Analytical Characterisation of Material Corrosion by Biofilms

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Abstract
Almost every abiotic surface of a material is readily colonised by bacteria, algae, and fungi, contributing to the degradation processes of materials. Both biocorrosion and microbially influenced corrosion (MIC) refer to the interaction of microbial cells and their metabolic products, such as exopolymeric substances (EPS), with an abiotic surface. Therefore, biofouling and biodeterioration of manufactured goods have economic and environmental ramifications for the user to tackle or remove the issue. While MIC is typically applied to metallic materials, newly developed and evolving materials frequently succumb to the effects of corrosion, resulting in a range of chemical reactions and transport mechanisms occurring in the material. Recent research on biocorrosion and biofouling of conventional and novel materials is discussed in this paper, showcasing the current knowledge regarding microbial and material interactions that contribute to biocorrosion and biofouling, including biofilms, anaerobic and aerobic environments, microbial assault, and the various roles microorganisms’ play. Additionally, we show the latest analytical techniques used to characterise and identify MIC on materials using a borescope, thermal imaging, Fourier transform infrared (FTIR), atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray photoelectron microscopy (XPS), X-ray diffraction (XRD), optical and epifluorescence microscopy, electrochemical impedance spectroscopy, and mass spectrometry, and chemometrics.

Keywords Biocorrosion · Biofilm

1 Introduction

The processes by which microbial organisms accelerate the corrosion rate of materials by microorganisms are generally referred to as biocorrosion, or more specifically, microbially induced corrosion (MIC) [1–3]. Materials that undergo microbial colonisation typically undergo chemical reactions, which are cathodic or anodic, or the establishment of differential oxygen concentration cells in a localised electrolytic environment. The economic cost of biocorrosion has been estimated to be at least 20% for material corrosion, amounting to a direct cost of 30–50 billion dollars per year globally [4]. For example, Michelangelo’s statue of David underwent cleaning measures for the first time in 500 years and cost €165k [5]. The French have injected some $250 million into a corrosion clean-up and protection of the Eiffel Tower in 1989 [6]. Thus, corrosion researchers and engineers have been interested in preventing biocorrosion for structural materials for decades. Numerous strategies, including biocides, cathodic protection, beneficial bacterial biofilms, and
protective coatings, have been proposed to combat biocorrosion [7].

MIC was first reported in the twentieth century by Gaines, R.H. (1910), who discussed the inherent corrosion causes of steel [8]. Further investigation was then intensified by von Wolzogen Kuhr and Van der Vlugt in 1934, in an attempt to interpret MIC using electrochemistry, whereby they proposed the theory of cathodic depolarisation to explain the role of sulphate-reducing bacteria (SRB) in the anaerobic MIC of steels [9].

Essentially, the cathodic depolarisation offered a classical mechanistic model for the explanation of MIC, where hydrogen is removed from the cathodic surface (iron), leading to the cathodic depolarisation where sulphate is reduced to sulphite [1–3, 10–13] (Fig. 1). The generation of hydroxide ions, which creates iron sulphide and hydroxide products, forces more iron to be dissolved at the site of the anode. The mechanistic approaches were only undertaken in the early 1960s; apart from these two efforts, publications are scarce. Any slight reference to MIC was made involving underground corrosion of iron structures by bacteria. An excellent review of these mechanistic behaviours has been recently published by [7].

2 Metal Corrosion

Corrosive processes for metals can occur in numerous forms, and classification of these processes can be addressed based on three primary factors [11, 14–16]:

(i) The mechanism of corrosion involving the electrochemical effects or direct chemical surface reactions.
(ii) The appearance of the corroded surface corrosion is either uniform, and the surface will corrode at the same rate over the whole surface, or it can be localised, in which only small areas become affected.
(iii) The nature of the environment the corrosion can be classified into two categories: ‘dry’ or ‘wet’.

Corrosion can be classed through physical appearance, either by the naked eye or microscopic techniques. The morphology of the attack forms the basis for some classification. Figure 1 illustrates a schematic classification of the common types of corrosion, which includes the non-biological (referring to corrosion) and biocorrosion (which involves the microorganisms). Non-biological corrosion refers to corrosion that does not involve microorganisms (i.e. bacteria, viruses, fungi).

Biological corrosion (i.e. biocorrosion) refers to corrosion that involves microorganisms. Biological corrosion occurs when microorganisms are present in a wet environment where they can feed on organic matter and produce acids.

Essentially, these corrosion types are evidently visible upon a surface of a material. However, some types of corrosion can fit into multiple categories, or some that are not so fitting into the category of corrosion. Each form of corrosion shall now be discussed in more detail. These points of discussion are always assumed to be in the aqueous state. However, corrosion can occur in other environments that cause the material to become stressed, for example, high temperatures, high pressures, and salinity. It is safe to assume that all materials are affected by uniform corrosion. Passive materials such as stainless steel (SS) or other high corrosion resisting alloys are normally subjected to a more localised form of attack. Metals can resist corrosion by forming passive films upon the surface. This can also develop when exposed to air, also existing through chemical treatments, and exemplified through nitric acid treatment on austenitic steels. If the film is left intact, the surface will generally not undergo any form of corrosion [4, 11]. Examples of constant attack would be tarnishing of silver, green patina of copper, and the rusting of steel.

