2019 Award Nomination
Title of Innovation:
Microfluidic MIC model: Biocide studies

Nominee(s)
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Category: Chemical treatment

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-micmodel1/

Summary Description:

Microbiologically influenced corrosion (MIC) occurs by complex interplay between microbial metabolism, corrosive compounds, and metal. MIC has been extensively explored using batch reactors or circulating loop systems or continuous stirred tank reactors (CSTR). Both batch systems and circulating systems can provide confounding results due to nutrient limitation and accumulation of corrosion products and waste products that affect microbial growth and biofilm formation. Further, CSTR requires large volumes of fluids. To overcome these disadvantages, we developed a novel microfluidic microbiologically influenced corrosion model, "Microfluidic MIC model: Biocide studies" (**Figure 1**), comprising of carbon steel coated glass slide bonded to a microchannel imprinted inside a transparent polymer, polydimethylsiloxane (PDMS). This flow model is a continuous once-flow-through unit similar to pipelines where MIC

is a major concern.

This flow model can be used to establish MIC environment in microchannels 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 viability (instead of planktonic cell viability) using confocal laser scanning microscopy (CLSM) and sessile adenosine triphosphate (ATP) assay and metal surface measurements using scanning electron microscopy (SEM) and optical profilometry. This flow model has been evaluated for its feasibility in the laboratory by conducting biocide efficacy studies using model bacteria associated with MIC. Biofilm viability determined using confocal microscopy was used as an indicator for effectiveness of biocide. Further, this flow model was also tested using produced water to establish biofilm growth and MIC in the microchannels. Biofilm viability determined using fluorescent microscopy and sessile ATP assay were used as indicators for comparing the efficacy of biocides. Hence, this flow model provides an ideal testing and evaluation method with cost and time savings for conducting biocide screening, efficacy, and dosage studies with process fluids for effective MIC mitigation.

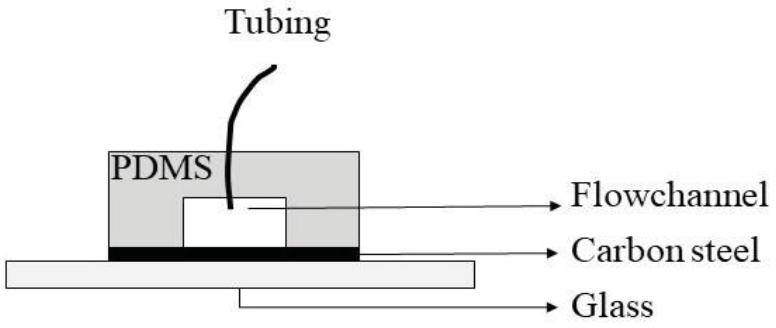

Figure 1: Schematic of the side-view of "Microfluidic MIC model: Biocide studies"

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: Biocide studies" for microbiologically influenced corrosion (MIC) testing and subsequent utilization of this flow model for laboratory evaluation of effective MIC mitigation strategies by conducting biocide screening, biocide efficacy, and biocide dosage studies. These flow models are fabricated by depositing metals/alloys on glass slides 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 for deposition and polydimethylsiloxane (PDMS) as the polymer for conducting MIC studies.

2. How does the innovation work?

The "Microfluidic MIC model: Biocide studies" can be fabricated by depositing various metals or alloys such as iron, copper, stainless steel, and carbon steel on glass. MIC environment can be simulated in this flow model by flowing cultures of model organisms isolated at MIC impacted field locations or flowing process fluids from MIC impacted field locations. A wide range of flowrates and environmental conditions can be utilized for mimicking the field conditions. After establishing MIC in the flow model, the system can be treated with various biocides to conduct preliminary biocide screening studies. Later, biocide efficacy and dosage studies can be conducted to determine the appropriate biocide dosage for MIC mitigation. Conducting biocide studies with biofilms of model organisms also helps investigate the differences in their modes of action.

The biofilms grown in this flow model can be tested for their viability instead of using the inaccurate planktonic testing methods. Biofilm viability can be evaluated by conducting live dead staining followed by confocal laser scanning microscopy. Live dead staining utilizes two stains, SYTO9 and propidium iodide such that live microorganisms (with intact cell membrane) stain green while dead microorganisms (with damaged cell membrane) stain red. Confocal microscopy helps obtain 3D structure of biofilms at high resolution distinguishing live and dead biomass. Next, quantification of live and dead biomass can be conducted using image analysis software such as ImageJ and COMSTAT. Additionally, biofilm viability can be evaluated using sessile adenosine triphosphate (ATP) assay for determining the extent of microbial activity in the biofilms through the presence of energy storage molecules, ATP.

