Understanding and Addressing Microbiologically Influenced Corrosion (MIC)

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OVERVIEW

Microbial life is everywhere. Microorganisms have been found inhabiting iced-covered lakes in Antarctica at -13°C and hydrothermal vents at the bottom of the ocean at 120°C [1]. Microorganisms have inhabited our planet for billions of years before plants and animals appeared. It was through their activities that higher forms of life could appear and thrive [2]. However, microorganisms can also be harmful and their activities can result, under certain conditions, in detrimental effects such as disease and damage to infrastructure. Industrial systems typically create new microbial habitats that can stimulate undesired microbial activities. A notable example of this is microbiologically influenced corrosion (MIC) which refers to corrosion of metallic equipment and structures caused or accelerated by microorganisms. These microorganisms are mainly bacteria and archaea, but microalgae and fungi can also be important contributors in certain environments [3, 4]. In Australia, MIC represents a common threat to the oil & gas, defence and marine industries which are major components of the national economy. Deterioration and corrosion due to microorganisms drives a worldwide market for microbial control that is worth billions of dollars annually.

MIC unquestionably has always occurred in industry; however, it remains poorly understood and recognized. In the past, reluctance among corrosion professionals to acknowledge the possibility for MIC and its potential to cause equipment failure led to it being considered of less significance, therefore research to advance its understanding was less well supported. However, this has drastically changed in recent years. It is now widely recognized that MIC contributes to at least 20% of the total cost of corrosion worldwide, 40% of internal corrosion and 20% to 30% of external corrosion on underground pipelines [5]. The formation of biofilms on metals and alloys can result in corrosion rates in excess of 10 mm per year (mm/y) leading to equipment failure before their expected lifetime and serious environmental damage [6, 7]. The 2006 trans-Alaskan pipeline failure, which was attributed to microbial corrosion where 200,000 gallons of crude oil leaked resulted in significant environmental pollution, lost production of two million barrels and a worldwide impact on oil price [7] is a prime example of such issues. Incidents like these have resulted in increased industry awareness and to increasing research and developments in the field over the past 10 years. The evolution of MIC knowledge throughout these years has been reviewed recently by Hashemi et al. [5].

We know MIC involves the interaction of electrochemical, environmental, operational, and biological factors that often result in substantial increases in corrosion rates to metals in specific environments. However, the complex and dynamic nature of these interactions have made detailed mechanisms elusive, i.e. the precise mechanism(s) of MIC is still being debated among corrosion specialists. The most challenging aspects of MIC is its lack of predictability and the fact that it does not produce a distinct type of corrosion or a unique morphology of corrosion damage, or any sort of mineralogical fingerprints. Although some investigators claim that certain metallurgical features are characteristic of a MIC mechanism, e.g. deep pits that are hemispherical, and cup-like in appearance with striation or

Figure 1 Microbiologically influenced corrosion of crude line to cargo storage in a Floating, Production, Storage and Offloading vessel (FPSO). Reproduced with permission from Machuca and Polomka (2018), Microbiology Australia 39 (3), 165-169.

contour lines, the evidence remains largely anecdotal. In addition, MIC generally occurs in conjunction with other corrosion mechanisms. These characteristics present challenges to identifying MIC as the mechanism of failure. In most cases, MIC morphologies are localized types of corrosion that manifest in pitting, crevice corrosion and under deposit corrosion (UDC).

MIC is driven by environmental conditions. Mechanisms that govern MIC in freshwater environments seem to be quite different to those observed in marine environments and to those in oil and gas production facilities [9-11]. Parameters such as temperature, flow conditions, pH, light and nutrients will favour the growth of particular species and restrain the growth and metabolism of others on a particular substratum. Particularly, the type of electron acceptors available in a system, e.g. sulphate, CO_2 and oxygen, are known to play a critical role in shaping the microbial community developed on the metal surface, its biofilm structure and the resulting MIC [12, 13]. In addition, the presence of chemical inhibitors and additives used by the industry to prevent scaling, fouling, microbial activity and corrosion will also create a selective environment for and against certain groups of microorganisms. Likewise, in low velocity systems and stagnant flow conditions, deposits and debris can settle providing an ideal niche for bacterial accumulation, growth and activity [14]. We have recently reported MIC under oilfield deposits from corroded pipelines which resulted in pitting rates greater than those obtained in the absence of bacteria [15]. Results indicated that bacterial extracellular polymeric substances, or EPS, on the metal surface created a diffusion barrier that entrapped chemical species within the deposit, which facilitated the formation of concentration cells on the metal surface. MIC under deposits have been reported to result in corrosion rates greater than 5 mm/y in Floating production storage and floating vessels (FPSO's) (Personal communication)[16]. A case study in West Africa, related the predominance of high-MIC risk bacteria *Thermovirga* sp. in solids deposited in separation vessels with high corrosion rates, which were 30 times higher than the expected range [17]. Wang et al., suggested a synergistic effect between MIC and under-deposit corrosion resulting in more severe localized corrosion [18].

