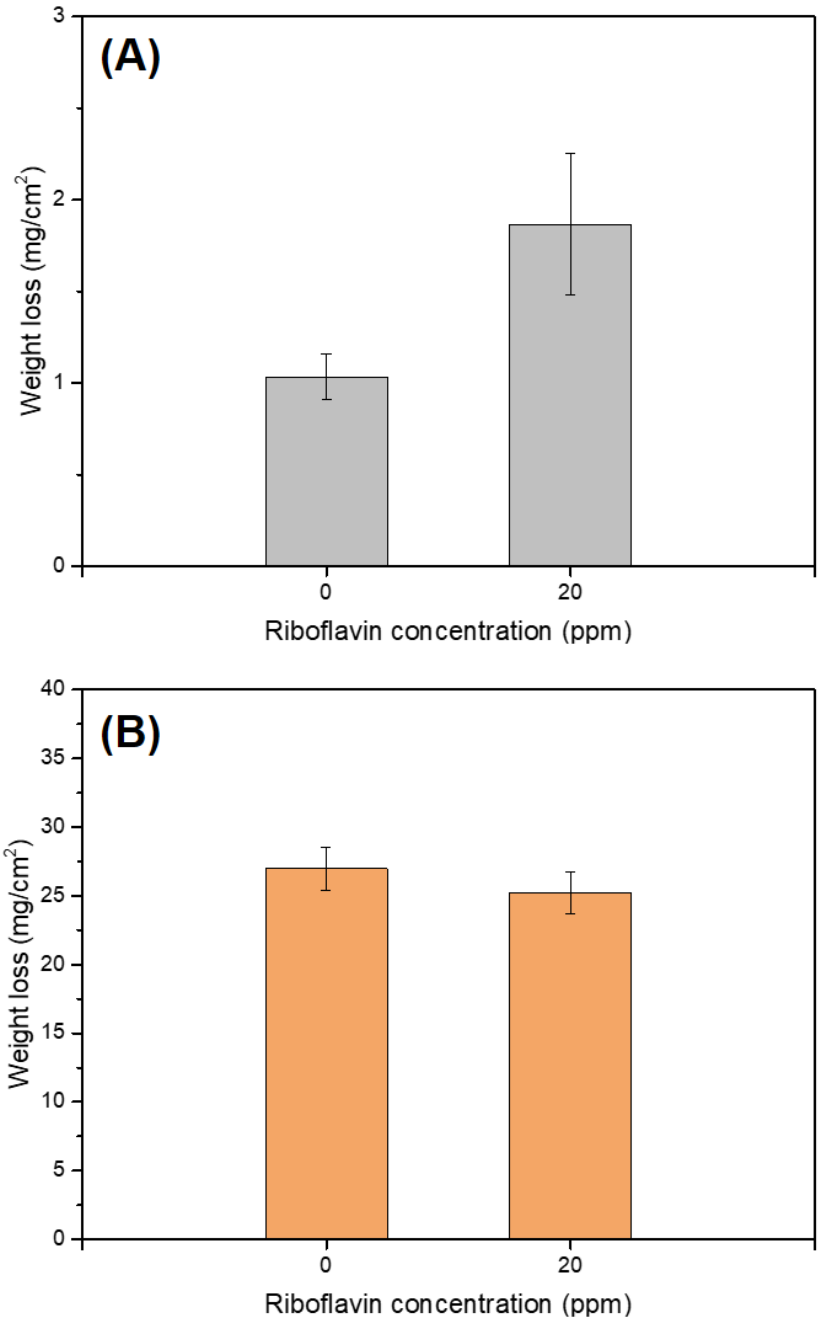

Figure 4. Weight losses of X65 carbon steel (A) and copper (B) after the 7-day incubation with and without 20 ppm riboflavin.

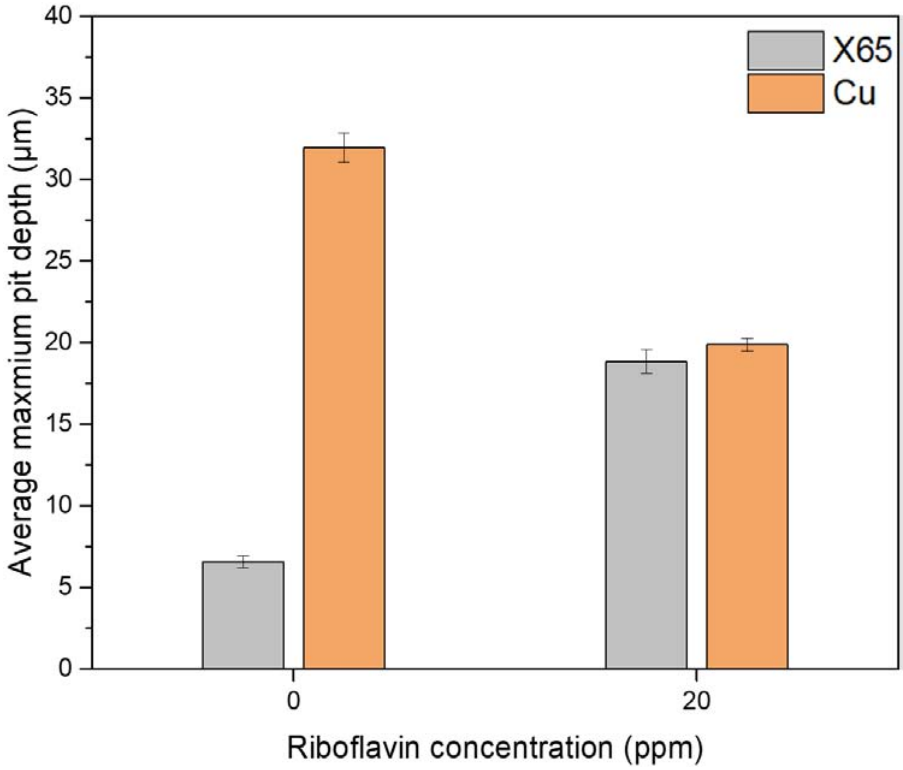

Figure 5. Average maximum pit depths of X65 carbon steel and copper after the 7-day incubation with and without 20 ppm riboflavin.

After the 7-day incubation, the maximum pit depths of six coupons from two replicate vials were measured using IFM to provide the average maximum pit depth for that condition after dropping the lowest one. Figure 5 shows that 20 ppm riboflavin greatly increased the average maximum pit depth on X65 from 6.6 ± 0.4 μm to 18.8 ± 0.7 μm, reflecting a 285% increase. In comparison, Figure 5 shows that 20 ppm riboflavin reduced the maximum pit depth on copper from 31.9 ± 0.9 μm to 19.8 ± 0.4 μm, reflecting a 38% reduction. The reason why riboflavin reduced pitting remains unknown. It is interesting to know that while the average maximum pit depth decreased for copper, the weight loss did not show a statistically significant decrease.

CONCLUSIONS

EET-MIC relies on extracellular electron transfer while M-MIC does not. The experimental results in this study demonstrate that electron transfer is a bottleneck in *D. vulgaris* MIC against X65, while it is not the case for *D. vulgaris* MIC against copper because the former belongs to EET-MIC, while the latter M-MIC. The data in this work clearly show that 20 ppm riboflavin considerably accelerated *D. vulgaris* MIC against X65, but not against copper. This work demonstrates that even with the same biofilm, two very different MIC mechanisms can occur. It also shows that SRB MIC weight loss against copper was at least one order of magnitude higher than that for carbon steel even though the SRB sessile cell count on copper was 10 times lower.

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