After analyzing the biofilm viability, the biofilms can be detached from the flow channel to expose the metal surface which can then be examined for indicators of corrosion. Techniques such as scanning electron microscopy (SEM) and optical profilometry can be utilized to monitor the topography and the roughness of the metal surface. These methods can also be used to calculate the pit depths on the metal surface and the pitting corrosion rate.

2. 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?

Laboratory MIC studies are extensively carried out using batch reactors where metal coupons are incubated in a static environment. Although batch reactors are easy to use, they do not represent pipeline flow environment where MIC occurs and exhibit several disadvantages such as accumulation of waste products, corrosion products and limited availability nutrients which further impacts microbial metabolism. Alternatively, flow systems such as, continuous flow systems, circulating flow loops, modified continuous stirred tank reactor have been developed. While these flow systems address some of the drawbacks of batch systems, they typically require large volumes of liquid. Further, circulating flow loops present drawbacks with waste product accumulation and nutrient deficit. To summarize, both the batch reactors and flow systems currently employed for conducting MIC studies have several disadvantages.

The batch reactors or flow systems currently employed for MIC studies establish MIC conditions using inoculum from the field systems associated with MIC (liquid/biofilm scrapings from pipelines) to investigate their impact on metal coupons and corrosion and evaluate the efficacy of biocides for field application. Most of the corrosion control studies still rely on using planktonic testing methods such as bug bottle tests or most probable number (MPN) methods although it has been established that biofilms mediate MIC and planktonic testing methods misrepresent and underestimate the microbial community.

The “Microfluidic MIC model: Biocide studies” presents a system with once-flow-through conditions (similar to pipelines) that utilizes minimal reagents for establishing biofilms and corrosion from process fluids from MIC impacted field locations. Also, this flow model can be used to evaluate biocide efficacy tailored to the process fluids and the corrosive conditions in that field system using biofilm viability (and not planktonic microbial counts) and indicators of corrosion on the metal surface. In short, this flow model addresses the gaps with current MIC testing methods by providing the right platform and evaluation method for conducting biocide screening, biocide efficacy, and biocide dosage studies.

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: Biocide studies" has been tested in the laboratory to evaluate and biocide performance using model organisms. Further, this flow model has been tested using produced water from an oil and gas platform in the Gulf of Mexico to establish biofilm growth and compare the performance of a newer biocide formulation to a traditionally used biocide using biofilm viability determined from sessile ATP assay and fluorescent microscopy. However, only the results from the laboratory study are included here.

Biofilms of *Shewanella oneidensis* (an iron-reducing bacterium commonly associated with MIC) were grown on carbon steel in this flow model by seeding the flow model at 30^{o}C with an overnight grown culture of *S. oneidensis*. Subsequently, a constant flow of Luria-Bertani growth medium (10g/l tryptone, 5g/l yeast extract and 15 g/l sodium chloride) was introduced at a flow rate of 0.25 mL/h. After 12 hours, the flow of growth medium was stopped and 400 ppm of two common oilfield biocides, glutaraldehyde and tetrakis-hydroxymethyl-phosphonium sulfate (THPS), were added to the biofilms. After 4 hours of biocide treatment, the live and dead cells in the biofilms were stained using SYTO9 (green) and propidium iodide (red), respectively. Stained biofilms were imaged using confocal laser scanning microscopy (CLSM) and analyzed for the percentage of live and dead cells to determine biocide efficiency. A difference in the biofilm viability was observed when treated with the two biocides (**Figure 2**). A 2.1-fold increase in the percentage of dead cells was observed with glutaraldehyde treatment while the percentage of dead cells with THPS treatment increased by 3.1-fold. This data suggests that THPS treatment is more effective than glutaraldehyde against *S. oneidensis* biofilms under the conditions tested.