Microorganisms can either enhance the rate of corrosion or slow it down by colonising the surface of a uniform corroding material in a manner that would either form protection across the surface or cause other corrosive effects, many of which are shown in Fig. 2. Microbe adhesion to surfaces is critical for many ‘ideal’ living circumstances, which include resistance to external effects such as temperature, pH, and even biocidal substances. Microorganisms and their metabolites and extracellular polymeric substances (EPS) produce biofilms because of this surface proliferation. Biofilms practically coat any material surface that encounters water. The homogenous nature of the material’s surface makes it a very suitable site for microbial colonisation. Uniform corrosion will usually entail through SRB, which may
form oxides of their metabolic processes and further enhance the rates of corrosion [17–19].

Pitting is one of the most destructive forms of corrosion and is one of the hardest types of corrosion to detect because of corrosion-based products often filling the holes [21, 22]. The pitting process is unpredictable, as the process is autocatalytic and often requires a varied amount of time to process. Once the pitting process begins to evolve, the length of time a material ‘holds up’ could be a matter of days (depending on the overall environment), so the failure of the material could occur suddenly [23, 24]. Pitting corrosion is different in the initiation stages, whereas crevice corrosion is initiated by a differential change in concentration of oxygen or ions surrounding the electrolyte [25–27], where the material alone initiates pitting. Microorganisms, in theory, could, therefore, enhance pitting corrosion in terms of their excretion products, which causes further and subsequent degradation to the material [28–30]. Further pits ensue across the surface of the material, where the natural pitting process will continue to propagate.

Crevice corrosion is another form of localised attack on a material, mainly occurring in areas of narrow spaces on metal–metal and non-metal–metal interfaces. Sometimes, the material is shielded in certain areas that are exposed to corrosives, which protects against this crevice action [31, 32]. The attack is the result of high concentrations of cells formed between the electrolyte and the crevice. Usually, the crevice will become oxygen starved, and the surrounding electrolyte will gain oxygen, causing this crevice system to exist. Therefore, the material within this crevice system is acting as the anode, and the exterior will act as the cathode, completing the redox system. An example of this would be SS that is corroding in a neutral aerated sodium chloride system. The anodic metal dissolution reaction within the crevice, $M \rightarrow M^{n+} + ne^-$, is balanced by the cathodic reaction on the adjacent surface, $O_2 + 2H_2O + 4e^- \rightarrow 4e^- \rightarrow 4OH^-$. This increase in $M^+$ ions within the material crevice results in an influx of chloride ions, resulting in a net neutralisation reaction. An acidic reaction is then established, whereby releasing a free acid through the hydrolysis of the metal chloride, in Eq. 1:

$$M^+Cl^- + H_2O \rightarrow MOH = H^+Cl^-.$$  \hspace{1cm} (1)

The free acid produced by the hydrolysis reaction keeps the pH below 2, while the pH outside the crevice remains neutral [33]. This microenvironment and pH state mean that the electrolyte present within the crevice has a high concentration of hydrochloric acid also containing metal chloride ions, which are dissolved at concentrations near-total saturation.

Briefly, in both the aerated and deoxygenated solutions, the anodic reaction is always metal dissolution as shown in Eq. 2:

$$M^0 \rightarrow M^{2+} + 2e^-.$$  \hspace{1cm} (2)

However, the reverse cathodic reaction depends on the solution conditions. For aerated solutions at neutral or alkaline pH, the main cathodic reaction is the reduction of oxygen as given in Eq. 3:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-.$$  \hspace{1cm} (3)

In addition, with all types of de-aerated solutions, as well as in aerated and acidic solutions, proton reduction occurs as seen in Eq. 4:

$$2H^+ + 2e^- \rightarrow H_2.$$  \hspace{1cm} (4)

When ferrous materials begin rusting, we know this to be the most common type of corrosion. In the presence of oxygen and water, the new chemical reaction for iron rusting can be expressed as in Eq. 5:

$$Fe^0 + 1/2O_2 + H_2O \rightarrow Fe^{2+} + 2OH^-.$$  \hspace{1cm} (5)

![Fig. 2 Schematic illustration showing the involvement of various microbes such as aerobic bacteria, anaerobic sulphate-reducing bacteria in the biocorrosion mechanism of 304 L SS (obtained with permission from [20])](image-url)
Microorganisms present in a crevice corrosion system can cause more severe effects as a differential oxygen system is established [34–36]. Having two different oxygen concentrations at two locations will cause a difference in electrical potential and consequently produce corrosion currents. Where a crevice exists, this gives the possibility of setting up these parameters, meaning that respiring microbial colonies become anodic, causing the surrounding areas to become cathodic. Some metals develop a natural surface film or oxide layer that is a natural barrier for the stresses that erosion–corrosion brings to them. The ability of these films to protect the metal depends significantly on the speed it takes for them to be removed [37–39]. Therefore, a more complex and increased dense layer yields better protection than removed by mechanical action through ‘wear and tear’ as shown in Fig. 3. A film that is too brittle or cracks is not thoroughly protected. It is the film or surface coating that SS relies on for their inherent corrosion resistance.

Consequently, the materials will be vulnerable to corrosion in some way. The erosion–corrosion of a stainless steel type 316 (18Cr–12Ni) from a pectin extraction plant vessel was increased as temperature increased [40]. This vulnerability brought about a modification in the hydrodynamics, causing steam to increase the rate of corrosion attack [41]. The erosion–corrosion property of the steam has a stripping effect that lowers a dissolved oxidant gas, thus, causing an increased level of corrosion in the column of the distillation unit [42].

