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Omics-based approaches and their use in the assessment of microbial-influenced corrosion of metals

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Abstract: Microbial-influenced corrosion (MIC) has been known to have economic, environmental, and social implications to offshore oil and gas pipelines, concrete structures, and piped water assets. While corrosion itself is a relatively simple process, the localised manner of corrosion makes *in situ* assessments difficult. Furthermore, corrosion assessments tend to be measured as part of a forensic investigation. Compounding the issue further is the impact of microbiological/biofilm processes, where corrosion is influenced by the complex processes of different microorganisms performing different electrochemical reactions and secreting proteins and metabolites that can have secondary effects. While traditional microbiological culture-dependent techniques and electrochemical/physical assessments provide some insight into corrosion activity, the identity and role of microbial communities that are related to corrosion and corrosion inhibition in different materials and in different environments are scarce. One avenue to explore MIC and MIC inhibition is through the application of omics-based techniques, where insight into the bacterial population in terms of diversification and their

metabolism can be further understood. As such, this paper discusses the recent progresses made in a number of fields that have used omics-based applications to improve the fundamental understanding of biofilms and MIC processes.

Keywords: corrosion; metabolomics; metagenomics; microbial corrosion; next generation sequencing.

1 Introduction

In recent decades, there has been a considerable amount of research published on the role of microorganisms in promoting corrosion. The majority of this work has been undertaken to address the problem of microbial-influenced corrosion (MIC) in offshore oil and gas pipelines and concrete structures, with some preliminary research on microbial/metal surface interactions in water pipes. As such, a number of extensive reviews have been compiled on MIC mechanisms over the past 20 years (Beech & Gaylarde, 1999; Edyvean & Videla, 1991; Flemming, 1994; Videla, 2003; Videla & Herrera, 2009) and, most recently, the review by Beech, Sztyler, Gaylarde, Smith, and Sunner (2014), to name a few.

It has been highlighted in these reviews that there is a growing number of researchers applying omics-based techniques for the characterisation and understanding of MIC, where omics-based techniques comprise: (meta) genomics (e.g. the analysis of genetic material recovered from an organism or environmental samples), transcriptomics (e.g. the analysis of ribonucleic acid (RNA) molecules, including messenger RNA (mRNA), ribosomal ribonucleic acid (rRNA), transfer RNA, and other noncoding RNA, produced by an organism or a population), proteomics (e.g. the analysis of proteins produced by an organism or population, and their function), and metabolomics (e.g. the analysis of the small chemical compounds produced and consumed by an organism or a population), or a combination of approaches. The application of these techniques has been demonstrated to be

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beneficial in MIC research due to the presence of causative biofilms that are heterogeneous in nature and involve multiple organisms comprising complex and variable structures; thus, their interaction with the metal surface to which they have adhered will be similarly complex and likely to vary within the biofilm. As such, the application of omics-based techniques, specifically metagenomic techniques (also known as shotgun genomics), enables researchers to identify and characterise the entire microbial population present and, in combination with complementary techniques, identify what that population is doing in terms of gene expression (i.e. transcriptomics), protein production (i.e. proteomics), and metabolism (i.e. metabolomics) (Figure 1).

2 Microbial-influenced corrosion (MIC)

Corrosion itself is a relatively simple electrochemical process that involves two matched electrochemical processes – the oxidation of the metal at the anode and a parallel chemical reduction process at the cathode. The electrochemical circuit is completed by a transfer of electrons from the anode to the cathode. Corrosion may be accelerated or retarded by encouraging or restricting either the anodic or cathodic reactions.

However, in understanding corrosion, a difficulty arises in that most corrosion takes place in a localised manner, meaning that the separation of anodes and cathodes is on a submillimetre scale, and secondly, unlike galvanic corrosion, electron transfer occurs within the conducting metal and cannot be measured. The only measurements that can be made are through physical evaluation of metal loss, oxide accumulation, or localised measurement of electrolyte composition and gradients

(i.e. pH, solution potential, cation/anion concentration). Hence, where biological activity interacts with the localised electrolyte near the metal interface, it becomes increasingly difficult to isolate the (i) causal factors leading to electrochemical corrosion, (ii) casual factors leading to biofilm growth, (iii) the organic and inorganic films resulting from localised corrosion, and (iv) the chemical and localised potential gradients resulting from biofilm growth (see Figure 2).

As a result of the overlapping consumption and production of chemical species, gaining a precise understanding of the mechanisms of MIC requires more sophisticated methods for being able to isolate microbial processes from physical electrochemical processes, hence the suitability of omics for such studies, as will be stressed throughout this paper.

Biofilm formation has been demonstrated to develop within relatively short periods of time through extracellular polymeric substance (EPS) excreted by the populating microbes (Characklis, 1981). Keevil (2004) described biofilms as a complex structure that consists of arrays of microcolonies encased in EPS but with significant channels between these stacks of microcolonies that facilitate diffusion. They are heavily hydrated gelatinous-like films that have been demonstrated to physically affect corrosion processes (Videla & Herrera, 2009) and, if heterogeneously distributed over the surface, can result in the establishment of permanent anodic and cathodic regions across the metal substrate. However, biofilms are highly reactive to the environment, and survival of the biofilm requires nutrient transfer into the interior, and thus, the film cannot behave as a complete barrier. This open structure means that biofilms can also incorporate oxide-based corrosion products and may also contain specific zones where the diffusion of particular chemical species is restricted. For instance, activity by one group of microbes may hinder or foster

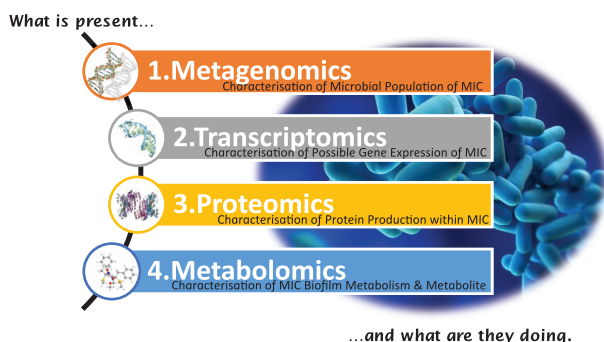


Figure 1: Application of omics-based techniques to understand and characterise biofilms and MIC.

