

Novel Multiport Flow-Column Corrosion Monitoring System (MFC) Revealed High Corrosion Rates by Corrosive Methanogenic Archaea

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ABSTRACT

Microbiologically influenced corrosion (MIC) is a complex and costly issue that is often attributed to the activities of sulfate-reducing bacteria (SRB) mainly because of their abilities to produce hydrogen sulfide. Other groups of MIC-related microorganisms, including methanogenic archaea (MA), are often underrepresented due to inadequate monitoring techniques. The main MIC mechanism of MA is through the direct uptake of electrons from the metal surfaces since their metabolic product, methane, is unreactive. To accurately study their MIC potential, a versatile multiport flow-column (MFC) was developed. The MFC revealed an extensive corrosion rate profile for the corrosive MA, with a maximum corrosion rate of 0.38 mm/yr and an average corrosion rate of 0.17 mm/yr at neutral pH. In comparison, the corrosive SRB in the MFC reached an average corrosion rate of 0.1 mm/yr. At low pH, the average corrosion rate of MA was 0.17 mm/yr but with a maximum of 1.57 mm/yr. Surface analyses of the corrosion layer showed that iron carbonate is not the sole corrosion product of methanogen-induced MIC (Mi-MIC). Overall, this study highlighted the importance of using environmentally relevant study techniques to accurately depict the corrosion potential of MIC, especially for Mi-MIC.

Key words: Microbiologically influenced corrosion, anaerobic microorganism, environmental simulation, microbial modelling, methanogenic Archaea, sulfate-reducing bacteria

INTRODUCTION

Microbiologically influenced corrosion (MIC) is a complicated and interdisciplinary challenge concerning a wide range of industries. The complexity of MIC is due to the interdependencies between a number of factors: associated environmental conditions, materials, fluidity, stresses and the microbial communities (*in-situ* or introduced) involved¹. Both aerobic and anaerobic microorganisms are involved in MIC¹. However, anaerobic MIC-related microbes are the main culprits for submerged or buried metal infrastructures and are therefore the most problematic, as they require more time and effort for inspection, maintenance and repair. Several key players were identified and investigated, including members of sulfate-reducing bacteria, thiosulfate-reducing bacteria, acid-producing bacteria and methanogenic archaea¹⁻⁵. Sulfide-producing microorganisms (SPM) received the greatest number of interests due to the negative impacts they pose on the industry, i.e. reservoir souring⁶.

The importance of SPM in the industry led to well-defined MIC models and testing methods⁷⁻¹¹. The two main types of corrosion mechanisms were also defined mainly based on the activities of SPM: indirect MIC and direct MIC^{5,12}. Indirect MIC is defined as the degradation of material as a consequence of microbial metabolic activities, i.e. production of sulfide by SPM or acetic acid by acetogens¹³. Direct uptake of electrons from the metal surface through pili, cytochromes or other electroactive proteins i.e. hydrogenases, is commonly referred to as the direct MIC⁵. Though the study of MIC is well established for SPM, it is still perplexed by other contributing factors, i.e. environmental conditions. For example, under elevated salinities, the chloride ions will influence the deposition and structure of FeS on the metal surface^{14,15} and consequently may affect the corrosion process of SPM. This is because FeS is a conductive corrosion product and it is believed that MIC can still occur despite FeS deposition on the iron surface.

The corrosion mechanism for other groups of anaerobic microorganisms are even less well-defined, particularly for methanogenic archaea (MA). Methanogens are strictly anaerobic and produce non-corrosive metabolic products, i.e. methane. The lack of reactive metabolic products indicates the corrosion mechanism of MA is through a direct pathway. The results of studies suggest that a certain group of corrosive methanogens (hydrogenotrophic) exhibit a special "MIC-island", which is a unstable genomic island that encodes for a hydrogenase and the corresponding genes for the secretion of this hydrogenase¹⁶. The secreted hydrogenase leads in contact with a metal surface to the production of hydrogen and support the growth of the corrosive MA¹⁶. Other corrosive MA, such as *Methanobacterium*-affiliated IM1 had higher methane production growing on iron in comparison to H₂ supplemented environments, indicating a strong adaptation of this organisms to metal as electron donor¹⁷. Similarly, a corrosive strain of *Methanosarcina* isolated from the Baltic sea could not use the provided H₂ for methanogenesis, but instead can only use electrons from the iron surface¹⁷. However, MA was so far classified to the "low to moderate" corrosion risk categories because of the low reported corrosion rates (< 0.065 mm/yr)¹⁸. One reason for the low corrosion rate can be explained by the inertness of methane but the other important aspect is the inadequacy with the MIC-testing method. Improper testing methods will lead to the production of unrealistic corrosion products. It is believed that siderite (FeCO₃) is the most common corrosion product associated with MA¹⁹. It is considered to be an insulating compound rather than conductive^{20,21}. But the nucleation and crystallization of siderite is dependent on a number of factors, including fluidity, temperature, pH and salinity²⁰ and this is difficult to simulate with the currently used laboratory methods.