Erosion corrosion of a material is linked to the aggravation of the corrosion process [44, 45]. For instance, if microorganisms are present in a system whereby erosion–corrosion is prevalent, the microbes can attach to the eroded surfaces of the material, thrive, and excrete by-products that could enhance the overall corrosion process. In a comprehensive review by Cheung and Gu [43], the authors summarise the mechanisms of hexavalent chromium detoxification by microbes (Fig. 4). This process, in turn, has some benefit to the erosion–corrosion system; if microorganisms are allowed to build up, thin films will be beneficial in coating the points where erosion occurs, as they are small and localised [36]. This localisation of erosion, in turn, protects the material from the corrosive electrolyte, thus, reducing the problem temporarily to the survival or timescale of the biofilm [46, 47].

Some methods of prevention that could be employed to combat this erosion problem are choosing materials less susceptible to erosion, better the design of the system to which the materials will be employed, limiting enrichments that the materials are exposed to, and employing surface protective materials coatings [48–50].

Dealloying is known as selective leaching, parting corrosion, or selective dissolution and is an essential electrochemical process associated with alloys formed by components with different noble characters (with varied standard equilibrium potential) [51, 52]. Nobler component phases, formed during the dealloying process, play essential roles in stress corrosion of cracking metallic surfaces, but selective dissolution can be utilised via suitable technologies [53, 54].

In the dealloying process, one of two mechanisms can occur: alloy dissolution and re-plating the cathodic element or selective dissolution of an anodic alloy component. Both cases leave a spongy and porous metal, and all aspects of shape, rigidity, strength, and ductility are sacrificed. As with other types of corrosion discussed, the prime cause of the selective leaching effect is the galvanic reaction between the elements. Examples of alloy combinations and elemental leaching are described in Table 1.

3 Analytical Methods in MIC Characterisation

Many methods have been used over the last few decades to measure and determine corrosion rates. Ever since the first report of corrosion in 1910 until the present, techniques have developed and evolved. Some of these methods have not been explicitly designed for biocorrosion analysis and have merely been adapted. In this section, we report the state-of-the-art characterisation techniques for MIC and the processes of corrosion.
Fig. 4 Use of borescope images to show a (left) iron oxide and (right) clear. b Sample borescope images of manganese-oxide borescope images. A value of 1 was assigned if manganese oxide was removed or replaced with iron. The yellow colour is the abiotic iron-oxide replacement of the manganese oxide (Color figure online). Reproduced with permission from CCC Rightslink, Soil Society of America

Table 1 Alloy combinations and elemental leaching

| Alloy                  | Environment                                                                 | Element and dealloying term       | References |
|------------------------|-----------------------------------------------------------------------------|-----------------------------------|------------|
| Brass                  | Many waters, incredibly stagnant, or low aeration                          | Zinc (dezincification)            | [55]       |
| Grey iron              | Soils, most waters, engine blocks, and low tensile strength required housing | Iron (graphite corrosion)         | [56]       |
| Aluminium bronzes      | Hydrofluoric acid, acids containing chloride ions, non-sparking environments | Aluminium (dealuminification)     | [57]       |
| Copper nickels         | High heat flux and low water velocity (e.g. condenser tubes—refineries). Offers exceptional resilience to corrosion of seawater | Nickel (denickelification)        | [58]       |
| Gold alloys with silver or copper | Sulphide solutions, human saliva                                            | Copper, silver                    | [59]       |
| Medium and high carbon steels | Oxidising atmospheres, hydrogen at high temperatures                        | Carbon (decarburisation)          | [60]       |
| Iron-chromium alloys   | High-temperature oxidising atmospheres                                      | Chromium, protective film         | [61]       |
| Nickel–molybdenum alloys | Oxygen at high temperatures                                                | Molybdenum                        | [61]       |
3.1 Borescope

A borescope is an optical-based device, which enables online visual inspection of the internal surfaces of pressurised vessels and reactors. Chiavari and co-workers implemented a borescope to measure visible corrosion in historical tubes of a pipe organ. This measurement was achieved using a 37.5 cm borescope with a digital camera attachment [62]. Borescopes currently available do not support high-resolution inspection or automation. A recent study proposed a hyperspectral imaging (HSI) probe for corrosion monitoring that enables high-resolution scanning and automation [63]. Compared to RGB images, the proposed HSI modality captures reflectance at multiple wavelengths from multiple spatial points on the sample and provides an enormous amount of spectral detail. A key research area that has been developed as a strategy for automatically detecting corrosion on aeroplane structures using images taken by a borescope and also using deep neural networks (DNNs) has been reported by [64]. The authors demonstrate that a robust model could be developed using a dataset that included D-Sight Airplanes Inspection System (DAIS) photos from various lap joints on Boeing and Airbus aircraft. With a precision of more than 93%, this study proved that the use of DNNs can help reduce uncertainty caused by operator tiredness or insufficient training. This technology enables rapid, non-contact corrosion inspection, and the flexible fibre probe, which functions similarly to a borescope, can be utilised to inspect difficult-to-access locations. Although primitive in the determination of corrosion or indeed rates of MIC on a material, the borescope has been taken to advanced levels. For example, Scott et al. [65] used the borescope to create images of corrosion of metals in soils which bacteria were facilitating. Indicator of reduction in soils (IRIS) is an important technology for identifying hydric soils. However, the method does not allow real-time analyses. The authors-built sample images that were scanned and then given a value of 1 if iron oxide was removed (Fig. 4). The method can be paired with Wi-Fi and, thus, continuous monitoring applied to the system. The authors set out to measure the rates of microbial influence and identify the bacteria responsible for the removal of the metal oxides—however, this proved difficult.