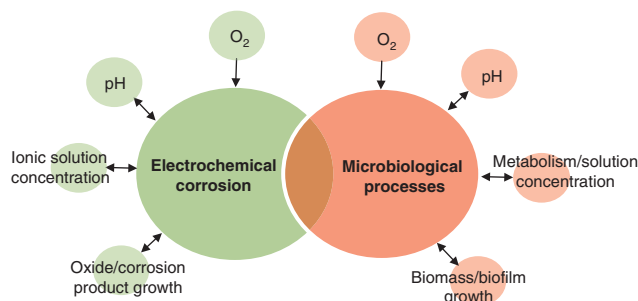


Figure 2: What comes first, the “chicken or the egg?” Causal and resultant factors relating to “electrochemical” corrosion and microbiological changes.

the development of others (Zuo, 2007). Thus, there can be a competition between sulphate-reducing bacteria (SRB) and iron-reducing bacteria (IRB) so that IRB may decrease SRB activity (Potekhina et al., 1999). In contrast, acid-producing bacteria, as well as being corrosive in themselves, may provide nutrients and environmental conditions conducive for SRB growth (Jack, Rogoz, Bramhill, & Roberge, 1994).

The major impact of biofilms and microbes on corrosion occurs through changing the rates of either anodic or cathodic reactions. Before moving on to detail the impacts and potential impact of omics, it is worth reviewing some of the current understandings of MIC and how biofilms and microbes can influence anodic and cathodic reaction rates.

2.1 Diffusion

If the reactants and/or products of anodic or cathodic processes cannot move freely to and from the metal surface, corrosion rates can be either diffusion limited or concentration limited. Videla and Herrera (2009) emphasised how the respiratory processes of microorganisms may modify the oxygen concentration at the metal interface. If the biofilm covers the whole surface, this may lead to a reduction in the cathodic reaction rate and decreased corrosion or, alternatively, if the coating is patchy, is likely to create a potential gradient that encourages localised corrosion. Edyvean and Videla (1991) illustrated the latter scenario where biofilms were shown to accelerate corrosion by forming oxygen cells in association with tubercles in potable water pipework. In addition, it is reported that aside from oxygen gradients, chemical and pH gradients can also be created within biofilms and can drive corrosion processes. For instance, pH differences within microalgal/bacteria films have been demonstrated to exceed nine units (Terry & Edyvean, 1986).

2.2 Polarisation past the pitting or breakdown potential

Where either the anodic or cathodic reaction rates are altered consistently over time, the potential of the underlying metal may be polarised. Polarisation can have a large impact on metals that are prone to pitting and rely upon oxide passivation to avoid corrosion. For instance, Dexter, Duquette, Siebert, and Videla (1991) suggested that the presence of a biofilm was able to shift the corrosion potential of from the passive zone to the pitting region.

2.3 Cathodic reaction rates

The cathodic reaction may be affected in a number of ways, as already mentioned; the oxygen concentration may be increased or decreased both by direct consumption (Zuo, 2007) or production of oxygen or due to the formation of barriers (by bacteria) to oxygen diffusion. Additionally, the production of acidic metabolites can increase cathodic processes through both the dissolution of protective films and through the supply of oxidising agents (i.e. protons) that encourage cathodic processes (Edyvean & Videla, 1991). Alternatively, Little, Ray, and Pope (2000) proposed that SRB may enhance cathodic reaction rates by removing atomic hydrogen from the cathode and thus avoid concentration polarisation limitations. Herrera and Videla (2009) found that the preexposure of steel to *Vibrio alginolyticus* had a profound effect on the free corrosion potential, shifting the free corrosion potential to a more negative value. This suggests a decreased cathodic reaction rate and enabled the creation of an anaerobic environment that allowed SRB growth and an even more significant negative shift in potential.

If metal oxides are conductive, they may support cathodic reactions, or, in the case of iron in anaerobic conditions, the redox reaction of Fe(III) to Fe(II) may act as the cathodic reaction (Stratmann, 1990). In environments with a high sulphur content or containing SRB, a protective layer of FeS may form but is, like most inorganic protective layers, highly dependent upon pH (Shoesmith, Taylor, Bailey, & Owen, 1980). Other reports have suggested that FeS can act as a cathode and support the anodic dissolution of iron that is not protected by corrosion products (Edyvean & Videla, 1991). For the case of Fe(III) to Fe(II), microbes such as *Shewanella oneidensis* that enhance this process can have two contradictory effects: it could reduce the overall cathodic current by decreasing the iron reduction pathway, or it may disrupt the passive layer formation of Fe(III) oxides and therefore increase corrosion (Herrera & Videla, 2009).

2.4 Anodic reaction rates

Microbes may affect the rate of the anodic reaction in a number of ways. Acid metabolites can dramatically lower the local pH. Terry and Edyvean (1981) indicated that the pH differences can be up to nine units. Furthermore, Edyvean and Videla (1991) demonstrated that light/dark cycles in photosynthesis and respiration can produce daily cycles of up to 3.5 units. Sulphur-oxidising bacteria (SOB) (genus *Thiobacillus*) can produce up to 10%

sulphuric acid with pH <2 (Edyvean & Videla, 1991). Nitrogen-utilising bacteria, microfungi, and microalgae can also produce acids (Edyvean & Videla, 1991). Such local acid regions can dissolve any protective films and accelerate the anodic reaction.

The slime produced by iron reducers may absorb iron, and such absorption can have diverse effects depending on the state of the oxide/biofilm. It could limit oxide formation, which would promote corrosion. On the other hand, it could also limit the acidification caused by the hydration of metal ions, which can lead to local acidification and pitting.