The microbial ecology of a system is a critical component to consider when assessing the potential mechanisms for MIC. Microbial communities with distinct capabilities commonly inhabit oil production facilities [19]. These microorganisms are typically strictly anaerobic (oxygen is not present in oil production systems), thermophilic and exhibit slow growth and enhanced resistance to stress. MIC mechanisms in these conditions are usually related to CO_2 and H_2S corrosion and electron acceptors such as sulphate, CO_2 and nitrate are critical in supporting such mechanisms. In marine environments, conversely, oxygen is a key player, which not only drives the corrosion reactions but also the metabolic activities of colonizing species and thus, the resulting biofilm composition and structure [20, 21]. Marine bacteria typically identified in corroded equipment correspond to mesophilic species. Marine biofilms on steel materials can also contain other seawater organisms such as algae (e.g. diatoms) and barnacles [21]. However, the structure and composition of marine biofilms (and surface concretions) on steel materials depend on water depth; deep waters have different nutrient, microbial and dissolved oxygen content and exhibit lower temperature and higher pressure when compared to shallow waters [22]. There is no light in the deep ocean which restricts the potential activity of photosynthetic organisms that can contribute to oxygen and organic carbon generation within biofilms if light is present [23].

MIC is also affected by material (substratum) composition. Carbon steel is the most common material of construction due to its commercial attractiveness. However, carbon steel is susceptible to MIC. In offshore oil and gas production systems, for instance, carbon steel is prone to MIC at even low water cuts (ratio of water vs. total fluids produced) under the right combination of nutrient availability, microbial population density and temperature. Corrosion resistant alloys (CRA) as opposed to carbon steel are more resistant to MIC despite having similar susceptibility to biofilm formation [21]. This is particularly the case for highly-resistant alloys that contain higher concentrations of alloying elements such as chromium, nickel, molybdenum and nitrogen [24]. However, in lower grade CRAs such as 304 and 316 and even 2205 duplex stainless steel both the breakdown of passive films and the propagation of localized corrosion can be greatly affected by microbial activity [25]. The phenomenon of ennoblement of CRAs, a shift of the corrosion potential (E_{corr}) in the noble or anodic direction, is associated with microbial activity [21, 26]. The significance of this phenomenon lies in its influence on the susceptibility to corrosion of anode materials in galvanic couples and the initiation and propagation of localized corrosion [27, 28]. There is a particular risk for CRAs with MIC when exposed to environments were oxygen is present or when oxygen can ingress as a result of contamination, such as during poor water flooding of subsea pipelines [29]. MIC of CRAs under oxygen-free conditions appears to be of less concern. However, this is a topic that remains under debate and requires more systematic investigations.

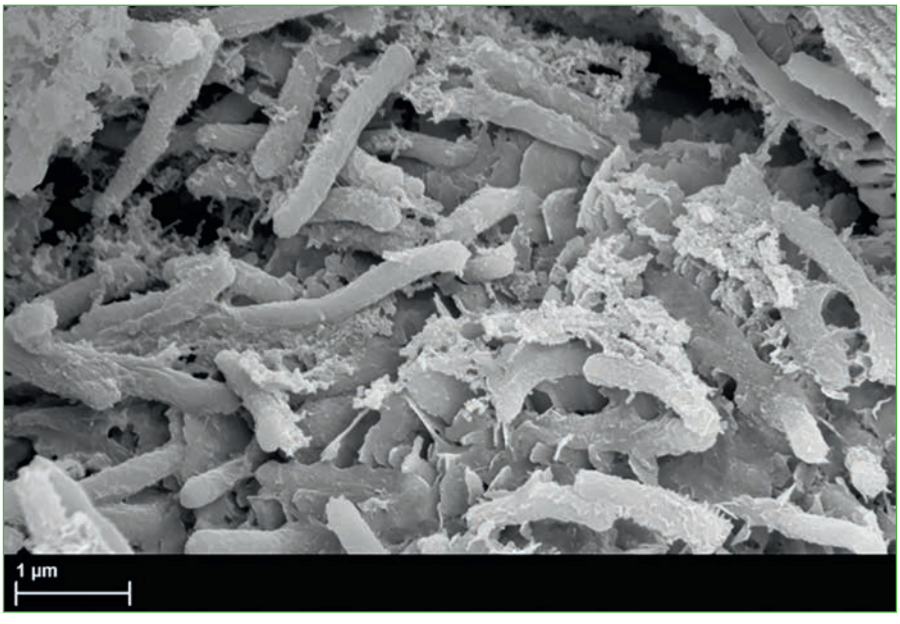

Figure 2 Scanning electron micrograph of microorganisms on corroded carbon steel (AISI 1030).