3.2 Thermal Imaging and FTIR

Thermographic imaging is a valuable tool for detecting less visible forms of corrosion. It has the advantage of scanning vast areas with high throughput to provide a more comprehensive view of simple structures. The method is fast and non-intrusive but does not offer a cost-effective way to quantify low levels of corrosion. At low levels, the images lose clarity, and layered structures provide a problem. Mabrouki and colleagues investigated vibro-thermographic methods for detecting fatigue cracks in steel [66]. This technology is close to that used in electromagnetic shakers or ultrasonic burst actuators. The study focused on the efficient use of thermal imaging technology to predict fatigue cracks. Another work employed a dual-band infrared tomography system to seek out aircraft imperfections and hidden corrosion [67]. Also, the infrared thermography was used to investigate the fatigue performance of structural steel under the laboratory and actual corrosive environments [68]. This system offers more excellent resolution in imaging and, therefore, better detection of surface corrosion.

An excellent use of thermography was demonstrated for concrete by Vishwakarma et al., where the authors used lock-in thermography (LT) phase angles and amplitude images were compared between unexposed and exposed concrete specimens to characterise deterioration under biofilms. Fly ash-modified concrete showed least pH reduction and least density of total heterotrophic bacteria, SRB and SOB in the biofilms. In order to carry out the method, the authors used that a CEDIP Silver 420 camera was used for the study with thermal resolution of 25 mK. Two halogen lamps of 1000 W power each were used as optical light source. A function generator and an amplifier were used for generating sinusoidal stimulation. The samples were kept at a distance of 40 cm from the camera [69].

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR–FTIR) has been used to quantify rates of dissolution of thin metals. Changes to the atomic layers of thin metal can be measured using ATR–FTIR by non-destructive means and in real time. For example, Bremer and Geesey used ATR–FTIR to monitor bacterial interactions on copper in both static and flowing conditions [70]. Additionally, the use of FTIR to study material degradation in the presence of microbes was used by Nasrazadani et al., where FTIR was used to identify concrete degradation due to biogenic sulfuric acid attack. Concrete samples were exposed to electrolytes containing bio-species in laboratory-simulating field conditions and FTIR spectra of the reacted concrete were analysed for concrete phase formation. Streptomyces sp. was subsequently isolated and identified using 16S RNA sequencing which was then used as the biogenic medium to simulate MIC in the concrete samples which was then analysed using FTIR [71].

3.3 Atomic Force Microscopy (AFM)

AFM was first used in 1986 and is considered a foremost tool in nano-imaging and manipulation [72]. It has become a ubiquitous method in terms of the ability to characterise a surface at nanoscale, showing topographies that covers the show. It is an exact technique that provides topological and compositional information about a wide range of materials. These can include living cells, ceramics, or high-strength
steel. This is useful in understanding how microorganisms interact with a substrate, where they attach, and how they further colonize. Although AFM is not commonly used in corrosion studies, it has been successfully used for in situ electrochemical studies. Other authors performed an in situ electrochemical atomic force microscope to investigate pitting corrosion on Cu films [73].

AFM has two main modes of operation, contact and non-contact. Contact mode is the basis for all AFM techniques in which the probe tip is in physical contact with the sample surface of the material. While the tip scans along the surface, the sample topography induces a vertical deflection of the cantilever, producing high-resolution surface topographical images [74]. Corrosion measurements were performed using AFM in both modes (contact and non-contact) to measure levels of corrosion on stainless steel SUS304 deposited with sulfuric acid [75]. An excellent example of work showing high-resolution AFM of MIC was demonstrated by Xu et al. [76]. This work shows high-resolution topological imaging of bacteria that are attached to steel surfaces. The AFM work showed not only single-cell SRB with EPS production but also SRB clusters [76]. AFM can also be used to assess various corrosion inhibitors such as non-ionic surfactants. Mu and Li investigated the inhibition action of Tween-20 with sulfuric acid on SS [77]. Olivares-Xometl et al. performed similar work where imidazolines and amide precursors derived from oleic acid were evaluated as a corrosion inhibitor on steel, which can be seen from the AFM images that the corrosion inhibitors formed a film across the surface of the steel [78]. More recently, Yuan and co-workers have used AFM to evaluate the biofilm colonisation dynamics of Pseudomonas NCIMB 2021 and Desulfovibrio desulfuricans ATCC 27774 on 304 stainless sheets of steel [79]. This was achieved by measuring the forces and interactions of the tip of the biofilm and the stainless-steel coupon surface.

Electrochemical atomic force microscopy (EC-AFM), a subfield of scanning probe microscopy (SPM), is capable of high-resolution imaging of substrate topography (Fig. 5) [80]. It has been expanded to a diverse variety of research areas by continuous improvement since its inception. The presence of an electrolytic cell and a potentiostat enables real-time observation of the sample surface’s topographical changes. EC-AFM is used in situ corrosion research because electrically conductive samples are not required. EC-AFM operates similarly to AFM, with the instrument can be controlled in either constant height or constant force mode. The imaging method is commonly classified into different operating modes based on the interaction force between the tip and the sample, including touch mode, non-contact mode, intermittent tapping (tapping mode), torsional resonance mode, and peak force-tapping mode. The first mode is touch, in which the probe’s tip is often in contact with the sample’s surface, and the force between the tip and the sample is repulsive. Due to the softness of the silicon nitride cantilever and its high resonance frequency, which prevents vibration instability, a silicon nitride probe is often used in touch mode. Although the contact mode offers superior resolution and scan speed, it can cause damage to the sample’s surface.

In conclusion, AFM is a very versatile technique with an array of use in biocorrosion studies. The method has some drawback; due to its sensitivity, it is susceptible to environmental interferences, i.e. vibration. The technique is also very time consuming due to its accuracy and results often must be treated for errors that exist.