2.5 The role for omics

Corrosion is the result of a series of chemical, physical, and (micro)biological processes leading to the deterioration of materials. The mechanisms of MIC and MIC inhibition are not completely understood because they cannot be linked to a single biochemical reaction or specific microbial species or group (Kip & van Veen, 2015). Corrosion is influenced by the complex processes of different microorganisms performing different electrochemical reactions and secreting proteins and metabolites that can have secondary effects. Information on the identity and role of microbial communities that are related to corrosion and corrosion inhibition in different materials and in different environments is scarce. As some microorganisms are able to both cause and inhibit corrosion, we pay particular interest to their potential role as corrosion-controlling agents. One avenue to explore MIC and MIC inhibition is through the application of omics, where insight into the bacterial population in terms of diversification and their metabolism can be understood. Table 1 provides a summary of omics-based techniques applied to the investigation of MIC, preceded by a discussion of their use and benefits. It should be noted, it is not the intention of the authors to provide a review of the different analytical platforms applied in omics-based research. The focus of the review presented is to highlight the application and benefits of omics-based techniques in the assessment of MIC of metals.

3 Application of omics-based techniques to characterise MIC and biofilms

Metal corrosion is one of the main causes of damage to petroleum pipeline systems, resulting in great economic

losses to the industry. Around 40% of the internal pipeline corrosion in the gas industry has been attributed to MIC (Chakraborty, DasGupta, and Bhadury, 2014). Through the use of metagenomics and transcriptomics, MIC biofilm communities can be studied at both their compositional and functional levels. A number of traditional techniques, such as clone libraries and genetic fingerprinting, along with more recent metagenomics and transcriptomics, are being used to characterise and understand MIC biofilms (Chakraborty et al., 2014). Genetic fingerprinting techniques share a common trait of generating profiles of microbial communities based on direct analysis of polymerase chain reaction products amplified from environmental DNA (Muyzer, 1999). Over the years, microbial ecologists have used a number of techniques that produce community fingerprints based on either sequence polymorphisms or length polymorphisms. The community profiles from different samples can be compared using computer-assisted cluster analysis (i.e. chemometrics). In general, genetic fingerprinting techniques are rapid and allow simultaneous analyses of multiple samples. Since fingerprinting approaches have been devised to demonstrate effects on or differences between microbial communities and do not necessarily provide direct taxonomic identities, these approaches are specifically significant in the context of assessing the impacts of abiotic and biotic factors on microbial communities in contaminated sites (Chakraborty et al., 2014).

4 MIC biofilm genome and gene expression

Metagenomics was developed in the first decade of the 21st century and laid the foundation of all the omics-based techniques. Metagenomics revolutionised microbial ecology research, where the collective microbial genome collected from a sample is analysed and reconfigured to provide knowledge of the community's diversification (Handelsman, 2004). Transcriptomic tools are used to gain functional insights into the activities of microbial communities by studying their mRNA transcriptional profiles (Pascault et al., 2015).

Advances in high-throughput next-generation sequencing (NGS) (Jiang et al., 2008) technology for direct sequencing of environmental DNA (i.e. shotgun metagenomics) are transforming the field of microbiology (Ahmed et al., accepted for publication; Gomez-Alvarez, 2014). NGS technologies are now regularly being applied in comparative metagenomic and transcriptomic studies,

Table 1: Summary of omics-based techniques applied to the investigation of MIC.

Industry/Context	Material	Omics technique	Summary	References
Wastewater	Concrete sewer	Metagenomics Transcriptomics	Taxonomic and functional characterisation. Abundance of SRB and SOB bacteria. Enrichment of genes associated with metal resistance.	Gomez-Alvarez, Revetta, and Domingo (2012)
Electricity transmission tower	Steel	Metagenomics	Characterisation of diverse population comprising archaea, fungi, and several bacterial groups.	Vincke, Boon, and Verstraete (2001)
Marine environments	Carbon steel	Metagenomics (and culture dependent techniques)	Culture-dependent techniques resulted in 31 isolates. Metagenomic technique resulted in 160 clone sequences comprising filamentous fungi, yeast and bacteria.	Sette et al. (2010)
Oil production facility	Carbon Steel	Metagenomics	Characterisation of diverse population comprising bacteria and archaea.	Usher, Kaksonen, and MacLeod (2014)
Extra heavy crude oil	Carbon steel	Metagenomics Metabolomics	Dominant group was methanogenic archaea. Characterisation of diverse population comprising thermophilic sulfidogenic anaerobes and mesophiles.	Lenhart et al. (2014)
		Metagenomics	Hydrocarbon metabolites detected. Molecular identification, characterization. Phylogenesis of novel ascomycetes (<i>Wickerhamia</i> spp., <i>Cladosporium</i> spp. BM-103) and basidiomycetes (<i>Rhodotorula</i> spp.)	Naranjo et al. (2015)
Food industry	AD0-aluminium alloys	Ionomics	Degradation of crude oil resulting in to further degradation of carrier pipelines and storage tanks. Fungal mediated corrosion of AD0 alloys within 60-day incubation.	
Wood	Chromated-copper arsenate (CCA) preservative-treated wood	Metabolomics	<i>Alternaria alternata</i> fungus caused maximum corrosion, mediated by several exometabolites. Disposal of CCA-treated wooden poles due to toxic preservatives such as hexavalent chromium.	Smirnov, Belov, Sokolova, Kuzina, and Kartashov (2008) Sierra-Alvarez (2007)
Aerospace industry	AA2024-T3 aluminium alloy	Ionomics	Brown rot fungi (<i>Antrrodia vaillantii</i> , <i>Poria placenta</i>) caused an oxalic acid-mediated leaching of chromium and copper up to 52.4% and 15.6%, respectively.	Lee, Little, Ray, and Stropki (2012)
Various	Carbon steel/aluminium	Metabolomics	Consortium of <i>A. niger</i> and bacteria caused heavy leaching of Cr ⁶⁺ preservative coating on aluminium alloy to form nontoxic, elemental Cr. Fungal-mediated degradation of metals. Mitosporic fungi such as <i>Chrysosporium merdarium</i> , <i>Penicillium cyclopium</i> , <i>Arthrimum phaeospermum</i> , <i>Cladosporium herbarum</i> , and <i>A. niger</i> caused mild to severe deterioration in carbon steel and aluminium alloys in 30-day incubation period. The likely mediators were indicated as organic acids, indole alkaloids, trichothecenes, amides, etc.	Lugauskas et al. (2009)
Cooling water system of nuclear reactor	Carbon steel	Metagenomics	Characterisation of SIR such as <i>Desulfovibrio</i> , <i>Desulfomicrobium</i> , and <i>Desulfosarcina</i> .	Balamurugan, Hiren Joshi, and Rao (2011)
Household water pipes	Copper	Metagenomics	Characterisation of diverse population comprising bacteria. Mainly <i>Alpha</i> , <i>Beta</i> , and <i>Gamma</i> proteobacter species were isolated, including <i>Acinetobacter</i> , <i>Sphingomonas</i> , <i>Chryseobacterium</i> , and <i>Methylobacterium</i> species.	Pavissich, Vargas, González, Pastén, and Pizarro (2010)
Energy emission towers	Carbon steel/zinc	Metagenomics	Bacterial and fungal communities were identified. Mainly <i>Acinetobacter</i> and <i>Stenotrophomonas</i> bacterial species and fungal genera such as <i>Capnobotryella</i> <i>Coniosporium</i> and <i>Fellomyces</i> were found.	Oliveira et al. (2011)