Several MIC mechanisms have been described including direct and indirect mechanisms. Comprehensive review papers on MIC mechanisms are available elsewhere [30, 31]. For decades, specific microbial groups such as sulphate-reducing bacteria (SRB) and acid-producing bacteria (APB) have been identified as the main culprits of MIC and their metabolites, sulphide and organic acids, respectively, have been directly associated with corrosion. Such MIC mechanisms, in which corrosion is accelerated by production of corrosive microbial metabolites, are known as chemical MIC, or CMIC, and have been reviewed extensively in the literature [32, 33]. However, the list of microorganisms that can influence corrosion is constantly growing. Particularly with the advent of new sequencing technologies that allow identification of non-culturable species present in corroded equipment that have not previously been associated with corrosion.

In recent years, novel MIC mechanisms involving direct electron transfer (DET) between bacteria and steel have been revealed and are now the focus of a great deal of scientific work [34]. This mechanism is known as electrical MIC, or EMIC. Typically, corrosion rates obtained in laboratory experiments involving CMIC mechanisms are much lower than those reported in field. It appears that one of the reasons for this persistent outcome is that most laboratory experiments involving SRBs generate H_2S and iron sulphide films that are protective and uniformly distributed on the surface of a test coupon [32]. This scenario cannot be compared with field conditions were in metres of pipe only a few pits are present (small anodes) with large areas outside the pits being covered by sulphide films (large cathode), which results in greater corrosion rates. However, studies involving EMIC mechanisms have shown that DET can result in corrosion rates as severe as those reported in field (>5 mm/y), which suggests that this novel mechanism can be significant in industrial systems [34]. Hitherto, only a few species have been demonstrated to have the ability to induce EMIC. However, only a few studies have looked into natural biofilm consortia and the potential of different microbial populations to carry out DET has not been fully investigated. Methanogens represent a group of microbes of increasing interest in MIC despite not being associated with corrosion via production of a corrosive metabolite (methane is not known as a corrosive gas) [35-37]. Methanogens have not been widely detected in corroded facilities since they are very difficult to grow under laboratory conditions. However, DNA based analysis has shown that methanogens are widespread in oilfield environments and are typically detected in biofilms associated with corrosion. MIC research involving methanogens has demonstrated that these microorganisms can accelerate corrosion via EMIC mechanisms [38].

A concerning aspect in the understanding of the MIC risk is the typical assignment of specific metabolic functions to individual microbial populations. One example is the assumption that the sole presence of SRB species is enough evidence for sulphide-associated corrosion. Microorganisms are significantly versatile, with the potential to use different metabolic pathways than those commonly attributed to them, depending on the environmental conditions and the availability of nutrients [39]. For instance, the detection of fermenting microbes, which are widely distributed in oil production facilities, is typically associated with the risk of acid corrosion. Some of these microorganisms are known to be able to reduce sulphur compounds such as elemental sulphur and thiosulphate as electron-sink reactions that prevent accumulation of inhibitory concentrations of the fermentation product molecular hydrogen [40, 41]. This will result in sulphide production; therefore, some fermenting microbes can also become sulphide-producing bacteria. Likewise, some microorganisms can switch from one electron acceptor to another if the first one becomes depleted and another one becomes available [42]. Therefore, assigning specific microbial populations to a specific corrosion mechanism or associated risk can result in erroneous interpretations.

In addition, transposing a field environment to a laboratory environment to investigate MIC is one of the biggest challenges in the field. The effect of feeding nutrients in the laboratory at concentrations and compositions different to the field fluids on the growth of biofilms, and the activity of bacteria in the biofilm as well as the influence that the nutrients may have on the corrosion and MIC process, remains a big limitation [43]. Investigators have shown that test media can have an impact on bacterial attachment and the outcome of MIC tests [44, 45]. We studied SRB biofilm growth on carbon steel and its inhibition by biocides in three different media, i.e. produced water inoculated with active SRB, raw produced water containing field bacteria and Postage B medium inoculate with active SRB [46]. Results indicated that biocide efficiency was affected by growth medium. Overall biofilms formed in Postgate B medium exhibited more resistance to biocides compared to biofilms formed using produced water from field. This could be due to the fact that Postgate medium is very nutritious and results in the formation of more robust biofilms. Also, Postgate B medium contains yeast extract (YE) which is a very complex nutrient source that contains organics, proteins and vitamins. YE can form complexes on the surface and potentially react with biocide compounds. The potential impact of YE on the biotic reactions in MIC experiments has been discussed previously [47]. Ideally, MIC experiments should be designed to use field samples directly, or synthetic medium of very similar composition, and to replicate the field environment (temperature, material, gas atmosphere, etc) to grow and study biofilm activity and its inhibition on metals.