### 3.4 Scanning Electron Microscopy (SEM)

SEM is a technique that is paramount and routine in determining biofouling and biocorrosion on a substratum. SEM has been used in many scientific papers and has become a susceptible and highly effective technique in identification, analysis, and characterisation [81–93]. For examples, field emission scanning electron microscopy (FESEM) and AFM were used to characterise the dimensional morphology of the SS after pitting [94]. It was shown that the bacterial adhesion increased with the pH and temperature, which significantly increased the surface roughness of the SS. SEM and AFM analyses showed cracks and dislocations on the surface of stainless steel underneath the attached bacteria, which suggested a direct role of biofilm in corrosion induction.

The technique is often coupled with energy dispersive spectroscopy (EDS) and has been extensively used to demonstrate bacteria in corroded areas with the determination of surface chemistries from MIC [95–97]. The elemental chemistry of the base metal can also be determined using the EDS tandem with SEM. The preparation of biological material requires manipulation and fixation with some samples requiring dehydration or critical point drying because the SEM operates under high vacuum [98, 99]. Also, non-conducting samples, inclusive of biofilms, must be coated with a thin film of metal before the specimen can be analysed, usually known as ‘sputter-coating’. If the sample was uncoated, a build-up of local electrons would occur, this is known as sample ‘charging’, and the imaging is regarded as unusable owing to localised charge effects superimposed over the detail of the image. EDS can be used to determine the elemental composition of a surface; this can only be achieved before sputter coating. EDS samples are collected from a specific area, removed from the sample chamber, sputter coated, and then returned to the sample chamber. This can cause problems as more than often, the sample will not be in the same place, so reproducibility in the position is often difficult. Sample preparation for SEM, inclusive of...
solvent removal, and drying can decrease the areal surface by the biofilm, as Little et al. [100].

Some shortcomings of SEM and EDS come in the form of peak overlap in the data and spectral data output. For example, spectral peaks for sulphur overlap with those of spectral peaks for molybdenum, thus, highlighting that for materials and corrosion aligned with MIC can be problematic. In addition, the characteristic peak for manganese coincides with the secondary peak for chromium highlighting that that data need to be carefully analysed. In order to reduce these limitations, wavelength dispersive spectroscopy can be used to resolve overlapping EDS peaks.

Another area of SEM that is becoming more widely used is ‘environmental scanning electron microscopy’ (ESEM). The method is fast and accurate imagery can be obtained based on less vacuum and more appropriate imaging without the need for exhaustive sample preparation. The technique uses unique secondary electron detectors to form high-resolution images at pressures ranging from 0.1 to 20 Torr. Charging is dissipated into a gaseous form in the chamber.

Fig. 5 A SEM micrographs showing the surfaces of steel substrates (a) prior to and (b) following biofilm and corrosion by-product removal. For 90-day incubation, these substrates were initially incubated in the synthetic saltwater containing Bacillus sp. Following that, the surface was cleaned of biofilm and corrosion by-products.

B Atomic force microscopic images showing the surface topography of the steel plates after submerging in artificial salt water for 90 days (a) before and (b) after biofilm removal, demonstrating the apparent pitting of the steel samples following biofilm removal (permission obtained from https://www.mdpi.com/2079-6412/10/10/983/htm)
at these pressures, enabling direct observations of uncoated or non-conductive samples [101].

Greggerová and co-workers used SEM as a technique for the identification of corrosion in concrete [102]. They state that it is an effective tool in analysing deteriorating material properties, mentioning that concrete is being attacked by sulphate and carbonation. Although this is true, consideration should have been given to microorganisms attributing to the further corrosive action surmounted by biological means instead of pure chemical processes.

In short, SEM offers detailed structural topographical imagery. It can visualise flaws in materials but also highlight any microorganism attachment upon a substrate, especially in biocorrosion. Sample preparation is relatively simple and does not require a lengthy preparatory approach. The method has some disadvantages such as sample charging, the ability of reproducibility is limited and time consuming.

3.5 X-Ray Photoelectron Spectroscopy (XPS)

XPS has been routinely used for quantitative surface composition analysis. Relative elemental concentrations are determined by comparing intensities of the characteristic signals [103]. XPS is excellent at quantitatively analysing outermost passive layers on a surface and has evolved as a predominant technique in biofilm analysis. Greggerová and co-workers used XPS to determine concrete composition from the Charles Bridge in Prague, Czech Republic [102]. This could be useful in determining an initial and final sample point and seeing the changes in composition and applied to further biocorrosion samples or environments.

Yang and co-workers have analysed the surface morphology of magnesium alloys, and XRD was applied to monitor the biocorrosive characteristics for medical application [104]. X-ray photoelectron spectroscopy was used to study the interactions of an exopolymer-producing bacteria, Burkholderia sp., with polished AISI 304 SS substrates (XPS) [105]. Another excellent example of how XPS is an excellent technique in determining what compounds and effects are causing biocorrosion.

XPS, unlike SEM and EDX, produces far lower kinetic energies for Auger electrons and X-ray-excited photoelectrons than those of fluorescence X-rays generated by the electron beam in a SEM. This lower energy produces meaningful information when they are not attenuated by surface layers; namely the photoelectrons and Auger electrons from a few monolayers of the top of the surface are characteristic elements and molecules in these monolayers [106].