which provide the data for functional annotations, taxonomic comparisons, community profile, and metabolic reconstructions.

Gomez-Alvarez et al. (2012) analysed the whole metagenome to determine microbial composition and functional genes associated with biomass harvested from sections of a corroded wastewater pipe. Taxonomic and functional analysis demonstrated that approximately 90% of the total diversity was associated with the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Furthermore, the biofilm was found to have an abundance of SOB and SRB. Gomez-Alvarez et al. also demonstrated, combined with transcriptomics, an enrichment of genes associated with heavy metal resistance, virulence (protein secretion systems), and stress response in the biofilm analysed.

Vincke et al. (2001) investigated the genetic fingerprint of the microbiota on corroded concrete sewer pipes using denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments. The DGGE profiles of the bacterial communities present on the concrete surface changed as observed by shifts occurring at the level of the dominance of bands from noncorroded places to the most severely corroded places. By means of statistical tools, it was possible to distinguish two different groups, corresponding to the microbial communities on corroded and noncorroded surfaces, respectively. Characterisation of the microbial communities indicated that the sequences of typical bands showed the highest level of identity to sequences from the bacterial strains *Thiobacillus thiooxidans*, *Acidithiobacillus* spp., *Mycobacterium* spp., and different heterotrophs belonging to the α -, β - and γ -Proteobacteria, Acidobacteria, and Actinobacteria (Vincke et al., 2001). In addition, Santo Domingo et al. (2011) investigated the microbial composition of concrete biofilms within wastewater collection systems using metagenomics molecular assays. They found that α -, β -, γ -, and δ -Proteobacteria represented 15%, 22%, 11%, and 4% of the clones, respectively, in the 2457 sequences analysed. Bacteria implicated in concrete corrosion were found in the clone libraries while archaea, fungi, and several bacterial groups were also detected using group-specific assays, which highlights that concrete sewer biofilms are more diverse than previously reported (Santo Domingo et al., 2011). However, this diversity was not the case for corrosive biofilms in oil pipelines analysed with DGGE (Neria-González, Wang, Ramírez, Romero, and Hernández-Rodríguez, 2006).

Sette et al. (2010) profiled the fungal community structure found in Brazilian energy transmission towers that displayed signs of corrosion and/or biofilm formation using both culture-dependent and independent

methods. In total, 31 isolates comprising 10 filamentous fungi and 21 yeasts were recovered from enrichment cultures, comprising *Aspergillus fumigatus*, *A. niger*, *Candida albicans*, *C. pseudointermedia*, *C. tropicalis*, *Cryptococcus laurentii*, *Debaryomyces nepalensis*, *Exophiala dermatitidis*, *Fusarium* spp., *F. solani*, *Paeclomyces lilacinus*, *Trichoderma citrinoviride*, *T. longibrachiatum*, and *Pichia guilliermondii*. Metagenomic analyses based on 160 clone sequences revealed 30 operational taxonomic units (OTUs) comprising 20 OTUs of filamentous fungi and 10 OTUs of yeasts. The majority of OTUs were related to the genera *Capnobotryella*, *Cryptococcus*, *Devriesia*, *Fellomyces*, *Fusarium*, *Kockovaella*, *Panaeolus*, *Rhodotorula*, *Sirobasidium*, *Sporobolomyces*, *Strelitziana*, and *Teratosphaeria*. While the fungi identified are ubiquitous fungi commonly found in other environments, some have been related to MIC of metals. Furthermore, Sette et al. (2010) emphasised the need for complementary approaches to assess the microbial assemblage of unusual environments, stating that not one single approach was sufficient to characterise a community effectively.

Usher et al. (2014) investigated marine rust on carbon steel in order to determine the importance of various microorganisms in corrosion. Microorganisms were imaged (Figure 3), identified, and enumerated by pyrosequencing. The analysis demonstrated the presence of a diverse population of bacteria and archaea. However, the dominant group was methanogenic archaea, representing 53.5% of all sequences. One methanogenic species, *Methanococcus maripaludis*, comprised 31% of sequences and can significantly increase corrosion rates by extracting electrons directly from steel (Usher et al., 2014). Furthermore, Mohan, Bibby, Lipus, Hammack, and Gregory (2014) analysed hydraulic fracturing source water and wastewater produced during fracking using metagenomic and metabolomic techniques. While not related to corrosion, the metabolic profile revealed a relative increase in genes responsible for carbohydrate metabolism, respiration, sporulation and dormancy, iron acquisition and metabolism, stress response, and sulphur metabolism in the produced water samples from a diverse microbial population (Mohan et al., 2014). These results suggest that microbial communities in potable water have an increased genetic ability to handle stress, which has significant implications for infrastructure management, such as biofilm control and combating MIC.

