2019 Award Nomination

Title of Innovation:
Microfluidic MIC model: EIS, microscopy

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Category: Testing

Coatings and Linings	Instrumentation
Cathodic Protection	Testing
Materials Design	Integrity Assessment
Chemical Treatment	Other—fill in

Dates of Innovation Development: From Jan 2015 to May 2017

Web site: https://sites.google.com/tamu.edu/m-micmodel2/home

Summary Description:

A holistic understanding of microbiologically influenced corrosion (MIC) requires investigation of the underlying microbiological, metallurgical and electrochemical mechanisms. MIC studies have been conducted largely using batch reactors or large scale continuous circulating loops or continuous stirred tank reactors (CSTR) that require enormous quantities of fluids or have disadvantages with nutrient insufficiency and corrosion product accumulation which disrupt biofilm growth. To effectively comprehend MIC mechanisms while overcoming these drawbacks, we developed a novel microfluidic microbiologically influenced corrosion model for EIS and microscopy (Microfluidic MIC model: EIS, microscopy) that is amenable to dynamic and integrated measurements of biofilm physiology and electrochemical impedance (**Figure 1**). This flow model simulates once-flow-through setup of pipelines and comprises of a two-metal

electrode system with carbon steel (corroding electrode) and titanium (non-corroding, counter electrode) deposited on glass and bonded to a transparent polymer, polydimethylsiloxane (PDMS) with imprinted microchannels.

This flow model can be used to establish MIC environment using corrosive process fluids or cultures of model organisms in a continuous flow setting while using minimal reagents. This flow model further enables measurements of biofilm growth dynamics using confocal laser scanning microscopy (CLSM), electrochemical reactions using electrochemical impedance spectroscopy (EIS), and metal surface measurements using scanning electron microscopy (SEM) and optical profilometry. This flow model has been assessed for its feasibility in the laboratory by simultaneously monitoring biofilm biomass and EIS using cultures of bacteria (modified to express fluorescent protein) associated with MIC and a correlation was observed between these two. Further, the use of equivalent circuit fitting leads to calculation of the corrosion rate through polarization resistance. Hence, the "Microfluidic MIC model: EIS, microscopy" model provides an ideal testing platform with cost and time savings for simultaneously monitoring all the aspects of MIC (corrosion reactions, biological changes and metal surface variations) for obtaining fundamental understanding of MIC.

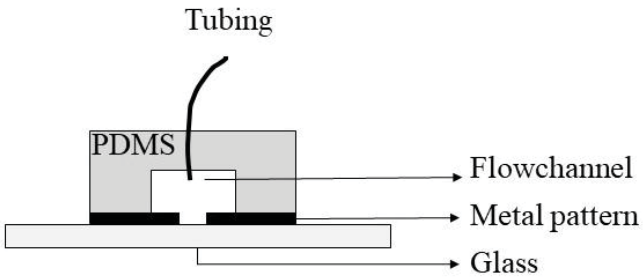

Figure 1: Schematic of the side-view of "Microfluidic MIC model: EIS, microscopy" for simultaneous monitoring of MIC using electrochemical impedance spectroscopy and confocal microscopy

Full Description:
(Please provide complete answers to the questions below. Graphs, charts, and photos can be inserted to support the answers.)

1. What is the innovation?

The innovation outlines the development of a novel microfluidics based flow model, "Microfluidic MIC model: EIS, microscopy" for microbiologically influenced corrosion (MIC) testing by integrating simultaneous measurements of biofilm physiology and electrochemical impedance and endpoint measurements of metal surface topography. This flow model is fabricated by depositing corroding metals/alloys on glass slides in parallel to non-corroding metals/alloys and then bonding the metal deposited glass slides with microchannels molded within a transparent polymer. Specifically for this work, we used carbon steel as the corroding alloy and titanium as the non-corroding metal for deposition. Polydimethylsiloxane (PDMS) was used as the transparent polymer for conducting MIC studies.

2. How does the innovation work?

The "Microfluidic MIC model: EIS, microscopy" can be fabricated by depositing corroding metal or alloy (such as carbon steel) and non-corroding metal (such as titanium) and patterning them as parallel rectangular bands on glass. This flow model enables simultaneous and integrated readout of corrosion reactions, biofilm physiology, and metal surface changes. Cultures of model organisms isolated at MIC impacted field locations or process fluids from MIC impacted field locations can be used for establishing MIC in the flow model. The impact of various hydrodynamic factors (such as flow rate, shear), biochemical factors (such as availability of certain nutrients or quorum sensing molecules), environmental factors (such as temperature, gas composition), and metallurgical factors (such as composition of corroding alloy) can be evaluated on the biofilm formation, subsequent biofilm growth, interaction of biofilms with other corrosive agents and metal, electrochemical reactions, and sustenance of MIC.