Juzeliunas et al., measured the rate of corrosion on zinc and aluminium in a two-year long study using fungi. Aspergillus niger, a filamentous ascomycete fungus, was isolated from the metal samples exposed to marine, rural, and urban sites in Lithuania, Europe. Al and Zn samples were subjected to the influence of A. niger under laboratory conditions in humid atmosphere for two years. The use of electrochemical impedance spectroscopy (EIS) ascertained microbiologically influenced corrosion acceleration (MICA) of Zn and MIC of Al. The surface analysis was carried out by XPS where the surface sputtering by ionised argon showed significant content of organic carbon within the layer of the material. The highest content of organic carbon was found on the top of the corrosion product layer (ca. 40 at.%), which comes from the dehydrated microorganisms and their metabolites. The noticeable concentrations of organic carbon were found to be in deeper levels (8–10 at.%) which have to be considered as the result of the organic acid penetration into the corrosion product layer, the authors provided this evidence as they did not find the carbon in the XRD data, highlighting that the multi-instrument analysis principles are imperative to corrosion studies [107].

3.6 Optical Microscopy (OM) and Epifluorescence Microscopy (EM)

OM is likely to be the oldest technique in visually characterising the level of corrosion that a material has suffered in more depth. Few researchers utilised this method to analyse bio-based films and immediately use SEM to characterise a surface. OM can evaluate biological-based material microstructures and has been done by Veiga-Santos et al. [108]. The technique offers excellent visual imaging of the sample, including colouring and hue depth, with the only limitation is that it cannot directly measure forces that biological samples exert on surfaces, such as bacterial populations upon a substrate. This limitation can be overcome if combined with other techniques, for example, with AFM, which can measure forces of attachment by using the cantilever as a force measurement [109]. Another form of microscopy closely linked to OM is EM, and this will document numbers of bacteria present upon a surface and enumeration. EM has been used to evaluate the distribution of cells on a surface but requires a certain level of sample preparation. The sample needs to be fixed and stained (Fig. 6). It is often hard to distinguish individual cells within densely populated regions on the sample and can often be impossible to image with corrosion-based samples as the stain and products are impenetrable.

Confocal scanning laser microscopy is emerging as a combinational approach to distinguish material and MIC. For example, in a study by Arun et al., the MIC of UNS S32750 super-duplex stainless-steel joints fabricated was investigated using different welding methods. The authors introduced the sample into a medium inoculated with Macroccocus equiperccius isolated from a marine environment. The study found that when CLSM and AFM
could readily characterise the topography and formation of pits in the corroded samples. The authors successfully used CLSM for surface morphologies and the width of the pits of the SS materials which were then turned into 2D and 3D interpretations created when in combination with AFM [52].

Interestingly, a material known to be relatively solid and robust was found to corrode when subjected to *Pseudomonas aeruginosa*. For example, Khan et al., found that MIC of pure titanium when studied using CLSM demonstrated pitting corrosion. The pitting corrosion was investigated with the help of CLSM, and the largest pit depth found on Ti surface immersed in *P. aeruginosa* was 1.2 μm. The study highlighted that Ti was not immune to MIC caused by *P. aeruginosa* [110]. CLSM is fast becoming a method of analysis for pitting corrosion with many authors already using the microscopic method [111].

### 3.7 X-Ray Diffraction (XRD)

XRD is widely used in works concerning corrosion. It works on the principle where X-rays are scanned to the surface of the sample, and a signature relay is measured, giving a signature for each element present. Reyes et al. investigated

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**Fig. 6** Fluorescent micrographs showing the biofilm formation of *Bacillus* sp. on the steel surfaces which were incubated after a-1 1 day, a-2 1 week, and a-3 1 month and side view images b-1 1 day, b-2 1 week, and b-3 1 month. Cross-section micrographs showing the thickness of formed biofilms on steel substrates (adapted with the permission from https://www.mdpi.com/2079-6412/10/10/983/htm)
the MIC of copper pipes at a low pH. This was to simulate the drinking water conditions in a typical rural home [112]. They took various sections of the water system’s copper pipes and analysed them using XRD. The analysis told them that the biofilms were growing around malachite that had formed due to oxidation of the copper pipe. Dinu et al. monitored biomedical alloys for the growth and deposition of unwanted bacteria; hydrogen was tested as an anodizing element, and analysis was performed using XRD, another use of characterisation using XRD [113]. Gebert and co-workers analysed structural states of corroding NiMnGa alloys for memory applications [114], with XRD. Christina-Cardona and co-workers utilised XRD to characterise native microorganisms that inhabited two coal types [115]. XRD was used to measure the depletion of pyrite peaks in the sample, thus, illustrating that microorganisms assisted in the breakdown of the coal samples.

Calle and co-workers performed leaching studies upon copper pipe in a household-plumbing system [116]. Microorganisms were deemed problematic in adding to the corrosion problem through biofilm attachment. XRD was used as a means of determining copper leachate. This needed to be further resolved with X-ray absorption spectroscopy, which then identified the species of copper released on the flux of the copper piping.

In a study by Khouzani et al., MIC on pipelines from a petrochemical plant was investigated. The authors utilised several techniques; however, they showed that the typical XRD patterns of corrosion products, taken from the surface of the pipe, could be successfully evaluated. The results showed that iron was present in the biofilm and on the material, showing that the biofilm was using the pipe system to facilitate corrosion. The authors showed a typical XRD pattern of corrosion products, confirming the formation of a significant amount of oxide–hydroxide (FeOOH), iron oxide, and iron taken from the surface of the pipe. The results confirmed the formation of a significant amount of sulphide. The latter is a typical corrosion product when sulphate-reducing bacteria are the controlling microorganism [117].