With sequencing costs decreasing, NGS is enabling an increasing number of laboratories to taxonomically (and functionally) classify a wide range of the organisms that are present, and the extension of these techniques to

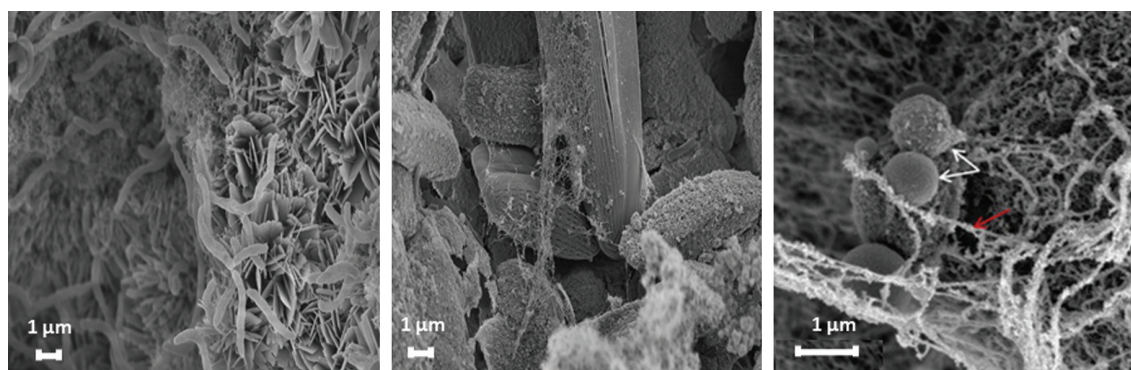


Figure 3: Scanning electron microscopy (SEM) images of microorganisms in marine rust tubercles sampled from carbon steel. After Usher et al. (2014). Note: the white arrows highlight microorganisms in the marine rust tubercles sampled from carbon steel. Extracellular polymeric substances (EPSs) are indicated with a red arrow. Panel A is a micrograph of carbon steel pipe (control), while panels B and C are micrographs of marine tubercles.

assess microbiological communities that influence corruptions is a logical step (Douterelo et al., 2014).

5 Proteomics and the emergence of matrix-assisted laser desorption/ionisation-time of flight

Proteomics is a discipline focused on the identification of proteins, and metaproteomics is defined as the characterization of the entire protein complement of a microbial community (Douterelo et al., 2014). Proteomics has been successfully used to investigate microbial community functions in marine environments, freshwater ecosystems, and biofilms from an acid mine drainage (Douterelo et al., 2014). Wikieł, Datsenko, Vera, and Sand (2014) studied the metabolic activity and biofilm development of *Desulfovibrio alaskensis* correlated to electrochemical response of carbon steel surfaces. While carbon steel was protected from the microbial attachment and exposed to metabolic products, only one spike in corrosion potential was recorded and coincided with increased sulphide concentrations during biofilm formation and ultimately resulted in corrosion rates that did not exceed 0.05 mm/year. Furthermore, 150 proteins were detected in the EPS matrix responsible for biofilm formation and maturation (Wikieł et al., 2014).

As previously stated, MIC is greatly influenced by the resident microbial community at any one site. Sites experiencing MIC are generally colonised by multispecies biofilms rather than a single species of bacteria (Li, Whitfield, and Van Vliet Krystyn, 2013). Thus, investigation of the microbes involved in the corrosion process becomes a

crucial parameter for adopting a suitable control strategy. Identification of microbes responsible for MIC is generally performed using conventional phenotypic methods that utilise culture-specific growth media and biochemical tests or by molecular methods such as 16S rRNA gene sequencing and the more recent metagenomics (Gomez-Alvarez, 2014; Gomez-Alvarez et al., 2012) and metabolomic approaches (Beale, Dunn, & Marney, 2010; Beale, Morrison, Key, & Palombo, 2014). Phenotypic methods are not always considered reliable and often require interpretation by skilled personnel. On the other hand, molecular methods of identification are much more reliable but can be expensive, time consuming, and difficult to interpret and not readily incorporated into routine laboratory microbiological analysis (Jadhav, Seviar, Bhav, & Palombo, 2014; Van Veen, Claas, & Kuijper, 2010).

A recent proteomics-based microbial identification approach that has gained increased popularity for its rapidity and cost-effectiveness is matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry (MS). Although MALDI-TOF MS was developed in the late 1980s, it is only in the last decade that it has emerged as a revolutionary technology in microbial diagnostics for the identification of different aerobic and anaerobic bacteria, yeasts and fungi (Anderson et al., 2012; Böhme et al., 2011; Griffin et al., 2012; Reich, Bosshard, Stark, Beyser, & Borgmann, 2013; Wieser, Schneider, Jung, & Schubert, 2012). Microbial detection using MALDI-TOF MS is also referred to as “whole cell” MS since the entire cell is analysed with minimal or no preextraction. In this technique, a single bacterial colony from solid culture media is spotted onto a target plate and overlaid with a matrix solution (containing solvents such as ethanol, acetonitrile, and trifluoroacetic acid). The solvents in the matrix penetrate the cell wall and make the inner proteins

available for crystallisation. A nitrogen laser is then utilised to ionise the microbial proteins (mainly ribosomal proteins); these ions subsequently travel through the TOF tube under vacuum until they reach the detector to generate characteristic spectra (Croxatto, Prod'hom, & Greub, 2012; Welker & Moore, 2011). The test spectra are then compared to commercially available reference databases such as MALDI Biotyper (Bruker Daltonics, Bremen, Germany) and VITEK MS (bioMérieux, Marcy l'Etoile, France), which include spectra obtained from known reference strains (Tani et al., 2012) (Figure 4). The main advantages of using MALDI-TOF MS in routine diagnostics is that it is a simple, cost-effective, rapid, and reliable technique that can be automated and incorporated into high-throughput diagnostic laboratories. More importantly, it is a single system that can be used for detecting multiple microbes.

MALDI-TOF MS has been extensively used for the identification and subtyping of different clinically significant microbes (Murray, 2010). Other than routine identification from solid culture media, this technique has been used to detect bacteria from more complex samples such as urine (Ferreira et al., 2010), positive blood cultures (Dran-court, 2010), stool samples (Sparbier, Weller, Boogen, & Kostrzewa, 2012), and, more recently, from different food

matrices (Jadhav et al., 2014). Some of the other applications of MALDI-TOF MS also include determination of antibiotic resistance in bacteria (Knox et al., 2014), analysis of specific protein biomarkers, and the determination of secondary metabolites (Vávrová, Matoulová, Žová, & Šedo, 2014). Whilst there have been numerous studies highlighting MALDI-TOF MS as a powerful tool for identification of clinical pathogens, few studies have investigated its application in the detection of environmentally important microbes, such as the ones involved in MIC.

