

A disposable electrochemical biofilm/MIC test kit to detect MIC and to assess efficacy of biocide treatment of biofilm

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ABSTRACT

Microbial biofilms are behind microbiologically influenced corrosion (MIC). Currently, all the so-called MIC test kits on the market are actually microbe test kits. Our new disposable miniature electrochemical biofilm/MIC test kit based on 10 ml serum vials with solid-state electrodes fills the gap to provide corrosion information such as abiotic corrosion vs. MIC, and MIC by electron-harvesting biofilms vs. MIC by corrosive metabolites. It can also test antimicrobial efficacy. An electrochemical workstation is used as a base station to perform various electrochemical scans such as linear polarization resistance, electrochemical impedance spectrometry and potentiodynamic polarization scans to provide near-real time transient corrosion data after a fluid or sludge is injected into the vial. This work presented a prototype with two different solid-state electrode choices. Three different methods to distinguish MIC from abiotic corrosion, and two different methods to distinguish MIC by electron-harvesting biofilms from MIC by corrosive metabolites. Anaerobic *Desulfovibrio ferrophilus* IS5 (a highly corrosive sulfate reducing bacterium) biofilm, aerobic *Pseudomonas aeruginosa* biofilm and an anaerobic corrosive oilfield biofilm consortium were used as examples. Tetrakis hydroxymethyl phosphonium sulfate (THPS) biocide was used as an example for assessing biocide efficacy. The patent-pending technology is currently undergoing field-testing.

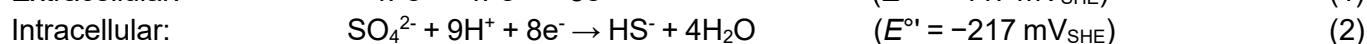
Key words: *Biofilm sensor, biofilm kit, corrosion sensor, MIC, biocide efficacy*

INTRODUCTION

Corrosion has been a major issue in many industries, especially the oil and gas industry¹⁻³. Nearly 25% of failures encountered in oilfields can be attributed to corrosion⁴. CO₂ corrosion and acid corrosion are common types of abiotic corrosion. CO₂ corrosion, also known as sweet corrosion or acid gas corrosion, of carbon steel has been extensively studied with the aim of reducing damage economic losses^{5,6}.

Microbiologically influenced corrosion (MIC) is an important branch in corrosion. Various microorganisms such as bacteria, archaea, fungi, and microalgae can cause MIC through their metabolic activities or metabolites⁷⁻⁹. Among them, sulfate reducing bacteria (SRB) are notorious and most prevalent for causing the most aggressive MIC^{10,11}. Other bacteria such as nitrate reducing bacteria (NRB) were also found to be corrosive against carbon steels, stainless steels^{12,13}.

When SRB utilize extracellular electrons from Fe⁰ oxidation for sulfate reduction, the electrons are transported from outside the SRB cells to the cytoplasm with the help of biofilm extracellular electron transfer (EET)¹⁴. This kind of MIC is known as EET-MIC (extracellular electron transfer-MIC)¹⁵,



SRB can also corrode via M-MIC (metabolite-MIC), mainly because of the highly reactive sulfides. SRB MIC of Fe(0) at circumneutral pH is not caused by sulfides as demonstrated before¹⁶. However, this is not the case for SRB MIC of Cu. Cu is not energetic enough to supply electrons directly for sulfate reduction. However, H₂S can corrode Cu with H⁺ as the electron acceptor, because the corrosion product Cu₂S is extremely insoluble^{17,18}. This makes the following corrosion reaction's Gibbs free energy change rather negative,



In this case, H⁺ at neutral pH becomes a feasible electron acceptor (oxidant) in Cu corrosion because the corrosion product Cu₂S has an extremely low solubility¹⁹.

Riboflavin (RF) and magnetite nanoparticles (MNPs) are electron mediators that can accelerate EET^{18,20}. They have been used to accelerate EET in *Desulfovibrio vulgaris* MIC of carbon steel. Thus, they are used to distinguish EET-MIC from M-MIC such as SRB MIC of Cu^{18,21}. They can also be used to distinguish EET-MIC from abiotic corrosion, because the latter does not rely on cross-cell wall electron transfer (i.e., EET).

