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On the need for more realistic experimental conditions in laboratory-based microbiologically influenced corrosion testing

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ABSTRACT

Microbiologically influenced corrosion (MIC) encompasses a complex suite of processes that can result in costly and dangerous acceleration of corrosion rates. However, the complexity of MIC, and the wide range of conditions in which it occurs in the natural environment, has resulted in researchers performing tests under a wide range of laboratory conditions. This scattergun approach can potentially lead to ambiguous results and can compromise the ability to compare data from different tests. Therefore there is a need for the corrosion community to develop more realistic and standardised approaches to laboratory-based testing. This paper seeks to illustrate the challenges by examining a range of test parameters that can influence the results of MIC studies, using *Escherichia coli* and carbon steel as a model system. The experimental parameters considered here include test media composition, immersion temperature, immersion time and medium replenishment. It is shown that each of these parameters can exert a significant effect on the outcome of the study, thus supporting the contention that more realistic testing procedures are required to develop a better understanding of MIC processes and ultimately allow predictive management of MIC.

1. Introduction

Microbiologically influenced corrosion (MIC) is a general term used to describe a multitude of changes to corrosion processes that can occur due to the presence of microbes (Little and Lee, 2007; Videla, 1996). The most commonly known outcome of MIC is a rapid increase in corrosion, where rates of attack of up to several millimetres per year are not uncommon. This severe degradation is often unexpected and can have costly and dangerous outcomes (Wade et al., 2011). The study of MIC is challenging as it involves a complex mix of science and engineering, and spans diverse disciplines including materials science, chemistry and microbiology. There are also a wide range of potential microbes and mechanisms involved that makes providing a simple generalised explanation of MIC extremely difficult.

Moreover, the unpredictability of MIC makes it difficult to study in the natural environment. Therefore, laboratory-based tests are one of the key methods used to provide a better understanding of the mechanisms involved in MIC. They can also be used to study the susceptibility of different materials to MIC as well as the effectiveness of mitigation strategies. While efforts have been made in the past to develop standard analytical methods for diagnosing MIC attack in actual field conditions (e.g. NACE, 2004; 2006) at present there are no standards or guidelines for performing laboratory-based MIC tests. Indeed, laboratory-based MIC studies reported in the literature use a wide range of test conditions, which makes it difficult to compare results. The lack of standards in such a multidisciplinary field creates an additional problem in that basic knowledge in one discipline is not necessarily known or fully understood by those with a different academic background. This can inadvertently lead to unintended consequences where the selection of specific test conditions, while not the actual focus of the work, directly affects the outcome and interpretation of the test.

There are a number of factors that can affect laboratory-based MIC tests such as:

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Nutrients and metabolism:

- Different nutrients/test media components can change metabolic by-products and/or processes that can affect corrosion processes (e.g. levels of carbon sources, iron ions and yeast extract present in test media). There have been quite a number of publications on this topic (e.g. Adams and Farrer, 1953; Booth et al., 1966; Chen et al., 2014; Fonseca et al., 1998; Javed et al., 2015; King et al., 1976; Obuekwe et al., 1987; Rodin et al., 2005; Videla et al., 1998; Xu and Gu, 2014).
- When designing a test, a key question should be what is the purpose of the specific test medium being used? For example, is it to try and simulate what is happening in the "real world" (e.g. Spark et al., 2016) or to look at a particular aspect of metabolism related to MIC?
- The test arrangement used (e.g. batch, fed-batch, continuous) is likely to affect microbial metabolism, and thus corrosion rates.

Other nutrient/test media effects:

- Test media can also affect/interfere with measurement techniques used with MIC (e.g. yeast extract interferes with electrochemical measurements (Lee and Little, 2015; Webster and Newman, 1994)).
- Test media can also influence abiotic corrosion processes taking place at the same time as MIC which will influence the overall result (e.g. some components of test media are corrosion inhibitors (Dexter, 1988; Javed et al., 2014; Stott et al., 1988)).