Biofilm growth dynamics can be monitored real-time by modifying the microorganisms to express fluorescent proteins and subsequently utilizing confocal laser scanning microscopy to obtain biofilm 3D structures and quantifying biofilm biomass using image analysis software such as ImageJ and COMSTAT. Additionally, biofilm viability can also be evaluated by employing live dead staining methods to distinguish live and dead cells followed by confocal microscopy to obtain 3D biofilm structures and quantified biofilm biomass or using sessile adenosine triphosphate (ATP) assay for determining the extent of microbial activity in the biofilms through the presence of energy storage molecules, ATP.

Electrochemical reactions occurring can be monitored by connecting the flow model to a potentiostat using carbon steel as the working electrode and titanium as the counter and pseudo-reference electrode. Several electrochemical assessment methods such as electrochemical impedance spectroscopy (EIS) and open circuit potential (OCP) can be utilized to decipher electrochemical changes occurring due to microbial metabolism and corrosion reactions. OCP is commonly used to qualitatively measure the extent of corrosion. On the other hand, data from EIS can be used to represent Bode and Nyquist plots for obtaining both qualitative and quantitative information on corrosion reactions. For example, an increase in impedance values over time in Bode plot indicates the formation of corrosion product layer and an increase in Nyquist plot diameter correlates to formation of corrosion product layer. In a corroding system, the Nyquist plot diameter is expected to decrease and the impedance values are expected to increase. Furthermore, using the data from Bode and Nyquist plots, any electrochemical system can be fitted to an equivalent circuit of resistors and/or capacitors based on the nature of the system. Equivalent circuit fitting of the system can help obtain the polarization resistance (inversely related to corrosion current and corrosion rate) that gives information on corrosion rate of the system.

After investigating the biofilm growth and their impact on electrochemical reactions, the biofilms can be separated from the channel exposing the metal surface that can then be inspected for any signs of corrosion. Scanning electron microscopy (SEM) and optical profilometry can be employed to observe the topography of the metal surface and identify any regions of metal loss or pits to further estimate pitting corrosion rate.

3. Describe the corrosion problem or technological gap that sparked the development of the innovation? How does the innovation improve upon existing methods/technologies to address this corrosion problem or provide a new solution to bridge the technology gap? Currently employed laboratory MIC setups are batch reactors simulating static environments or recirculating loops and continuous stirred tank reactors simulating continuous flow environments. These setups result in issues with accumulation of waste and corrosion products and inadequate nutrient availability impacting microbial metabolism. Also, most of these setups are not once-flow-through units and require large quantities of fluids if they mimic once-flow-through conditions. Hence, studies conducted under such environments do not accurately represent MIC conditions occurring in pipelines.

These MIC setups typically use biofilm swabs from pipelines impacted with MIC or process fluids collected these pipelines as inoculum for simulating MIC and investigate MIC mechanisms by conducting dynamic measurements of only electrochemical changes and end point measurements of biofilm physiology and metal surface changes. Such analyses are insufficient

and present a technological gap as they do not account for temporal changes occurring in the system.

To decipher these temporal interactions between electrochemical, microbiological, and metallurgical aspects, “Microfluidic MIC model: EIS, microscopy” enables simultaneous and integrated measurements of electrochemical and microbiological variations with endpoint measurements of metal surface changes. Furthermore, this flow model mimics pipeline flow conditions while using limited quantities of process fluids or laboratory grown microbial cultures for establishing and investigating MIC. Hence, this flow model addresses the gaps of current MIC setups and additionally provides an integrated readout of microbiological and electrochemical changes to fundamentally investigate MIC as well as evaluate the impact of hydrodynamic, biochemical, environmental, and metallurgical factors on MIC.

4. Has the innovation been tested in the laboratory or in the field? If so, please describe any tests or field demonstrations and the results that support the capability and feasibility of the innovation.