BIOFILM: THE COMPLEX CULPRIT BEHIND MIC

Regardless of the particular reactions and processes governing MIC in different environments, there is agreement among researchers that biofilm formation is the critical step in MIC [48]. Thus, MIC is not only the result of complex corrosion reactions but also, the result of a series of flawless events involved in the construction of a biofilm, the most intricate, highly-organised and successful mode of life for microorganisms. Biofilms consist of communities of microorganisms attached to the metal surface and embedded in self-produced EPS, proteins, lipids and extracellular DNA [49]. However, far from this simple explanation, biofilms represent a set of properties, functions and structures that are highly dynamic and imperative for the long-term survival of biofilm cells. Within biofilms, a variety of interactions including chemical and electrical signals, social cooperation, differentiation and highly controlled expression of genes and proteins, occur as a response to exposure conditions [50]. Even though biofilms are comprised of individual bacterial cells, it is recognized that microbes within biofilms exhibit different properties than planktonic cells and that their response to environmental cues is the result of community interactions that cannot be predicted by only studying free-living bacteria or single-species biofilms [50]. In addition, biofilms are multi-layered so stratified cell-cell and cell-medium interactions on the metal surface are also expected.

Biofilm-surface interactions are also spatially heterogeneous and can result in localized gradients and microenvironments across the surface. This implies that more than one microbial mechanism has to be involved in MIC and that different mechanisms can occur simultaneously on metal surfaces. Synergistic and antagonistic (competing) relationships can emanate among biofilm species, which can in turn affect the structure and function of biofilms. Therefore, it is not a steady state "corrosion product" formed on the surface but a dynamic system that responds to its environment (nutrient availability, hydrodynamic shear, temperature, etc.). An interesting feature of these biofilms is that metallic ions can become adsorbed in biofilms preventing microbial exposure to toxic compounds [48]. This means that localized precipitation of metals can take place within biofilms and can potentially affect the surface electrochemistry, e.g. creating galvanic cells on the surface. This can represent one of the most important yet understudied mechanisms of MIC.

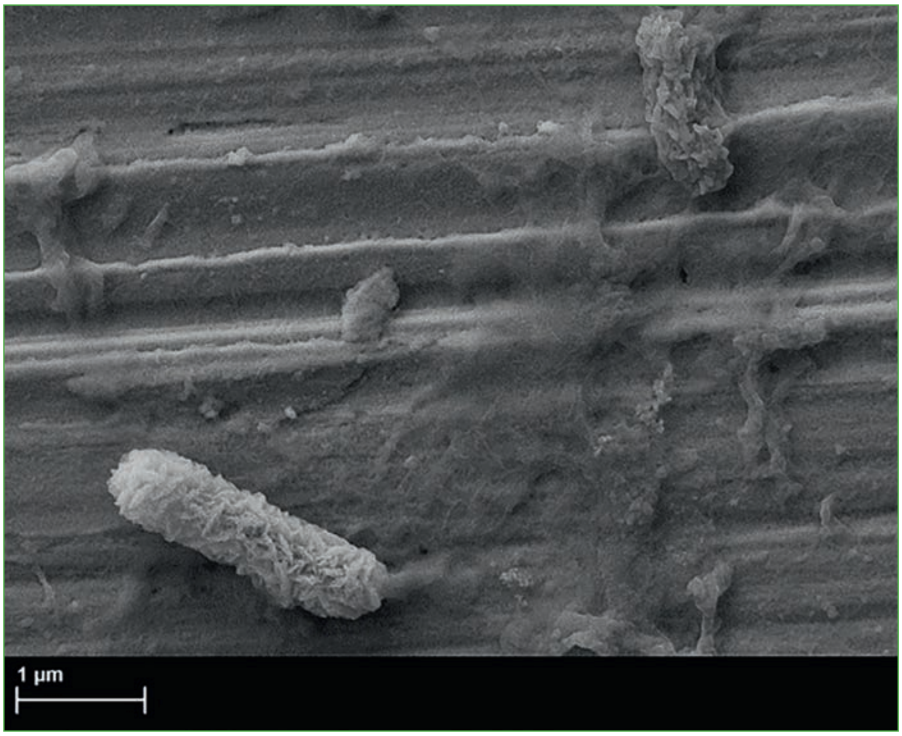

Figure 3 Scanning electron micrograph of a microbial cell secreting EPS on the surface of stainless steel at the early stages of biofilm formation.