The most popular approach to mitigate MIC is biofilm mitigation mainly by using biocides and scrubbing (e.g., pigging)²². A biocide typically has no impact on abiotic corrosion unless the biocide chemical has non-biocidal functions. Therefore, biocide addition can potentially distinguish abiotic corrosion from biotic corrosion. THPS (tetrakis hydroxymethyl phosphonium sulfate) is known as a broad-spectrum biocide widely used in the field and in lab tests^{23,24}.

There is a third way to distinguish MIC from abiotic corrosion based on the fact that a living biofilm is behind MIC, and it responds to electrochemical tests in a special way. Tafel scan is a popular tool for both

abiotic and biotic corrosion research^{25–27}. The orthodox Tafel scan method involves separate scans of the anodic and cathodic curves, which are performed on two replicate working electrodes (WEs). Each scan starts from the open circuit potential (OCP) (i.e., zero external voltage), extending to -200 mV (cathodic) vs. OCP and to $+200$ mV (anodic) vs. OCP (or higher if stainless steel passive film failure needs to be detected). To minimize the need for WEs in MIC, it is a common practice to scan continuously from the most negative potential to the most positive potential on a single WE, which is known as continuous upward scan. However, continuous upward scans in MIC introduce considerable distortions which often manifest as compression in the cathodic region and elongation in the anodic region (in voltage ranges), accompanied by a large deviation (typically around 50%) in corrosion current density (i_{corr}) compared to the orthodox scans. These distortions and i_{corr} deviation are attributed to insufficient time for a biofilm on the WE to adapt to the extreme externally-applied voltage (usually -200 mV vs. OCP)²⁸. In comparison, the orthodox scan method, which starts each scan from OCP (i.e., no external voltage), allows time for the biofilm to adapt while the continuous upward scan method does not. It was found that even with the same WE, repeated half-scans starting from OCP did not cause large Tafel curve distortions and i_{corr} deviations²⁸. Because an abiotic WE (no living biofilm involved) is far less prone to Tafel scan distortions and i_{corr} deviation using continuous upward scan method, the continuous upward scan comparison with dual-half scans can be used to distinguish abiotic corrosion from MIC.

In corrosion studies, weight loss and pitting rates are direct evidence for corrosion severity. Hanging coupons are placed inside pipelines or storage tanks to obtain weight loss and pitting data in field operations. Electrochemical tests such as LPR (linear polarization resistance) and Tafel scans are also widely used as a powerful tool to verify coupon weight loss results, especially in lab tests. EIS (electrochemical impedance spectroscopy) scans can provide additional information about charge transfer and mass transfer in the MIC process^{29,30}. In various MIC studies, electrochemical test results were consistently found to support weight loss results^{31–34}. Electrochemical data were also used in biocide efficacy studies and found to be consistent with weight loss reduction and sessile cell reduction trends^{35,36}. The percentage in the reduction of i_{corr} or increase in R_p (polarization resistance from LPR) can be used as biocide efficacy or corrosion inhibition efficiency, and compared with weight loss-based and sessile cell reduction-based efficacies³⁶. Among these electrochemical scans, LPR takes the least amount of time (e.g., as short as 2 min). Compared to coupon tests which only give cumulative results at the end of the corrosion period, electrochemical tests provide transient corrosion data. Therefore, the application of electrochemical tests makes near real-time corrosion rate measurements possible. For electrochemical tests, a typical 3E (3-electrode) setup consists of a WE (working electrode), a CE (counter electrode), and an independent (i.e., dedicated) RE (reference electrode). A typical RE needs an electrolyte solution, which makes it not suitable for a robust disposable MIC sensor. Thus, it is desirable to develop a solid-state RE. To simplify an MIC/(biofilm) test kit, a proper CE electrode can be chosen to serve as both CE and a pseudo-RE (p-RE) so the kit only has two connectors with the other connector being WE.