Some of the microbial groups linked to MIC include SRB, methanogens, sulphur-oxidising bacteria, nitrate-reducing bacteria, and iron-oxidising bacteria (Li et al., 2013). Genera representing the above-mentioned groups that have been studied using MALDI-TOF MS include *Pseudomonas* (Anderson et al., 2012; Seng et al., 2013; Vithanage, Yeager, Jadhav, Palombo, & Datta, 2014), *Desulfovibrio* (Barreau, Pagnier, & La Scola, 2013; Justesen et al., 2012; Seng et al., 2013), *Desulfotobacterium* (Krader & Emerson, 2004), *Methanothermobacter* and *Methanococcus* (Krader & Emerson, 2004), and *Sphingomonas* (Seng et al., 2013). Since the commercial databases were originally developed for the identification of clinically significant pathogens, they have incomplete representation of environmental microorganisms (Kopcakova et al., 2014). Thus, it is possible that this technology, in its current format, may show limited success for identification of microbes implicated in MIC.

In a study conducted by Vávrová et al. (2014), MALDI-TOF MS was used to identify anaerobic bacteria obtained from biofilms in a brewery environment. Initially, 47% of isolates were able to be identified to the species level. Following creation of an in-house database for relevant bacteria that were absent in the commercial database, the speciation rate increased to about 85%. A similar approach was used by Ziegler et al. (2015) for identification of root nodule soil bacteria. After the development of a rhizobia-specific in-house database, identification of the bacteria was found to be in complete congruence with phylogenetic analysis. The above-mentioned studies highlight one of the main advantages of this technology – enabling the user to update the database with microbes more relevant to the type of analysis. Such in-house databases for bacteria linked to industry-specific MIC can be created and could also be exchanged between laboratories. Addition of more reference spectra to existing databases will result in more reliable identification of microbes responsible for or implicated in MIC.

When investigating MIC, it is important to understand all aspects of microbial biofilms. Cells in biofilms have been found to secrete certain extracellular polysaccharides

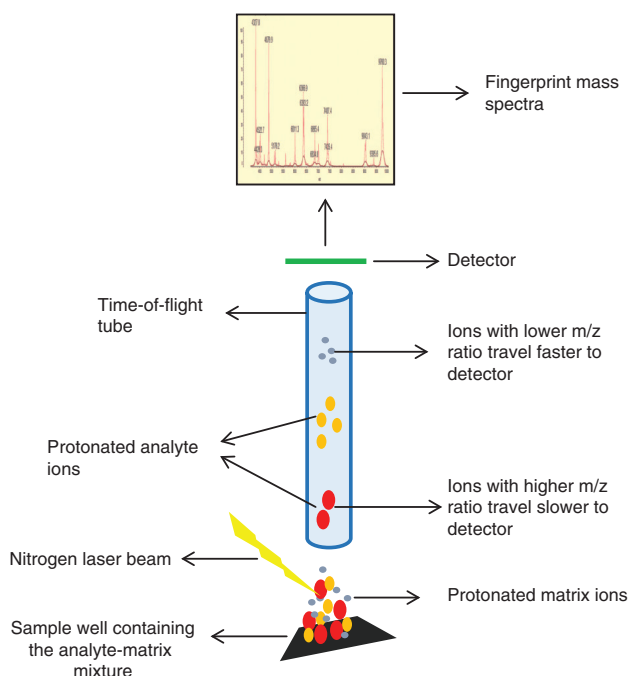


Figure 4: Schematic representation of MALDI-TOF MS mechanism. Note: The analyte-matrix mixture is bombarded with a nitrogen laser beam, which ionises the analyte (red and orange circles) and the matrix ions (blue circles). These ions travel to the detector based on their relative mass to charge (m/z) ratio.

(Belinky, Flikshtein, Lechenko, Gepstein, & Dosoretz, 2003), which serve as a binding component to provide a structural framework to the biofilm. EPS is known to vary with the type of microbial communities found within the biofilms. In an interesting study, Hasan, Gopal, and Wu (2011) used MALDI-TOF MS for the direct analysis of EPS produced by a biofilm formed on an aluminium surface exposed to sea water. The MIC was attributed predominantly to a fungus after characterising a specific polysaccharide “pullulan” in the biofilm. Thus, not only can this technology be used for microbial identification, but it can also be used for the direct analysis of biofilms involved in MIC. This can greatly reduce the time of analysis required for detecting the type of MIC at a particular site.

6 MIC biofilm metabolome and metabolomics

Metabolomics is the identification of the metabolites that are produced and consumed via either physical and/or chemical metamorphosis in biological samples (Beale et al., 2010). Metabolomics has been applied to understand metal corrosion and metals in the environment (Beale et al., 2010; Jones et al., 2015), food contamination (Beale, Morrison, & Palombo, 2014), general fungal and yeast metabolism (Brown et al., 2013; Christensen & Nielsen, 2000; Larsson, Snoep, Norbeck, & Albers, 2011; Niklas, Schneider, & Heinzle, 2010; Si, Luo, Xiao, & Zhao, 2014), and fungal based biomass degradation (Karpe, Beale, Harding, & Palombo, 2015). Furthermore, quantitative and qualitative metabolic profiling and flux analysis methods have been used to indicate the consumption of various substrates and relevant product generation. These approaches would considerably improve the understanding of fungal/bacterial-mediated corrosion of various metals and their minerals/alloys/metalloids. Metabolomic approaches also provide an insight to important metabolic pathways in bacterial (Cortés-Tolalpa et al., 2014) and fungal (Si et al., 2014) systems. These approaches have potential to improve fungal processing of metals and their related metalloids, alloys, and minerals.