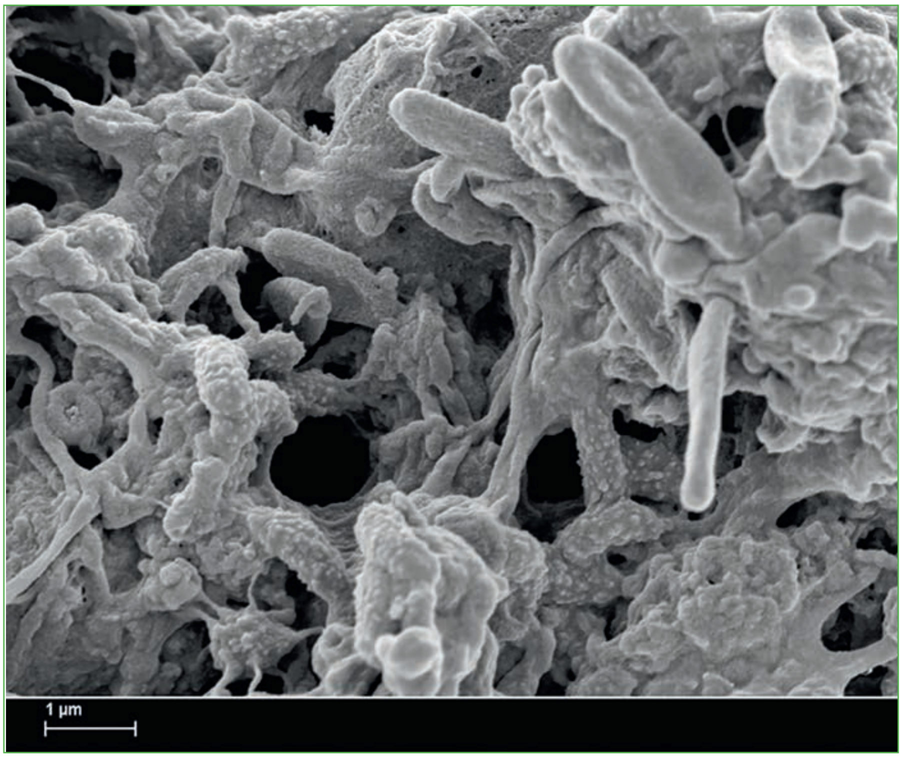

Figure 4 Scanning electron micrograph of complex mature biofilms on stainless steel.

Understanding the effect of nutrients on MIC represents another challenge. This is because even though nutrients are known to be essential to microbial reproduction and activity, microbial cross-feeding within biofilms can be used to overcome nutrient scarcity in a particular system [51]. For instance, microbes can exchange metabolic by-products in biofilms and interspecies electron transfer can provide pathways for energy generation; even dead cells can provide nutrients to other cells. In addition, microorganisms can enter into dormancy and viable but no-cultivable states which results in survival in times of starvation [52]. Likewise, binding sites within biofilms, including both anionic and cationic exchangers, can facilitate the entrapment and storage of substances for future consumption by cells in the biofilm. In terms of hydration, it has been demonstrated that the development of biofilms prevents dehydration (retaining water and preventing evaporation) which has been proven critical to withstand desiccation periods [50].

Undoubtedly, the most detrimental aspect about biofilms and perhaps one of the most difficult challenges about MIC control is the fact that biofilms provide a physical barrier that protects microbes against anti-microbial components. It is known that the efficiency of a chemical biocide is significantly reduced in the presence of biofilms [53]. The more mature a biofilm is, the more difficult to inactivate and inhibit. Different microbial mechanisms have been described that lead to isolation of toxic antimicrobial compounds within biofilms to prevent these toxic compounds from reaching and harming microbial cells [54]. The problem lays with this process resulting in sub lethal concentration of chemicals reaching biofilms cells, which can result in enhanced resistance of the microorganisms to chemical treatments.

A NEW ERA OF MIC DIAGNOSIS AND ASSESSMENT

It has always been difficult to establish a direct association between biological activity and corrosion failure as MIC does not produce a unique type of corrosion. For this reason, MIC is often misdiagnosed. MIC investigations are complex and involve interdisciplinary science that includes microbiology, chemistry, metallurgy, and corrosion. No single analysis can be used to diagnose MIC or to measure the risk of MIC in a particular system. Typical analyses to investigate MIC include microscopic examination and chemical and microbiological characterization of corrosion products and fluids [55]. Importantly, microbiological data should be gathered from more than one method, and interpretation of results requires the integration of this information within the context of field conditions. In the case of failure analysis, it is important to demonstrate a spatial association of the biological components with the observed corrosion, i.e. a biofilm or bacteria should be present on the metal surface at the point where corrosion took place. An association between the type of corrosion products formed and the predominant active bacteria present in the corroded area should also exist.

An accurate diagnosis of MIC involves strict sampling, storage and preservation regimes, comprehensive laboratory analysis and a good understanding of the operation process, material performance and corrosion mechanisms. For decades MIC assessment has relied on the use of standard culture medium and most probable number (MPN), or serial dilution technique, for detection and enumeration of corrosion-related microbes in a particular system [56]. However, standard culture techniques can only detect a minor fraction (0.1 to 10%) of the microorganisms in nature hence this method underestimates microbial diversity and provides an inaccurate assessment of the microbial community in a system if used alone [57]. It is also known that the mere isolation or identification of MIC related microorganisms (typically categorized based on their capability to produce corrosive metabolites) from a particular environment is not sufficient to relate these microbes with a corrosion problem. There are reports of systems where MIC remains insignificant despite detection of corrosion-related microbes in the system, i.e. the paradox of there being possible microbial contamination but no MIC activity remains (Personal communication) [16]. Likewise, microorganisms other than the ones typically associated with corrosion have also the potential to cause MIC. An additional limitation of cultivation techniques is that stress factors such as the presence of chemical treatments can lead to slow growth rates and a viable-but-not-culturable (VBNC) state or other forms of dormancy in microorganisms which will prevent them from being detected via MPN method despite being present in the system [58].