The “Microfluidic MIC model: EIS, microscopy” has been tested in the laboratory to obtain dynamic read out of electrochemical impedance and biofilm biomass using model organisms. *Shewanella oneidensis* (belonging to the iron-reducing class of microorganisms and commonly associated with MIC) was initially modified to express red fluorescent protein. Then, biofilms of *S. oneidensis* were grown in this flow model by seeding the flow model at 30^{o}C with an overnight grown culture of *S. oneidensis* and subsequently flowing Luria-Bertani growth medium (10g/l tryptone, 5g/l yeast extract and 15 g/l sodium chloride) continuously with 80 µg/ml of Kanamycin at a flow rate of 0.25 mL/h. These biofilms were imaged every 12 h using confocal laser scanning microscopy (CLSM) and analyzed for the biofilm biomass. Further, a potentiostat was also used to record electrochemical impedance spectroscopy (EIS) scans alongside microscopy images every 12 h. The schematic of the experimental setup is depicted in **Figure 2**. This experiment with *S. oneidensis* biofilms indicated a correlation between biofilm biomass and impedance spectra in the mid-frequency region (**Figure 3**).

Similarly, other studies can be conducted with various other laboratory grown microbial cultures associated with MIC or with process fluids to correlate impedance changes to biofilm growth dynamics. This knowledge can enhance our understanding of MIC mechanisms through insights on initiation and sustenance of biofilm growth and subsequent MIC. This knowledge of MIC mechanisms can be later used for successful mitigation of MIC.

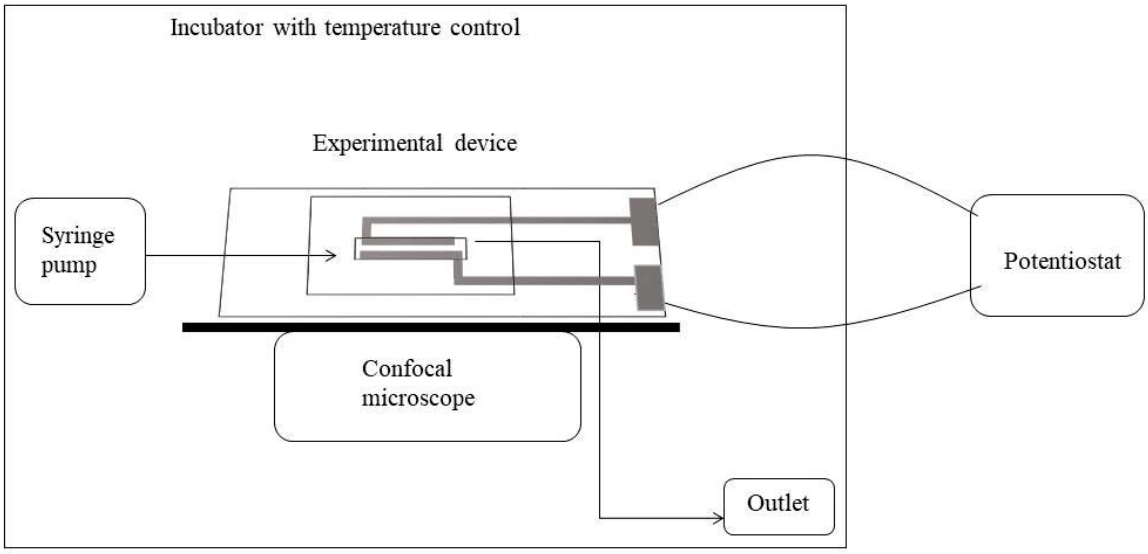

Figure 2: Schematic of experimental setup for "Microfluidic MIC model: EIS, microscopy"