Relatively few metabolomic-MIC studies have been reported. The role of corrosion products on MIC of carbon steel has been investigated by gas chromatography MS (GC/MS) (Liu, Xu, & Zeng, 2000), where S^{2-} and organic acids were found to destroy the protective layer and promote hydrogen permeation. Furthermore, GC/MS-based metabolomics has also been explored as a potential tool in monitoring MIC in copper pipes in water distribution systems

(Beale et al., 2010; Beale, Dunn, Morrison, Porter, & Marlow, 2012). It was found that the biofilm metabolites of bacteria-causing Cu pipe MIC comprised a combination of organic acids, amino acids, and lipids. These are common in biological metabolic processes, specifically those relating to soluble monomers and SRB substrates. In addition, the range of carboxylic acids released from the isolates (*Bosea*, *Methylobacterium*, *Paenibacillus*, *Sphingomonas*, and *Variovorax*) suggests that the corrosion potential of these biofilms varies, which would account for localised pitting corrosion commonly observed in metallic pipes (Beale et al., 2014). Kouremenos, Beale, Antti, and Palombo (2014) investigated the metabolic profile of *Pseudomonas putida* in potable water exposed to high and low doses of soluble and insoluble iron using liquid chromatography (LC) TOF-MS and identified metabolites that included nucleobases, nucleosides, dipeptides, tripeptides, amino acids, fatty acids, sugars, and phospholipids, significant as a response to exposure. While not directly related to MIC, the approach by Kouremenos et al. (2014) and the preliminary work of Beale et al. (2010, 2012) demonstrate the feasibility of GC- and LC-based metabolomics techniques to assess microbial populations exposed to metals or isolated from biofilms known to cause MIC in potable water networks. Furthermore, additional research on biofilms and biofilms known to cause MIC using other metabolomic based platforms, such as direct-injection MS, capillary electrophoresis-MS, Fourier transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy, has been described (Dunn & Ellis, 2005).

In a study by Booth et al. (2011), the difference in response to metal stress between sessile and planktonic bacterial populations was characterised. The planktonic population had an oxidative stress response to copper ion exposure, whereas the same species within a biofilm environment responded by shifts in exopolysaccharide-related metabolism. This finding suggests that microbial responses pertinent to corrosion are different between sessile and planktonic populations, and through more research using metabolomic-based techniques, linkages between the metabolite activity of both sessile and planktonic populations and their release of extracellular metabolites from a biofilm can be achieved. While biofilms, in general, and their influence on corrosion, in particular, have been subject to extensive research, there has been limited experimental work performed on the *in situ* characterization of the organic compounds within biofilms (Beech et al., 2014; Graeber, Boehm, & Kuever, 2014).

Fungi, especially the spore-generating species, have the ability to survive under variable environmental stresses. Similar to bacteria, fungi can metabolise a

number of carbon sources for survival, thus displaying a versatile nature of nutrient uptake. Fungi have been reported to grow in environments rich in heavy metals, such as mines and contaminated mine sites (Fogarty & Tobin, 1996; Gadd, 2007; Gadd et al., 2014).

A vast number of buildings, dams, and other constructions are based upon posttensioned concrete utilization. Stainless steel is heavily used to provide flexibility and support to this posttensioned concrete. However, to protect this steel from environmental factors such as humidity- and temperature-based corruptions, it is first coated with grease, which acts as a lubricant and corrosion protector. However, it has been recently reported that a number of fungi have the ability to utilise this grease as a source of nutrition, thus causing considerable contamination of this lubricant (Little, Staehle, & Davis, 2001; Lugauskas et al., 2009). The resultant fungal contamination causes the production of several organic acids. These acids cause considerable pitting in stainless steel over a long period, as evident from Figure 5 (De Leo, Campanella, Proverbio, & Urzi, 2013; Ehrlich, 1997).

It has been recently reported that *Aspergillus flavus*, *Penicillium commune*, *F. solani*, and *Acidomyces acidophilus* displayed the ability to metabolise OVOLINE 71C grease and produce numerous organic acids (De Leo et al., 2013). The acids were observed to cause pitting in stainless steel within a period of 10 months. The level of corrosion was equated with that caused by H_2SO_4 at pH 3. Although the authors reported that 10 months of fungal-mediated corrosion was not considerable, longer periods would be able to cause significant damage to the steel. Similar results were observed by earlier workers investigating metal corrosion by different microbes (Emde, Smith, & Facey, 1992). In this instance, the authors observed that water distribution system pipes had numerous fungi present in the corrosion pockets. A considerable proportion of those organisms were reported as the yeast *Rhodotorula*, which was considered as one of the causal organisms for pipe corrosion (Emde et al., 1992).

Vanadium is a metal that has found increased usage in various industrial applications such as stainless steel and sulphuric acid manufacturing (Moskalyk & Alfantazi, 2003). In addition, an estimated 110,000 metric tonnes/year of vanadium is released in the air by burning petroleum products and mining (Anke, 2005). It has been observed (Ceci et al., 2014) that fungi such as *A. niger*, *Aspergillus ustus*, *Clonostachys rosea*, and *P. lilacinus* were able to colonise vanadate $[Pb_5(VO_4)_3Cl]$, causing pits and surface degradation. The degradation, especially by *A. niger*, caused precipitation of lead oxalate, which resulted in an increase in fungal tolerance towards toxic heavy metals such as