Molecular microbiological methods have received increasing recognition as essential identification tools for MIC assessment to overcome the limitations of cultivation techniques [59, 60]. A review of molecular microbiology techniques applied to MIC assessment is provided elsewhere [61]. In particular, next generation sequencing (NGS) allows accessing genetic blueprints that provides insight into microbial communities and their potential activities including identification of microbes that have not been cultured and studied before [62]. These methods are rapid and have become easily accessible and cost-efficient providing microbial diversity information from complex environmental systems [63, 64]. We can now reveal which organisms are present, what they are doing and how they are doing it.

Nevertheless, novel techniques come with new limitations and challenges. Environmental samples may contain in excess of 10,000 microbial species, giving genetic techniques the potential to produce large amounts of sequence data, which is complex to process and to discern which are the key organisms that are giving rise to the series of corrosion issues. These massive data sets can only be processed using advanced bioinformatics that require skilled and trained analysists and vast computational power. With novel, uncultured and numerous species being frequently detected in field samples, the potential complexity of the problem increases. These new species can potentially reveal new MIC mechanisms, and complex antagonistic or synergistic relationships among populations in a sample which are difficult to interpret in the context of corrosion risk. Reporting hundreds of species in a sample creates uncertainty and meaningless data for corrosion engineers. Even for experienced microbiologists, this type of data represents a challenge for interpretation since it is difficult to associate taxonomic groups to specific corrosion outcomes. We intend to overcome this limitation by conducting isolation of microorganisms from corroded equipment and field samples to sequence their genomes and further characterize their metabolic and MIC capabilities. There is a clear and present need for coordination and integration of such data into a national MIC database.

Other challenges involved in NGS analysis include bias due to the many processing steps in the generations of NGS data (including DNA extraction) and the susceptibility of samples to microbial composition changes during sampling, preservation and shipping prior to analysis in the laboratory [65]. Generating reliable and accurate data via NGS represents a top priority in the current environment that urges close communication and collaboration between sampling personnel, analysts and project leaders.

Whereas DNA provides information of all the microbial populations present in a sample, including those dead and inactive, gene expression and functional analysis based on RNA examinations provide a more accurate assessment of the active microbial component in a system. Previously, NGS of microbial 16S rRNA has been used to assess the diversity and composition of the active component of microbial communities [66, 67]. Novel metatranscriptomics analysis in combination with metagenomics is a revolutionary approach that will provide MIC scientists with better ways to understand complex MIC mechanisms and the microbial artillery involved in such process. This approach allows the identification of the expressed genes in microbial communities and the construction of metabolic profiles in a particular system including community metabolic shifts in response to environmental cues [68]. For instance, corrosion studies can be designed to assess community gene expression fluctuations associated with corrosion events such as pitting initiation, which can potentially lead to elucidation of novel genes and mechanisms involved in MIC. In this fashion, we will be able to elucidate how biofilms on metals develop, adapt and respond. The application of this novel, sophisticated molecular techniques in combination with localized electrochemical and surface methods will become the basis of leading-edge MIC multidisciplinary research, which will ultimately result in superior technologies for managing MIC. However, there is a need to develop improved protocols to process DNA and RNA from difficult samples such as iron concretions, field deposits, corrosion products and corrosion coupons with limited biomass and with excessive metallic ions and polymerase chain reaction (PCR) inhibitors that interfere with nucleic acid extraction and downstream analysis [69]. There is a big limitation regarding RNA analysis because of the greater susceptibility of RNA molecules to degrade and change. In particular, messenger RNA (mRNA), the type of RNA used to determine which genes are expressed in a cell at different stages of development and under different environmental conditions, undergoes an exponential decay with an average of 1.3 min at 37 °C [70]. There is also a requirement for high quality and quantity of RNA in a sample to be able to apply RNA methods to identify functional and gene expression profiles of the entire microbial community. If RNA analysis is applied to MIC diagnosis or monitoring purposes, strict protocols need to be followed by sampling personnel and the analysis needs to be performed by skilled analysts with advanced knowledge of molecular biology and bioinformatics.

Importantly, microbiological data should always be correlated to key performance indicators for a given system. That means, MIC risk assessment should always integrate results from both corrosion and microbiological analysis. There could be occasions where high-risk microbial species are detected in a system; however the system does not experience significant corrosion [71]. It is likely that such species remain inactive if control strategies and field operations are not conducive of microbial activity. In this case, MIC is not considered an immediate concern. However, care must be taken to ensure field operations remain under control and the microbiology of the system is not altered. This will allow cost savings on unnecessary excessive dosages of chemical treatments. However, this requires adequate industry and academic engagement to make sure the system is properly monitored. We are currently developing a MIC prediction tool in collaboration with industry that will integrate corrosion and microbiological data, considering not only high-risk species but also incorporating RNA-based microbial functional activity information for the first time. Data for the development of such improved prediction tool will be based on field and experimental observations from studying the relationship between the functional activities of microbial communities and their association with corrosion.