In addition, Khanfar and Sitepu recently reported a case study of MIC and using XRD as a source of phase analysis for deposits created in a refinery. The refinery environment was from crude cooler and reboilers which were then analysed for MIC using microbial, metallurgic, and special analyses and correlates the Rietveld quantitative phase analysis of high-resolution X-ray powder diffraction (XRD) data of scale deposits with microbe compositions. Therefore, rapid in-field microbiological assays could be carried out to assess the potential of MIC. Based on the results, it can be highlighted that the MIC investigation showed that total bacteria and SRB were detected in all sampling locations [118].

### 3.8 Electrochemical Methods

An electrochemical method is a recent technique introduced to detect and monitor corrosion [82, 119, 120]. Most of these methods are governed by change by electron transfer within a sample as the basis of detection. The techniques covered are linear polarisation measurements, electrochemical impedance spectroscopy (EIS), electrochemical noise (EN), and mass spectrometry (MS).

#### 3.8.1 Linear Polarisation Measurements

A linear polarisation technique can coherently measure the rate of corrosion based upon the Stern–Geary equation in Eq. 6 [121, 122]:

\[
I_{\text{corr}} = K \frac{\Delta I}{\Delta E},
\]

where corrosion current \( I_{\text{corr}} \) is deduced from corrosion test coupons [i.e. stainless steel (SS)], \( \Delta I \) is the change in current, and \( E \) is the change in potential. This is where the Tafel slope of \( K \) can be evaluated. There are limitations to this equation, with slight differences in the environment inflicting limitations in the accuracy of \( I_{\text{corr}} \) and \( K \). Therefore, it can only be achieved in specific environments but gives excellent results.

Linear polarisation can also be harnessed, measuring the resistance, and is a common electrochemical technique called linear polarisation resistance (LPR) [123]. LPR is an excellent chemical technique for measuring continuous rates of corrosion. Here, \( R_s \) (the solution resistance) between the corroding interface (i.e., the metal) and the reference electrode, and the polarisation resistance \( R_p \) is related to the corrosion rate. LPR requires a potential scan or stepping sequence that varies the probe’s potential concerning the corrosion potential \( E_{\text{corr}} \). The technique has been used across industrial sectors to monitor corrosion rates, which was historically constrained to aqueous environments. More recently, probe technology has advanced, and high resistance and atmospheric exposures can now be monitored [124]. Habib and Bourelo developed an optical sensor for the active detection of corrosion in 304 and 316 SS. This was achieved by basing the metre on 3D holographic interferometry to measure micro-surface dissolution, i.e., mass loss. This was principally achieved through LPR [125].

#### 3.8.2 Electrochemical Impedance Spectroscopy (EIS)

EIS has been applied to corrosion systems for over 30 years and is now a powerful tool for corrosion analysis [126, 127]. An advantage of using EIS over other laboratory techniques is the leeway of using small-amplitude signals without disturbing properties being analysed. EIS studies the system
response to the application of a periodic small-amplitude ac signal. EIS can determine the polarisation resistance \((R_p)\), which is related to the rate of corrosion. EIS also provides a solution resistance \((R_s)\) and mechanistic information and adds extrapolating to a steady-state condition. This is beneficial as it allows for improved corrosion rate estimations. Guo et al. compared weathering resistance steels and weathering steels that are contained in performance bridge steel [128]. EIS results showed that rust layers of the resistant steels were more significant than the weathering steels.

Wenger and co-workers investigated open-pit morphologies upon an AISI 430 steel electrode using EIS [129]. Pits were initiated by using a sulfuric acid solution containing sodium chloride, where it could be noticed that pit morphologies could be related to fluctuations in impedance data. Gabrielli and co-workers investigated pit propagation using EIS in iron [130]. The pit morphologies were different from Wenger et al., as they were initiated using a scanning electrochemical microscope method (SECM). This method brings about a means of generating localised and controlled amounts of chloride ions.

### 3.8.3 Electrochemical Noise

EN involves measuring the electrochemical transient noise produced by the corrosion process [131, 132]. This is accomplished by measuring the potential noise of the potential differences between two identical electrodes or by tracking current differences between two similar electrodes using a zero resistance ammeter [133]. EN was initially designed to monitor corrosion such as pitting and crevice. EN recognises conditions versus stability, i.e., low noise to random events of material’s lifetime. Mansfield and co-workers developed EN to monitor both the initiation and propagation of stress corrosion cracks [134]. They developed electrochemical impedance and noise measurements taken bi-weekly through remote control modem access at Key West, Florida, over 9 months of three polymers coated to SS. Analyses were performed in the time and frequency domains. Conclusions were drawn that through the electrochemical analysis, a polyurethane topcoat fared better than the latex coating [134]. All electrochemical methods are averaging techniques that work best when the chemical and electrochemical conditions on the metal surface are uniform and at steady state, this is important when understanding MIC on the surface of a material.

### 3.9 Spectroscopic Techniques

The continuous proliferation of new bacterial species presents microbiologists with an ongoing typing challenge. Although molecular biology techniques are essential for bacterial typing, they are often laborious, time consuming, and inevitably fail when dealing with very closely related organisms. When paired with chemometric approaches, spectroscopic techniques can be a feasible alternative to molecular methods in some instances, providing advantages in terms of analysis time and expense. For examples, a combination of ATR–FTIR and chemometrics revealed significant spectral differences below 1400 cm\(^{-1}\), which is frequently associated with phosphate and carbohydrates molecules [135]. PCA and HCA revealed three distinct clusters, each containing isolates from a single genus. Janbu et al. have used FTIRS and FTIR micro-spectroscopy to distinguish five Listeria species [136]. The performance rates of discrimination obtained were approximately 93 and 100%, respectively. The stepwise canonical discriminant analysis (SCDA) and partial least-squares regression discriminant analysis were used to obtain these findings.