Thus, to accurately depict the picture of methanogen-induced MIC or Mi-MIC, we developed an affordable and fully anaerobic (also for aerobic use) environmental simulation system that can be adapted to a wide range of applications. The system requires no further measures for maintaining the atmosphere (e.g. anaerobic) and can be installed and operated both on the laboratory bench and in incubators (with enough space). We present here the effectiveness of the system on studying Mi-MIC including the interdisciplinary approach through material-science-based investigations. Ultimately, we hope this system can shed new insights on how MIC should be tested in a laboratory setting.

EXPERIMENTAL PROCEDURE

Design and operation of the multiport flow-column (MFC)

Design of the MFC

The multiport flow-column (MFC) was designed to embody six different testing sections (Figure 1), with four sampling ports (Fig. 1: yellow stars above the black rectangle). The sampling ports are sealed with removable rubber stoppers to ensure the system will remain anoxic during operation. Sampling from the side ports are possible using sterile syringes. Through the sample ports, local parameters and changes in the chemical composition of the liquid phase can be determined, allowing conclusions or assumptions to be drawn as e.g. why some sections show increased MIC. Fresh medium is continuously connected to the columns, which the peristaltic pump will actively pump into the MFC. A separate chemostat system can be incorporated (Figure 1) before the MFC. The effluent bottle can be disconnected from the column for headspace measurements.

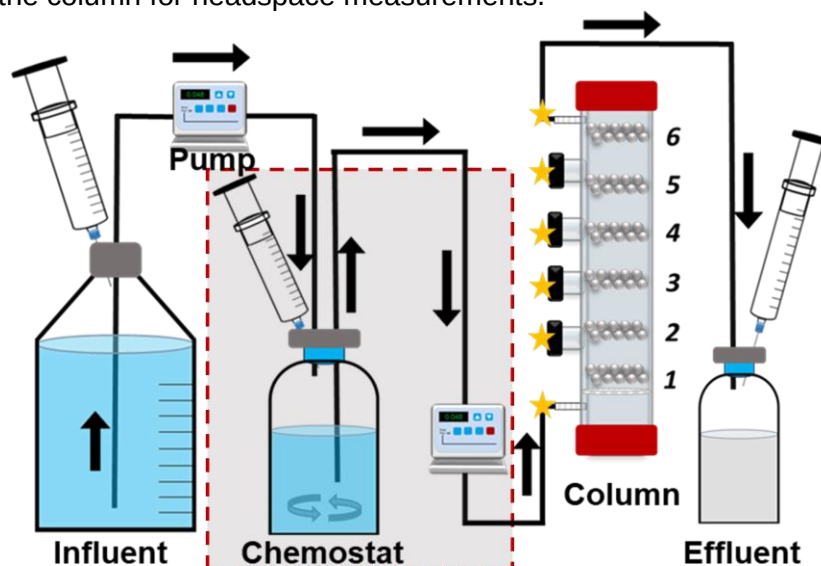


Figure 1 Design of the multiport flow-column (MFC). The grey box indicates an optional incorporation of a chemostat/continuous culture system. Arrows indicate the direction of the flow of the system and the yellow stars the different section. Figure was modified from An et al., 2020¹⁸.

Operating MFC for MIC studies

In this study, two types of microorganisms were tested: *Methanobacterium*-affiliated IM1 and *Desulfovibrio ferrophilus* IS5 (DSM 15579). Pre-cultures of *Methanobacterium*-affiliated IM1 and *D. ferrophilus* IS5 were established in anaerobic artificial medium (ASW)¹⁸ under N₂/CO₂ (80 %/20 %, v/v) headspace. The ASW media used for IS5 was additionally supplemented with 10 mM sulfate. Each culture also contained one piece of pre-treated (acid-sterilized) carbon steel coupon²² (99.5 % Fe⁰, 0.3 % Mn, 0.08 % C, 0.04 % P, 0.8 cm × 0.8 cm × 0.1 cm, Goodfellow, Germany). The pre-cultures and static cultures (for the comparison with the column system) were incubated under static conditions at 30°C for approximately one week.