MIC MITIGATION: FROM TOXIC BIOCIDES TO ECO-FRIENDLY APPROACHES

There are available treatments to restrict the activity of microbes and reduce the risk of MIC. The selection of a specific anti-microbial treatment will depend on the particulars of the system and what is permitted to use in the field environment situation. Typical treatments to prevent MIC include chemical biocides, chlorination, UV-irradiation, filtration, mechanical cleaning (e.g. pigging and flushing), velocity control, cathodic protection, protective coatings with anti-bacterial and corrosion inhibitor compounds and combinations of these treatments [72]. In order to set up a good microbial control program, the chemistry, microbiology and dynamics of the system need to be considered. Any selected control treatment should not affect the performance of the material under routine operations, i.e. it should not be corrosive and should not interfere with production. In the case of potable water systems treatments that present toxicity concerns and health hazards cannot be applied. Monitoring of the treatment efficiency using corrosion coupons, probes and sensors, as well as inspection and review programs that include recurrent chemical and microbiological analysis over the life of the asset are crucial to prevent MIC-induced failures [73]. It is also important to identify specific locations in the system where risks exist and where particular remediation strategies should be implemented. Establishing a multidisciplinary team involving industry (facilities operation) and academics with the right expertise in oilfield microbiology, MIC, chemistry, corrosion and materials is the ultimate approach for MIC management.

The main challenges in protecting equipment from microbial corrosion lies in how fast bacteria in fluids can form biofilms on the metal surface since biofilms afford protection from external harsh conditions and create a more sustainable environment for survival and proliferation. The great difficulty with eradicating microbial corrosion caused by biofilm-forming bacteria has received increasing attention over the last decades. It is recognized that once established on a metal surface biofilm cells are capable of resisting common antimicrobial regimes effective against planktonic bacteria [74].

Recently, we have studied the effect of a chemical biocide, one of the most widely used treatments to control microbial activity in industrial systems, against oilfield biofilms developed on steel under laboratory conditions (unpublished data). The biocide, evaluated at the manufacturer's recommended concentration, exhibited 99.9% killing efficacy against planktonic (free-living) cells but was only able to inactivate half of the population when the same cells were present in the form of biofilms (attached to the surface). The additional presence of deposits (acid-cleaned sand) on the surface, resulted in an even more resilient biofilm which was negligibly affected by the same biocide treatment, i.e. only 1-log reduction in the total number of biofilm cells was observed. Deposits and biofilms (embedded in EPS) resulted in a very robust bio-deposit that acted as a strong physicochemical barrier that prevented diffusion of chemical compounds through the biofilms. It has been demonstrated that bacteria in biofilms can resist antimicrobial compounds at concentrations up to 1000 times higher than those active on the same bacteria in the planktonic state. This supports the practise of implementing regular physical cleaning methods such as pigging, if economical and practical, to remove the biofilms from the steel surface. However, this is not always possible. In this area, we are currently studying the role of bacteria in under-deposit corrosion and the performance of corrosion inhibitors in the presence of microbial cells and deposits. It is widely recognized that corrosion inhibitors can adsorb onto deposits reducing inhibitor availability, thus leading to inadequate inhibition beneath the deposits [75]. In the presence of bacteria and deposits, complex organic deposits can be formed on metal surfaces and interact with an inibitor in a way that their inhibition perfomance is significanlty reduced.

In recent years, environmental concerns have led to legislation that encourages the replacement of toxic chemical compounds with environmentally-friendly antimicrobial treatments that are less harmful to the environment. Consequently, there is a growing interest in the application of green approaches to control biofouling and MIC. Most of these methods are being investigated and implemented within the water industry, e.g. wastewater treatment and potable water applications. However, these approaches may also be suitable for microbial control within the oil and gas industry. Microorganisms suspended in water for instance, may be controlled by the application of ultraviolet light (UV), which under certain conditions can be very effective. We studied the effect of filtration along with (UV-C) irradiation to mitigate MIC [21]. This approach showed promise in the mitigation of biofilm growth-associated effects on corrosion resistant alloys. However, the suitability of applying this method, particularly for long-term preservation, needs further investigation.

Further promising and exciting non-toxic approaches to mitigate biofilm formation include the application of antimicrobial surface coatings and recalcitrant surfaces [76, 77], membrane technologies to decrease the nutrient content in the water [78], a range of enzymatic and biochemical technologies [79, 80] and ultrasonic treatment [81]. Other natural compounds including herbal extracts, natural antimicrobial peptides and molecules of microbial origin have been studied. In particular, the potential of developing new molecules based on plant derivatives as inexpensive, eco-friendly alternatives to toxic chemicals for the control of MIC should be assessed. We have recently reported garlic extract (GAE) as an effective corrosion inhibitor which also exhibits antimicrobial activity [82]. GAE effectively reduced viable cells in multispecies biofilms developed on steels and was demonstrated to mitigate MIC on both carbon steel and stainless steels in conditions closely simulating hypersaline oilfield environments. Corrosion inhibition and anti-microbial properties of the GAE were attributed to organo-sulphur compounds present in GAE. Bacterial cooperative traits such as cell-cell signalling used to coordinate biofilm dispersal are also being explored for the control of biofilm-related problems [83]. However, the effectiveness and applicability of these approaches to industrial systems remains uncertain, mainly because of the unrealistic conditions used for their laboratory validation. In addition, many of these approaches fulfil their purpose as antifouling methods but remain inacceptable economically and environmentally. The use of these technologies for the preservation of oil and gas equipment has yet to be investigated.