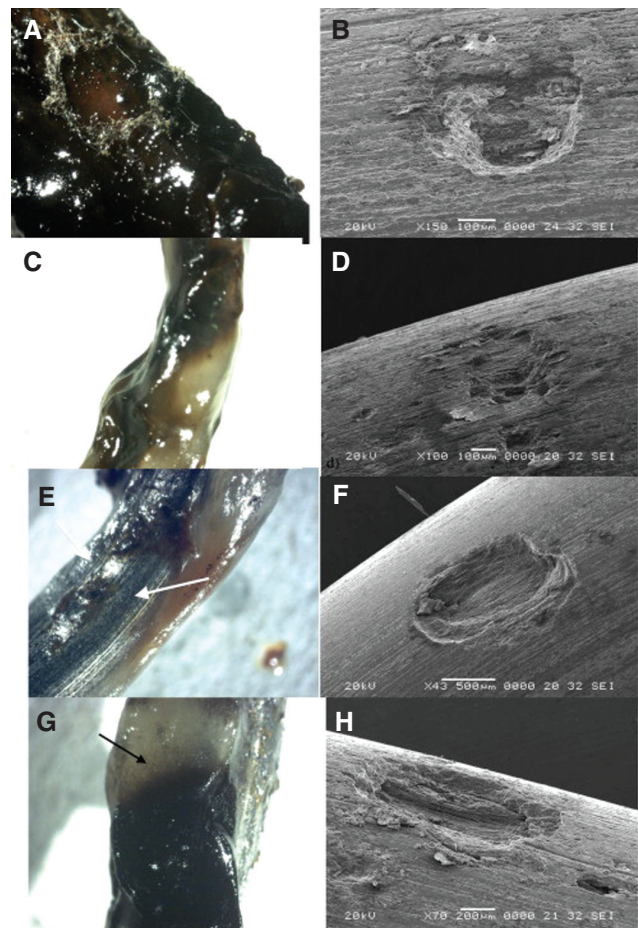


Figure 5: SEM images of fungal mediated corrosion of lubrication grease and stainless steel tendons used in building construction. Sourced from De Leo et al. (2013), with permission from Elsevier. Note: Photographs (A), (C), (E) and (G) show growth of *Aspergillus flavus*, *P. commune*, *F. solani*, and *A. acidophilus*, respectively. Photographs (B), (D), (F), and (H) show the pit formation on stainless steel by *Aspergillus flavus* ($\varnothing$ 400 μ m), *P. commune* ($\varnothing$ 350 μ m), *F. solani* ($\varnothing$ 1.2 mm), and *A. acidophilus* ($\varnothing$ 1.2 mm), respectively.

lead, vanadium, and their salts. According to the authors, this fungal degradation of vanadium minerals has numerous applications in stabilising and controlling various industrial (including nuclear) wastes.

The major metabolites generated by fungi to counter metal toxicities are organic acids, especially numerous metal oxalates. A large number of fungi excrete oxalic acid, which reacts with the surrounding metals, metalloids, or metal minerals to convert them to metal oxalates. This not only decreases the toxicity of these metals (allowing the development of tolerance in fungi) but also causes structural damages, which may become prominent after a considerable time (Gadd et al., 2014). While not desirable for industry trying to combat fungal mediated corrosion, this property has immense potential for the treatment of

heavy metal contaminated sites. The experiments conducted by several groups highlighted that the fungi such as *Aspergillus terreus*, *Trichoderma viride* (Joshi, Swarup, Maheshwari, Kumar, & Singh, 2011), and *Paecilomyces javanicus* (Rhee, Hillier, Pendlowski, & Gadd, 2014) have the ability to convert as much as 60 mg/g of lead (Pb) to nonhazardous minerals, such as pyromorphite. A similar process, termed *biomachining*, was developed and reported recently for tin (Sn), copper (Cu), aluminium (Al), and nickel (Ni) using *A. niger* (Jadhav & Hocheng, 2014).

Contrary to the above-mentioned experiments where various fungi have been used for metal or metal-based mineral corrosion, some experiments have also shown the utilization of fungal corrosive properties in restoration of metal and alloys. It has been proposed that fungi such as *A. niger* corrode copper to produce copper oxalate (Gharieb, Ali, & El-Shoura, 2004; Sayer & Gadd, 1997). Based on this principle, it was later reported that treatment of bronze coupons coated with *Beauveria bassiana* for 2 weeks at room temperature generated a coating of copper oxalate. This resulted in the prevention of further copper corrosion by environmental factors such as pollutants and humidity (Joseph, Simon, Mazzeo, Job, & Wörle, 2012). This aspect of fungal-based metal mineralization was also reported recently for the purpose of gold nanoparticle generation. It was observed that *Rhizopus oryzae* was able to reduce up to 130 μM HAuCl_4 to Au^+ and later to Au^0 in an intracellular mechanism within an incubation period of 12 h at pH 4. Gold nanoparticles generated during the fungal-mediated reduction process were calculated to be of about 15 nm diameter, thus indicating their application as biomedical nanoparticles (Das, Liang, Schmidt, Laffir, & Marsili, 2012).

7 Conclusion

The study of functional genes involved in metabolic pathways is essential when attempting to link microbial diversity with specific ecological functions. In the context of this review, better knowledge of the role that microorganisms play in MIC and MIC processes such as biofilm formation and corrosion is required and through the application of omics-based approaches is being realised. Enhanced knowledge through the application of omics-based techniques provides a holistic opportunity to measure respiratory processes that influence oxygen concentrations, metabolite (acidic) reaction kinetics, mechanisms, pathways, and characterising biofilm population diversity and dynamics. Furthermore, the application of multiomic approaches provides further potential of accelerated

understanding of these complex populations and processes through the combined assimilation of microorganism populations that are present and their activity, both localised and throughout a system.

The multiomic technology in MIC is novel as compared to various clinical applications. Although it is currently considered to be a nascent field, various potential applications resulting in considerable implications are either currently underway or are expected to appear on the scientific horizon in the near future. Until recently, a multiomic approach has not been applied in MIC and MIC-based research (Graeber et al., 2014). However, the research on concrete pipelines demonstrated the power of multiomics to identify key metabolic networks and gene functions within biofilms from corroded surfaces. It is expected that the exponential development in the overall omics knowledge, coupled with an rapidly developing technology in this field, will assist in exploration of various applications and related discoveries in the field of MIC and biofilm related research.

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Tim H. Muster is a senior research scientist in the Cities Program in CSIRO Land & Water. He has over 20 years of research experience in the scientific disciplines of colloid, surface, and electrochemistry, with over 65 refereed journal publications. In 2007, Tim was the recipient of CSIRO Young Scientist John Philip Award and has twice won the Marshall Fordham Best Research Paper of the Australasian Corrosion Association (2003 and 2005). More recently, Tim was the recipient of a CSIRO Julius Career Award for nutrient recovery from wastewater and leads research focussed on the effective management of urban and food production waste streams for the productive recovery of water, energy, and nutrients.



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Graphical abstract

David J. Beale, Avinash V. Karpe,
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**Omics-based approaches and their
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Review: Current assessment and potential application of omics-based techniques to understand and characterise biofilms and the microbial-influenced corrosion of metals.

Keywords: corrosion; metabolomics; metagenomics; microbial corrosion; next generation sequencing.