All components of the MFC (tubing, connectors, column, valves and packing material) were sterilized prior to assembly. The MFC was assembled and packed inside the sterile working bench. Extremely uniform carbon steel beads (20/section; Ø = 0.238 cm, 55.22 ± 0.026 mg each bearing; AISI 1010, Simplybearing, England) were used to measure corrosion rate distribution. The beads were weighed and sterilized prior to packing. To separate each section, sterile glass beads were used. Each biological treatment had 3 replicates and abiotic controls were duplicated¹⁸. Post assembly, each MFC was flushed with pure nitrogen gas for 3 minutes and sealed prior to medium-flooding. Sterile anaerobic

medium was flushed into the MFC, generating the 'wet weight'. Then, pre-cultures were directly inoculated into the sterile media-flooded MFC in section 1 (Figure 1). The amount of inoculum can be chosen freely, depending on the research question. In our case the initial inoculum volume was about 50% of the pore volume (half of the column volume, calculated by subtracting the dry weight from the wet weight). The MFC was set up in a standard incubator and could be operated in an aerobic atmosphere, as the system itself is airtight. The MFCs were incubated for 3.5 days (30 °C) without flow to allow the establishment of an initial biofilm. Post incubation, sterile anaerobic media were continuously injected into the columns at a flow rate of 0.048 mL/min¹⁸. Constituents of the effluent bottles were analyzed daily for methane and sulfide concentrations using the gas chromatography and the diamine method, respectively¹⁸. After 14 days of operation, the MFCs were section-wise disassembled, the beads were cleaned and weighed individually to calculate the final corrosion rates.

To test the effect of low pH on the growth and corrosion potential of IM1, an additional set of pre-culture, MFC and media at pH 6 were established. Another set of MFC established at neutral pH was used as a comparison.

Corrosion rate calculation

The corrosion rates of the individual carbon steel ball bearings were calculated using the weight-loss method with the following formula:

$$\text{Equation 1 CR} = 87600 \times \Delta W / (D \times A \times T)$$

Where the density (D; 7.85 g/cm³), surface area (A; 0.178 cm²/bead) and time (T; hours) were divided from the changes in weight (ΔW ; g). The final unit of the corrosion rate is in mm/yr, which was converted from cm/hr by multiplying it with the factor 87,600.

Surface analyses and visualization

Different types of surface characterization techniques were used to visualize the corrosion products of IM1. To analyze the possible corrosion products of IM1, the technique FIB-SEM was used. The FIB-SEM can generate a precise cross-section under vacuum using the focused ion-beam (FIB), which in combination with EDS can create a mapping of the different types of elements that were present. The FIB-SEM (FEI 200xP, Thermo Fisher Scientific, Germany) had an acceleration voltage of 30 kV (Ga⁺ ions) and the cross sections were sliced and imaged by SEM at a 52° angle to the surface (5 kV, secondary electron detector). Prior to FIB, the metal coupons were coated with gold (15 nm) to increase surface conductivity followed by a deposition of platinum (~5 nm) on top of the cross section to minimize surface damages from the ion beam.

In addition to FIB-SEM, carbon steel ball bearings from the MFC were imaged post-experimental using the SEM (12 kV, Zeiss EVO MA10, Thermo Scientific, Germany). With the SEM changes and damages on the surfaces of the beads were visualized. In addition, metal coupons extracted from the columns, were fixated using 2.5 % glutaraldehyde followed by ethanol dehydration¹⁸. The fixated coupons were used to identify and visualize the distribution of the microbial cells.

RESULTS

Corrosion rate distribution of *Methanobacterium*-affiliated IM1 at neutral pH

The corrosion rate distribution of the MFC inoculated with *Methanobacterium*-affiliated IM1 was not uniform between the different sections (Figure 2). Section 1, the inoculation region, had the highest average corrosion rates (0.17 ± 0.08 mm/yr)¹⁸, including beads that were extremely corroded (max: 0.38 mm/yr). Clusters of IM1 cells were also located on the carbon steel coupon extracted from the same site (Figure 3). The cells were located below the corrosion products of the coupon (Figure 3). The abiotic control of the same section had an average corrosion rate of 0.076 ± 0.027 mm/yr (Figure 2).