In this work, a disposable MIC test kit was presented for field applications which contains two solid-state electrodes. It is based on a common 10 mL serum vial for the injection of 5 mL to 7 mL of a field sample fluid or sludge to grow a biofilm on the WE surface. By using electrochemical scans employing a desktop or a battery-powered portable potentiostat, this MIC test kit can provide near real-time corrosion rate results and biocide efficacy data. Abiotic corrosion can be differentiated from biotic corrosion using one of the three different methods, and EET-MIC can be differentiated from M-MIC using electron mediators.

EXPERIMENTAL PROCEDURE

2. Materials and Methods

2.1 Bacteria, chemicals, and metals

Desulfovibrio ferrophilus (IS5 strain, DSM 15579) and an oilfield consortium (Consortium Ile) were grown in EASW (enriched artificial seawater) culture medium at 28 °C and 37 °C, respectively under anaerobic conditions. *Pseudomonas aeruginosa* was cultured aerobically in LB (Luria-Bertani) medium at 37 °C, respectively. The pH of each medium was adjusted to 7.0 using HCl (5% w/w) and/or NaOH solution (5%) before autoclave sterilization. EASW was sparged with filter-sterilized N₂ gas for 1 h for deoxygenation. L-cysteine was then added as an oxygen scavenger at 20 ppm (w/w) in the culture medium in an anaerobic chamber filled with N₂.

The composition (g/L) of EASW was: Na₂SO₄ 3.917, NaCl 23.476, NaHCO₃ 0.192, KBr 0.096, KCl 0.664, H₃BO₃ 0.026, SrCl₂·6H₂O 0.040, MgCl₂·6H₂O 10.610, CaCl₂·2H₂O 1.469, yeast extract 1.0, tri-sodium citrate (Na₃C₆H₅O₇) 0.5, sodium lactate (C₃H₅NaO₃) 3.5, CaSO₄·0.5H₂O 0.1, NH₄Cl 0.1, MgSO₄·7H₂O 0.71, Fe(NH₄)₂(SO₄)₂·6H₂O 1.38, K₂HPO₄ 0.05. The LB medium consisted of 10 g tryptone, 5 g yeast extract, 5 g NaCl, and in 1 L deionized water.

X65 carbon steel, 304 stainless steel (SS) and Cu (copper) (99.9% mass purity) were used in this work. Table 1 shows elemental compositions of X65 carbon steel and 304 SS. All metal coupons for WEs were polished to 600 grit. Anhydrous isopropanol (99% by volume) was used to sanitize electrode surfaces. Two different solid-state materials (code-named materials A and B) were tested as CE/p-RE.

Table 1. Elemental compositions (wt. %) of X65 and 304 SS (Fe balance).

Metal	C	Mn	P	N	Cr	S	Mo	Si	Ni	V	Nb	Co	Ti	Cu
X65	0.16	1.65	0.02			0.01		0.45		0.09	0.05		0.06	
304 SS		1.82		0.063	18.2		0.34	0.31	8.18			0.16		0.67

2.2 MIC test kit setup

The 10 mL MIC test kit consisted of a metal WE (1 cm² square work surface) and a solid-state CE/p-RE in a 10 mL anaerobic vial to form a disposable mini electrochemical glass cell (Fig. 1). For anaerobic tests, the vials were sealed with a rubber septum and aluminum cap. Silicone glue was used to seal the cap as an extra measure against possible gas leak. Sample liquids were injected into the MIC test kit with a needle.