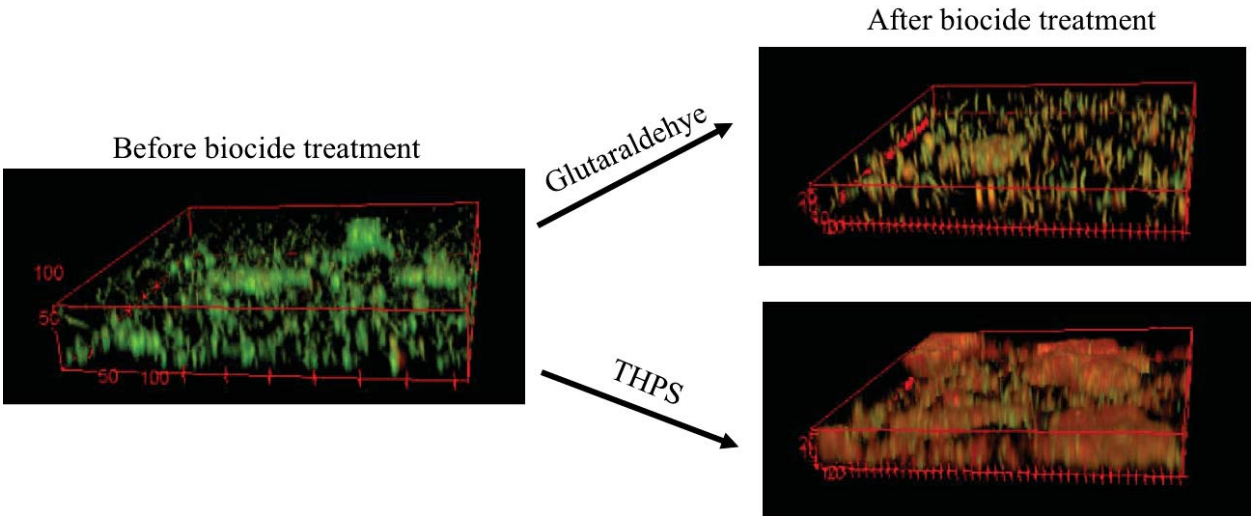

Figure 2: Comparison of *S. oneidensis* biofilm viability before and after biocide treatment with glutaraldehyde and THPS

Similar studies can be conducted with different biocide concentrations to determine the appropriate biocide dosage in addition to determining the right biocide. This flow model also allows conducting biocide efficacy studies in the laboratory to decide the biocide dosage to be applied in the field for effective MIC mitigation.

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: Biocide studies" provides a MIC testing platform simulating pipeline flow conditions while utilizing limited volume of process fluids collected from MIC impacted field locations. Due to these advantages, this model is an improvement over current MIC testing static and flow systems. Also, this model provides cost savings because it uses considerably lower amount of field waters for MIC testing. Further, this model offers time savings because the biofilms grow faster in the microchannels reducing the duration of experiment. Additionally, this model enables enhanced testing schemes by employing pertinent biofilm testing methods instead of the incorrect planktonic testing methods. Conducting the biocide screening and efficacy studies with field waters and relevant biocides in this flow model before field application helps control MIC efficiently. Hence, this flow model provides improved testing platform while utilizing enhanced testing methods resulting in effective MIC mitigation while simultaneously delivering cost and time savings.

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: Biocide studies" is not commercially available but has been used extensively for laboratory studies at Texas A&M University. Furthermore, a provisional patent 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 can be effortlessly modified to a high-throughput flow model to enable comparison of biocide efficacies. **Figure 3** depicts the PDMS layer of the microfluidic channel with a single cell inlet splitting into three microchannels for biofilm growth. This model can be used for comparing three different biocides that can be introduced through three separate inlets. While **Figure 3** shows three parallel channels, ~ 10 parallel microchannels can be

realistically fabricated so that multiple biocides can be compared. This high-throughput flow model enables comparison of efficacies of different biocides on microbial control required for MIC mitigation.

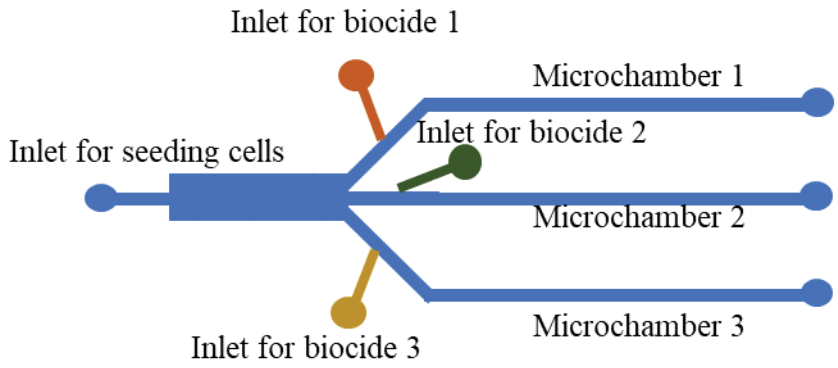

Figure 3: Schematic top-view of a high-throughput microfluidic flow model to compare biocide efficacies

In our work, these flow models were fabricated on a requirement basis only and venturing into mass production has not been the scope of our innovation. However, mass production platforms for microfluidic devices exist and have been successfully utilized for other applications. Alternatively, a setup similar to **Figure 3** can be used to reduce the total number of experiments required for biocide studies by increasing the throughput of the flow model.

The only challenge would be making this innovation commercially available by providing licensing rights to a company or group of companies. *MP*'s Corrosion Innovation of the Year Awards provides an excellent 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