As early as 1998, there were reports of FTIR being used to measure MIC by Hans-Curt Fleming and his team [137]. FTIR spectroscopy is suitable for the identification of microorganisms and presents a new addition to taxonomic and genetic characterisation methods. The FTIR analysis of bacterial isolates provides fingerprint spectra, allowing for more rapid characterisation of microbial strains and in complex matrices. When combined with the principles of attenuated total reflection (ATR), the FTIR–ATR technique can be used for the observation of biofilms forming directly on the interface of an ATR crystals such as germanium. Spectra can be acquired non-destructively, in situ and in real time. For example, Chapman et al. have recently been using FTIR and NIR systems to study microbial proliferation and reactions to antibiotics [138–141].

### 3.10 Mass Spectrometry (MS; Biochemical)

While many of the previous methods are used for imaging, MS can characterise complex molecular systems. More particular to corrosion analysis, secondary ion mass spectrometry (SIMS) has been used to determine the metal ion distribution in SS. Salaita (1995) reported using SIMS using a liquid metal ion source (LMIS) to achieve high-resolution chemical maps of the surface [142]. SIMS can yield images with high sensitivity and lateral resolutions of 100 nm or improved. The technique is primarily used for metal and inorganic materials. Severe limitations exist with SIMS, the first being that it is destructive to any organic surface layer, and second, extensive ionisation patterns make it challenging to analyse large molecules.

Matrix-assisted laser desorption/ionisation-mass spectrometry (MALDI-MS) was used to detect the polysaccharides produced in a complex biofilm composed of a mixed consortium of marine microbes. For example, corrosion of aluminium exposed to seawater caused by a marine fungus was observed, and the fungus was identified using
Suitable application of analytical techniques for both the detection and monitoring of MIC is critical to understand the mechanisms of microbial interactions with a given material and for selecting the correct inhibition, mitigatory, or control measures. This review provides the application of analytical techniques available for MIC measurement using ranges of methods such as electrochemical and surface analytical techniques. Conventional electrochemical techniques, such as corrosion potential ($E_{corr}$), redox potential, EIS, and micro-electrode techniques, are important for fundamental material and MIC studies. The emergence of an electrochemical quartz crystal microbalance has proved to be an important feature for the study of MIC. Microscopic techniques (SEM, ESEM, AFM, CLSM, confocal Raman microscopy) and spectroscopic analytical methods (FTIR, FTIR–ATR, NIR, and XPS) are also very important for the analysis of MIC and should be used in combination with methods. The heterogeneous characteristics of microbial consortia and use of special techniques to study their probable effects on the metal substrata are often difficult even without the issues of corrosion and material characterisation. The aim of this review is to motivate researchers to use a combination of new analytical techniques to perform practical measurements to generate meaningful data to calculate the impact of MIC and biofilms on materials. With this in mind, research will be able to progress in making more precision changes to materials to prevent MIC—for example, understanding the metabolite and often the biofilm features when interacting with a material will generate new approaches to mitigate the MIC issue, thus, producing new materials capable of resisting damage from microbes.

4 Conclusion and Future Perspectives

The long-term preservation of any material requires a holistic method for mitigation. Understanding the bio- and chemical environments that the material is immersed in will be a firm step in preventing and applying the correct countermeasures of fouling and biocorrosion. Without the aid of detection and monitoring, materials that are in contact with microorganisms will undergo rates of corrosion faster than ones that do. We have tried to capture the most important of these new trends in research and analytical methods of analysis.

Recent developments in the use of handheld systems will make it possible for online, site, and in-field measurements of MIC on materials. More attractive techniques using FTIR and NIR which are non-destructive will also play a key role in this area.

Electrochemical methods are still the gold-standard analytical method to measure the surface activity of kinetic and corrosion processes. The issue with electrochemical measurement is that the electrode surface tends to change because of the continuous electrode surface changes owing
to microbial adhesion and biofilm formation (biofouling). Noise analysis is the most typical method for the evaluation of MIC; however, its main disadvantage is the need for complex mathematical models to obtain real current and voltage values. Therefore, we recommend using tandem electrochemical techniques in order to see the corrosive interaction and deterioration of the material when measuring the MIC.

Biofilms formed in different environments which are measured either in situ, in the field, or in the laboratory on materials have been extensively investigated, and therefore, many techniques are already available for this reason. The methods of analysis provide qualitative measurements, while surface chemical techniques provide the quantitative material and composition changes caused by biofilm and MIC processes.

Fluorescence microscopy has opened the ability to visualise microbial cells on material surfaces. Once the appropriate dyes to stain the microbes are used, not only are all the microbes visible, but we also obtain information about the life cycle of the cell (e.g. live/dead). Supported by SEM, EDX can provide a quasi-3D image of single cells or colonies of cells as well as the EPS of the biofilm (although due to the preparatory process of SEM, this is typically dehydrated).

EDX can provide data on the change of composition caused by the presence of MIC. We have detailed many examples of this method for MIC determination, while the use of spectroscopic methods (FTIR and XPS) can explain why and how these methods support the understanding of MIC.

This review details many analytical techniques and explains the importance and relevance of parallel and combinatorial analytical techniques to obtain the information about MIC.

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Declarations

Conflict of interest The authors declare that there are no competing interests.

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