Other test conditions:

- The metabolism of many microbes is affected by the level of oxygen present in the test solution, as are abiotic corrosion processes. The sulphate reducing bacteria (SRB) are typically tested using anaerobic conditions, however many strains can survive in aerobic conditions (Lee et al., 1993, 2004) and some tests have shown that the presence of oxygen produces greater corrosion rates (Hardy and Bown, 1984). In addition, the method used to produce an anaerobic environment can alter the solution chemistry and microbiota over time (Lee et al., 2010).
- The test temperature can affect biotic and abiotic corrosion processes. While individual microbes have an "optimum" growth temperature, this is often quite different from the "real world" conditions and temperatures in which MIC is observed.
- In many field examples of MIC (e.g. piping and heat exchanger systems) the fluid of interest is subject to agitation/flow which can affect processes relevant to MIC. Therefore, the question arises as to whether fluid flow should be added to the test arrangement (Jhobalia et al., 2005).
- There are guidelines available for abiotic corrosion immersion tests that specify the volume of test media to use relative to the size of the test coupon (ASTM, 1999). For MIC tests the volume of test media may also affect metabolic processes of the microbes of interest (e.g. the availability of nutrients throughout the test duration).

Microbe(s) to use in study:

- A wide variety of microbes have been implicated in MIC and a range of different influences on corrosion have been reported. Even different strains of similar microbes (e.g. various species of SRB) have been shown to affect corrosion differently when exposed to the same test conditions (Beech et al., 1994; Bell and Lim, 1981; Booth and Wormwell, 1961; Gaylarde, 1992). Therefore, care needs to be taken in drawing general conclusions about particular groups of microbes and also with the level of identification of microbes when reporting MIC (i.e. the term SRB is used to cover a broad range of metabolically diverse bacteria and archaea (Enning and Garrelfs, 2014)).
- The majority of laboratory tests for MIC use a single microbial species which is unlikely to represent the complex communities and processes involved in the "real world". While single species tests may help to understand some of the fundamental processes of MIC, the use of multiple species (Jack et al., 1992; Steele et al., 1994) may be more representative. Alternatively, microbial consortia may be collected from the field, but with consequential limitations in test replication and the need for adequate identification of microbes involved.
- There are no standards for the number of bacteria to include in a particular test or how they should be prepared beforehand (i.e. what growth phase?).

Test duration:

- The various processes involved in MIC are likely to be time-dependant, so what length of time should tests be performed for (Beech et al., 1994; Lee et al., 2004; Stott et al., 1988)?

Test coupon:

- Various aspects of test coupon microstructure and composition have been found to affect microbial attachment as well as MIC (Javed et al., 2013a, 2016; Mara and Williams, 1972).
- Test coupon surface preparation (e.g. as supplied, polished), orientation in the test (e.g. vertical, horizontal) and even coupon size can also affect microbial attachment and MIC results (Beech et al., 1994; Chen et al., 2014; Lee et al., 2004; Mitik-Dineva et al., 2008; Wade et al., 2009).

From the preceding survey, it is clear that the effects of a number of experimental parameters have been appreciated in a wide range of contexts by various workers. However, to date we are not aware of any unified attempt to demonstrate the severity of the effects arising from these experimental parameters in a consistent experimental setting. Therefore this paper demonstrates, via experimental data, the effects of a variety of different laboratory test conditions on the initial attachment of bacterial cells and corrosion of carbon steel, investigated in the presence of *E. coli* bacteria as a model system. *E. coli* is a commonly used organism in a wide variety of microbiological studies, is relatively easy to work with, well characterised and readily available. In addition, there are a number of reports which have showed that the presence of *E. coli* can influence corrosion rates (Baeza et al., 2013; Javed et al., 2016; Nan et al., 2015). We draw on a combination of published and new results: the experimental conditions discussed here include test media composition (nutrient broth and minimal medium) (Javed et al., 2014), test duration (14 and 28 days), immersion temperature (21 °C and 37 °C) and the timing of test medium replenishment. It is important to reiterate that despite the effects of many of the aforementioned test conditions being known to influence microbial behaviour, for example by microbiologists, they do not appear to be considered in detail in many MIC testing studies found in the literature.

2. Materials and methods

2.1. Preparation of test media and bacterial culture

Nutrient broth (NB) and minimal medium (M9) were used to conduct bacterial attachment and corrosion studies. The compositions of the test media used are shown in Table 1. NB is a nutrient-rich medium that is used for growing a number of different bacterial species (Madigan et al., 2011) whereas M9 is a minimal medium which contains only essential salts and glucose as the sole energy source for bacterial growth. Both of the test media were