Regardless of the technology developed, it is important to assess their efficiency not only on planktonic bacteria but also on sessile bacteria to obtain information on the minimum biofilm eradiation concentration (M.B.E.C) [84, 85]. It is also important to understand that even though some of these methods can inactivate biofilms monitoring of such efficiency over time is very important to ensure MIC control is achieved in the long term. As discussed in previous sessions, microorganisms can develop diverse survival strategies when exposed to enduring stress conditions, which can result in mitigation approaches being only able to inactivate microbial cells but inefficient at killing them. The risk of this lies in the possibility that biofilm cells recover their activity very quickly in the event of treatment failure. In this context, it is also important to bear in mind that in difficult conditions, some microorganisms enter a dormant state in which they induce their metabolic functions to slow and eventually shut down completely. Then when the surrounding environmental conditions improve, they resume normal metabolism. In this fashion, we have recently studied thermophilic biofilms on carbon steel and the effect of exposing them to cold temperatures (10°C). This temperature is considered an aggressive environment for thermophiles, potentially resulting in lysis of biofilm cells. Results showed that even though thermophilic biofilms remain inactive (or exhibited extremely low metabolic activity) during exposure to low temperature, and even some biofilms cells died upon exposure to cold conditions, a significant portion of the biofilm cells remained live and were able to re-establish activity upon exposure to the original thermophilic conditions. Similar results were obtained by Weaver et al. [86] after evaluating the effect of desiccation on microbial activity. Microorganisms were exposed to long desiccation periods during which microbial activity was not detected. However, microorganisms were able to recover activity once they were in contact with water even after four months of desiccation highlighting the capabilities of microorganisms to withstand exposure to harsh environments.

CONCLUDING REMARKS

The oil & gas, marine and defence industries are a major component of the Australian economy. It is therefore essential to respond to the scientific challenge of MIC by developing and applying new knowledge to every aspect of our exploration and production processes. The application of advanced experimental and diagnostic methods in combination with high performance bioinformatics tools to MIC research would substantially improve our understanding of MIC processes. Notably, future MIC studies should focus on mixed-species, or community level biofilms, since most natural biofilms exist as very diverse communities. This is one of the most problematic issues involved in MIC research which has been traditionally conducted using planktonic microbes or single-species biofilms that do not represent the more complex naturally developed multi-species biofilms. Therefore, studies on the effect of antimicrobial substances on the biofilm phenotype are urgently needed. The future holds much promise for this area of research, where MIC researchers will increasingly be able to resolve, at the molecular and biochemical levels, interspecies relationships and mechanisms of cell-cell and cell-metal interactions and how these processes are associated with corrosion and its inhibition. In addition, MIC experiments should be designed to use the field environment, or the closest field simulating environment, to grow and study biofilms and MIC. The outcome of these studies will greatly enhance our understanding of the ecological factors that drive community function in industrial systems and move us towards developing appropriate management solutions.

MIC control has traditionally relied on the application of toxic chemical compounds incorporated into coatings or biocide products. Even though such chemical treatments have shown broad efficiency, environmental concerns regarding their application represent an ongoing challenge to industry, particularly with the advent of offshore developments and floating processing facilities where operations may involve disposal of chemical treatments to the ocean. Therefore, increased attention is being paid to develop and implement novel, eco-friendly approaches to mitigate MIC. These approaches include membrane technologies to remove critical nutrients, application of natural corrosion and antimicrobial inhibitors such as plant materials as well as peptides and molecules of microbial origin. Special attention should be paid to identifying new antibacterial molecules that specifically target the biofilm state, i.e. anti-biofilm substances. In this fashion, any alternative methods proposed for the control MIC should be screened on sessile bacteria (biofilms) to achieve relevant values on the minimum biofilm eradication concentration (M.B.E.C) before their actual implementation.

Concerning MIC management, establishing multi-disciplinary research collaboration involving academia and industry is critical to allow the development of integrated knowledge, improved MIC prediction tools and integration of these factors into effective decision-making and mitigation strategies that will protect the assets during their lifetime. It is important to be aware of the latest monitoring technologies available to monitor microbial activity and its inhibition in a system. Technologies such as NGS have become rapid, cost-effective and accessible for assessing microbial communities in industrial systems. Ensuring such data is collected and processed under rigorous protocols is imperative to develop reliable and valuable information to end users. Interpretation of NGS data remains a difficult task that entails more systematic industry-academia engagement and the establishment of a national MIC database

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