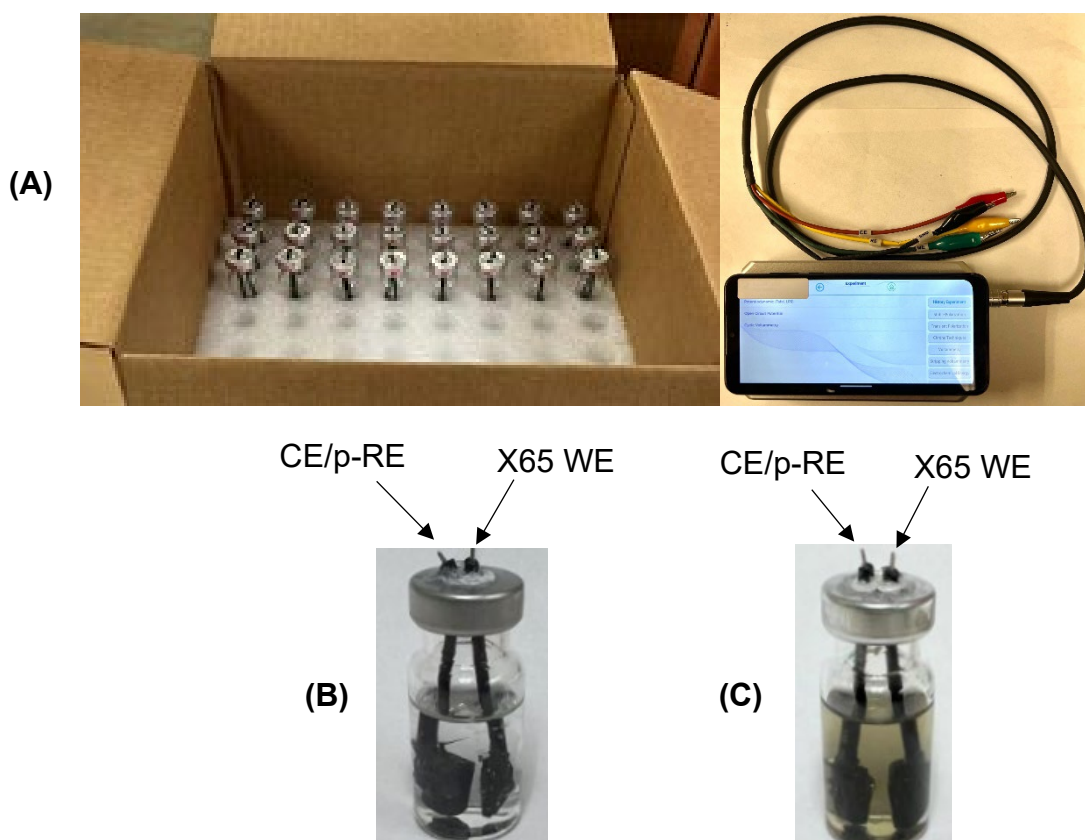


Fig. 1. (A) Multiple units of 10 mL biofilm/MIC test kit to be shipped out for field testing with electrochemical scans that can be performed using a battery-powered portable potentiostat (cell phone with app for it shown on top), (B) test kit setup for abiotic and (C) biotic tests with X65 WE and a solid-state material as CE/p-RE.

2.3 Tafel scans

Tafel scans were performed on each 10 mL electrochemical glass cell at 3 d (time needed to grow a mature biofilm on WE) of incubation at a scan rate of 0.167 mV/s. A PCI4/750 potentiostat (Gamry Instruments, Inc., Warminster, PA, USA) was used for measurements. Dual-half scans from 0 to -200 mV (vs. OCP) and 0 to +200 mV (vs. OCP) were followed by a continuous upward scan from -200 mV (vs. OCP) to +200 mV (vs. OCP) using the same 10 mL electrochemical glass cell.

2.4 Injection test

Injection tests were carried out at 3 d biofilm growth. LPR was scanned at a rate of 0.167 mV/s from the range of -10 to 10 mV vs. OCP every 20 min to get a stable R_p (polarization resistance) curve over 1 h. Then, RF or MNPs were injected into the cell to reach a final concentration of 20 ppm in the liquid. The vial was gently shaken for 3 min to disperse riboflavin or MNPs. LPR measurements were performed every 10 min to monitor R_p . After R_p became relatively stable for at least 30 min, THPS was injected to reach 100 ppm. The vial was again gently shaken for 3 min, and R_p was monitored every 10 min until it stabilized.