2017-12-15

Electrochemistry of Nanostructured Features on Steel Surface and the Applications

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Electrochemistry of Nanostructured Features on Steel Surface and the Applications

by

Yuan Li

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

GRADUATE PROGRAM IN MECHANICAL AND MANUFACTURING
ENGINEERING

CALGARY, ALBERTA

DECEMBER, 2017

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Abstract

Functionalization of pipeline steels through facile nano-techniques is valuable for industrial applications. In this research, the mechanistic aspects of steel corrosion at nanoscale has been studied in order to manipulate the development of corroded nanostructure on pipeline steel. High-performance nanocoatings capable of anti-bioadhesion and self-cleaning have been successfully developed on pipeline steels through facile electrochemical anodization methods.

When the X100 pipeline steel is either corroded or passivated in aqueous environments, the development of nanostructures on the steel surface highly depends on the early-stage corrosion behavior, where the thermodynamics and kinetics are affected by the conditions such as surface finish, electrolyte concentration, and electrochemical potential. The nanostructure on steel substrate shows a quick-response to changes of the conditions, either caused by exposure to corrosive electrolytes, or electrochemical potential. The surface features are under a non-equilibrium state lasting from hundreds to thousands of seconds, during which the corrosion processes of the steel were successfully characterized by topographic in-situ mapping through electrochemical atomic force microscopy. It is demonstrated that the corroded nanostructure on pipeline steel can be controlled through manipulating conditions in order to achieve various functions.

Nanostructured coating can be fabricated by anodization of pipeline steels in a concentrated alkaline solution. The nanostructure of the coatings can reduce the interactive force between microorganisms and the steel, resulting into anti-bioadhesion to sulfate-reducing bacteria (SRB) and *P. aeruginosa*. The photocatalytic property of iron oxides in the nanocoatings enables the release of toxic and oxidative reactive oxygen species (ROSs)
under light illumination, enhancing the anti-bioadhesion performance up to 99.9 % compared to bare steel. Further treatment by dipping ZnAc solution and annealing allows the formation of ZnFe$_2$O$_4$ in the nanocoating, improving the electrochemical stability of the nanocoating in corrosive environments while maintaining a high performance in anti-bioadhesion and self-cleaning of residual bacteria (up to 99.3 % of total coverage) on the steel.

*Keywords: Pipeline steel, Nanostructure, Corrosion, Anodization, Anti-bioadhesion, Photocatalysis*
Acknowledgements

I am incredibly grateful to Dr. Frank Cheng, my supervisor, for his unwavering support and encouragement throughout my Ph.D. years. He, as an excellent teacher and wonderful role model, has taught me so many invaluable lessons in the academy and enhanced my understanding of the world beyond academics, which will benefit my life all the time.

Thanks are also extended to the members of my group, Drs. Da Kuang, Qiang Li, Yuanchao Feng, Tao Liu and Shiqiang Chen, Yao Yang, Shan Qian and those whose names cannot all be listed here, for their help, valuable discussions, and close collaboration in this work. Sincere acknowledgment is also made to Dr. Cooper Langford of the Department of Chemistry and Dr. Gerrit Voordouw of the Department of Biological Science at the University of Calgary for permitting the use of photochemical facilities and biotesting equipment in their lab, respectively. I wish to express my deep gratitude to Dr. Michael Schoel, Anusha Abhayawardhana, Farbod Sharif and Josefina Perez Zurita de Scott for their sincere help in technical guidance.

This work was supported by Natural Science and Engineering Research Council of Canada (NSERC).
Dedication

To elders who led me

To age-likes who accompanied me
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# List of Symbols, Abbreviations and Nomenclature