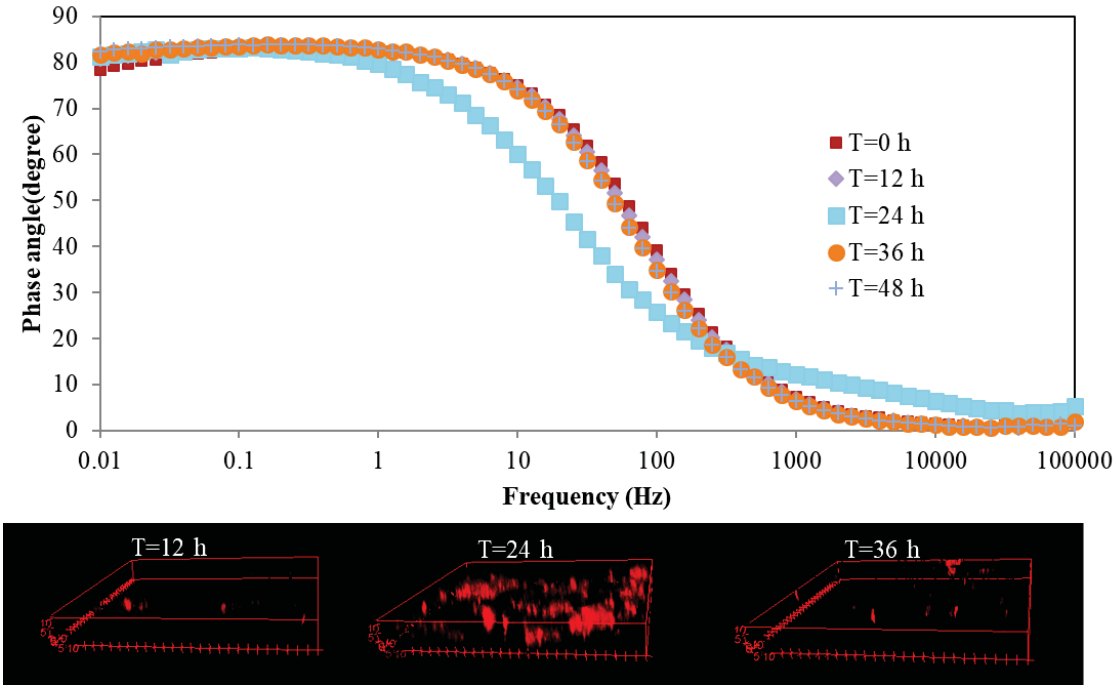

Figure 3: Bode plot of phase angle and frequency of a *S. oneidensis* biofilm experiment (top). Representative 3 D biofilm images of *S. oneidensis* (bottom).

5. How can the innovation be incorporated into existing corrosion prevention and control activities and how does it benefit the industry/industries it serves (i.e., does it provide a cost and/or time savings; improve an inspection, testing, or data collection process; help to extend the service life of assets or corrosion-control systems, etc.)?

The "Microfluidic MIC model: EIS, microscopy" provides an ideal MIC testing platform by simulating pipeline flow environments while utilizing limited reagents (process fluids from MIC impacted field locations or laboratory grown microbial cultures). This flow model is a superior testing platform over current static and flow systems due to the use of microchannels for establishing MIC. Also, this model incorporates an improved data collection process as it employs simultaneous measurements of confocal microscopy (biofilm growth dynamics or biofilm viability) and electrochemical impedance spectroscopy (corrosion reactions). Also, this model provides cost savings and time savings because it uses considerably lower amount of reagents and biofilms can be cultured quicker in the microchannels. A fundamental understanding of MIC mechanisms and interactions between the biofilms and corrosion reactions is crucial for successful MIC mitigation and can be addressed using this flow model. Hence, the M-MIC model provides improved testing platform while utilizing enhanced testing and data-collection methods resulting in increased fundamental understanding of MIC while simultaneously delivering cost and time savings. This enhanced understanding of MIC can be later used for successful corrosion-control strategies.

6. Is the innovation commercially available? If yes, how long has it been utilized? If not, what is the next step in making the innovation commercially available? What are the challenges, if any, that may affect further development or use of this innovation and how could they be overcome?

The "Microfluidic MIC model: EIS, microscopy" is not commercially available. However, it has been used for conducting laboratory investigation of MIC at Texas A&M University. Furthermore, a provisional patent relating to this innovation was filed in May 2017 and was converted to a full utility application in April 2018. This technology is currently available for licensing and can be commercially available once licensed.

This flow model uses a two-electrode system with the working electrode as carbon steel and counter and pseudo-reference electrode as titanium. Although this system has high resistance and the use of microelectrodes minimizes the changes in the counter electrode's potential, the counter electrode cannot always be reliably used as a reference electrode to measure the potential difference. Hence this flow model is currently being improved to modify it to a three-electrode system.

These flow models were presently fabricated on a need based approach and mass production of these flow models has not been the scope of our innovation. However, mass production platforms have been successfully utilized for the development of several other microfluidic devices. One challenges for this innovation would be making this innovation commercially available by granting licensing rights to a company. We believe that *MP*'s Corrosion Innovation of the Year Awards will be an ideal platform for publicizing this innovation.

7. Are there any patents related to this work? If yes, please provide the patent title, number, and inventor.

A provisional patent related to this flow model was filed in May 2017 and was later converted to a full utility application in April 2018. The details of the patent are:

Patent title: Microfluidic microbiologically influenced corrosion model

Patent number and details: PCT/US2018/030198, Full utility application April 2018

Inventors: Susmitha Purnima Kotu, Arul Jayaraman, Sam Mannan, Arum Han