<table>
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<tr>
<td>[OH(^-)]</td>
<td>The concentration of OH(^-) ions</td>
</tr>
<tr>
<td>'OH</td>
<td>Hydroxyl radicals</td>
</tr>
<tr>
<td>0-D</td>
<td>Zero-dimensional</td>
</tr>
<tr>
<td>1-D</td>
<td>1-dimensional</td>
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<td>2-dimensional</td>
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<tr>
<td>3-D</td>
<td>3-dimensional</td>
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<tr>
<td>A</td>
<td>The absorbance of XTT indicator measured at 470 nm</td>
</tr>
<tr>
<td>A(t)</td>
<td>The absorbance intensity after a period of illumination</td>
</tr>
<tr>
<td>A(_0)</td>
<td>The initial MB absorbance intensity</td>
</tr>
<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CE</td>
<td>Counter electrode</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscope</td>
</tr>
<tr>
<td>CPE</td>
<td>Constant phase element</td>
</tr>
<tr>
<td>CRA</td>
<td>Corrosion resistant alloys</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>(E_b)</td>
<td>Binding energy</td>
</tr>
<tr>
<td>ECAF M</td>
<td>Electrochemical atomic force microscopy</td>
</tr>
<tr>
<td>EIS</td>
<td>Electrochemical impedance spectroscopy</td>
</tr>
<tr>
<td>(E_k)</td>
<td>Kinetic energy</td>
</tr>
<tr>
<td>(E_{\text{photon}})</td>
<td>Photon energy</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>(F)</td>
<td>Faraday's constant</td>
</tr>
<tr>
<td>FAM</td>
<td>Formamide</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>Iron element with valence of two</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>Iron element with valence of three</td>
</tr>
<tr>
<td>Fe(^{2+})</td>
<td>Ferrous ions</td>
</tr>
<tr>
<td>Fe(_3)O(_4)</td>
<td>Magnetite</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscopy</td>
</tr>
<tr>
<td>Fig.</td>
<td>Figure</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>GDP</td>
<td>Gross domestic product</td>
</tr>
<tr>
<td>(\gamma)-Fe(_2)O(_3)</td>
<td>Maghemite</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck's constant</td>
</tr>
<tr>
<td>$i_{coor}$</td>
<td>Corrosion current density</td>
</tr>
<tr>
<td>$K_{sp}$</td>
<td>Solubility constant</td>
</tr>
<tr>
<td>$l$</td>
<td>The light path of cuvette</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>LPR</td>
<td>Linear polarization resistance</td>
</tr>
<tr>
<td>$m$</td>
<td>The number of sampling point on the y axis</td>
</tr>
<tr>
<td>M</td>
<td>Molar mass</td>
</tr>
<tr>
<td>MB</td>
<td>Methylene blue</td>
</tr>
<tr>
<td>MIC</td>
<td>Microbiologically influenced corrosion</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>$n$</td>
<td>The number of sampling point on the x axis</td>
</tr>
<tr>
<td>$O^{2-}$</td>
<td>Superoxide radical ion</td>
</tr>
<tr>
<td>OCP</td>
<td>Open circuit potential</td>
</tr>
<tr>
<td>OH$^-$</td>
<td>Hydroxyl ions</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Work function</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDM</td>
<td>Point defect models</td>
</tr>
<tr>
<td>$pO_2$</td>
<td>Partial pressure of oxygen</td>
</tr>
<tr>
<td>PSD</td>
<td>Power spectral density</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Take-off angle</td>
</tr>
<tr>
<td>$Q$</td>
<td>Passive film capacitance</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density</td>
</tr>
<tr>
<td>$R_a$</td>
<td>Average roughness</td>
</tr>
<tr>
<td>RE</td>
<td>Reference electrode</td>
</tr>
<tr>
<td>$R_f$</td>
<td>Film resistance</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>ROSs</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>$R_q$</td>
<td>Root mean square of roughness</td>
</tr>
<tr>
<td>$R_s$</td>
<td>Solution resistance</td>
</tr>
<tr>
<td>$S$</td>
<td>The surface area of the specimen exposed to solution</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated calomel electrode</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SRB</td>
<td>Sulfate-reducing bacteria</td>
</tr>
<tr>
<td>$t$</td>
<td>The illumination time</td>
</tr>
<tr>
<td>TA</td>
<td>Terephthalic acid</td>
</tr>
<tr>
<td>TA-'OH</td>
<td>2-hydroxy-TA</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>UV-visible diffuse</td>
</tr>
<tr>
<td>$V_m$</td>
<td>Molar volume of the substrate steel</td>
</tr>
<tr>
<td>wt. %</td>
<td>Weight percentage</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>XTT</td>
<td>2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide</td>
</tr>
<tr>
<td>ZnAc</td>
<td>Zinc acetate</td>
</tr>
<tr>
<td>$Z_{\text{range}}$</td>
<td>Height range</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>The absorbance coefficient of XTT-formazan</td>
</tr>
<tr>
<td>$\alpha$-$\text{Fe}_2\text{O}_3$</td>
<td>Hematite</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>Average change of the height of the substrate</td>
</tr>
<tr>
<td>$\Delta \varphi$</td>
<td>Change of electrode potential</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Excessive pressure resulted from local stress concentration</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>Time</td>
</tr>
<tr>
<td>$\Delta L$</td>
<td>Sampling distance</td>
</tr>
<tr>
<td>$\theta_E$</td>
<td>Emission angle</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
</tr>
<tr>
<td>$\lambda_e$</td>
<td>Mean free path of the electrons</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency</td>
</tr>
<tr>
<td>$N$</td>
<td>The number of data points</td>
</tr>
<tr>
<td>$f_x$</td>
<td>Spatial frequency in the x-direction</td>
</tr>
<tr>
<td>$f_y$</td>
<td>Spatial frequency in the y-direction</td>
</tr>
<tr>
<td>$y_i$</td>
<td>The height at the sampling position</td>
</tr>
</tbody>
</table>
Chapter One: Introduction

1.1 Research background

Since Richard Feynman introduced the concept of nanotechnology through the famous lecture, “There’s Plenty of Room at the Bottom” on December 29 1959 [1], the research and applications in areas such as nanomaterials, nanoengineering, nanomedicine, etc., have been booming for a half century, during which new fields are created and traditional industries are boosted. Along with nanoscale technologies came numerous noteworthy nanoscale materials, such as semiconductor quantum dots (the 1980s), and carbon nanotubes (1991), etc., all of which display promising nanoscale properties. The unique optical, interfacial, biological, chemical and electromagnetic properties of the nanomaterials are attributed to the nanostructures and composition. Applying nanotechnologies on conventional materials to achieve new functions, has been demonstrated to be a practical approach to develop innovative products, attracting increasing attention from researchers and industry communities.

Steels have been the most widely used engineering materials in current civilization due to their availability, economic benefits, and unreplaceable mechanical properties [2]. However, steels usually suffer from degradation by various mechanisms when exposed to aqueous environments. Particularly, corrosion and biofouling are two primary mechanisms resulting in detrimental effects on steel structures and facilities in a wide variety of industrial sectors, including oil/gas production and transportation, ships, aquaculture systems, heat exchangers, etc.[3, 4]. According to the current corrosion study in U.S., the direct cost of metallic corrosion is $276 billion on an annual basis, without the associated losses due to lost income and remediating expenses. This represents 3.1 % of the U.S.
Gross Domestic Product (GDP) [5]. For entire U.S. Navy fleet, it was estimated that the economic impact of biofouling was between USD $180 and 260 million per year [6].

At the same time, corrosion has been used to successfully fabricate nanostructures on metal substrates and foreign supports under controlled conditions [7]. This unique structure enables the metal new properties including anticorrosion and antifouling/anti-biofouling performance. Corrosion refers to the destructive attack of a metal through electrochemical reactions with the environments, causing disintegration of the metal into its constituent ions or further forms oxides or salts [8]. The processes, such as metallic dissolution and product precipitation, involved in corrosion can be used for nanofabrication. In a corrosion process where material dissolution is remarkable, i.e., the active corrosion system, the resulted nanostructure depends on the chemical and physical inhomogeneity of the materials over nanoscale. When product precipitation dominates the corrosion process, nanostructures can be achieved through manipulating conditions that control the nucleation and growth of corrosion products. Particularly, the naturally formed oxide layer on metals known as passive film is a 2-dimensional (2-D) nanostructure, which usually improves the chemical stability and mechanical properties of substrates. Anodization is a practical approach to enlarge thickness and tune nanostructure of oxide film, from which further functionalization becomes possible on conventional materials. However, the mechanistic relationship between corrosion conditions and the resulted nanostructure is yet to understand due to the complex interfacial processes and interacted parameters in corrosion.

To control facility failures induced by bacterial attachment and biofouling, high-performance coatings capable of anti-attachment of bacteria have been widely used due to their high efficiency and easy application [9]. However, biocides and heavy metal ions
contained in the coatings serving as the functional matters are usually toxic, impacting adversely the sustainability of environments and the ecosystem [10]. Novel surface engineering and nanotechnology have been developed for effective control of bacterial attachment and biofilm formation on metals. There are two main strategies, i.e., reducing the adhesive force between bacteria and the metal, and deactivating (killing) the attached bacteria by environment-friendly ways [11, 12]. In particular, deactivation (killing) of the attached bacteria by photocatalytic mechanism provides a potential alternative for surface engineering due to its environment-friendly mechanism for anti-microbial activity under light illumination [13-15]. However, most of the new coatings have been prepared on nonferrous metals such as aluminum and its alloys. There has been rare work conducted on pipeline steels, due to the high activity in various environments and chemical inhomogeneity of the steels, bringing with technical difficulty in nanostructure control.

To date, there has been limited work to develop effective coating technology capable of anti-bioadhesion and self-cleaning on pipeline steels. Development of anti-bioadhesion coatings with favored properties such as environment-friendly, easy or free of maintenance, long-lasting, facile preparation is expected to revolutionize the steel application with high reliability and sustainability.

1.2 Research objectives

The overall objective of this research is to study the mechanistic aspects of steel corrosion at a nanoscale scope, and to develop high-performance nanocoatings capable of anti-bioadhesion and self-cleaning on steels through facile electrochemical methods. Progress has been made in the following areas:
1) Investigate the mechanism and behavior of the early-stage corrosion of pipeline steels induced by nanoscale surface finishing.

2) Study the nanoscale topographic features of active corrosion and passivation of pipeline steel, and determine the corrosion mechanism based on the characterization of the evolution of topographic profiles.

3) Investigate the passive film growth on pipeline steel and its nanoscale structure at various passivating potentials.

4) Fabricate nanostructured oxide nanocoatings by anodization of pipeline steel for anti-bioadhesion.

5) Prepare nanostructured anodized nanocoatings on pipeline steel and characterize their photocatalytic property for anti-bioadhesion.

6) Enhance the applicability of anodized coatings doped with Zn for improved anti-bioadhesion activity and self-cleaning performance.

1.3 Main contents of the thesis

The thesis contains nine chapters, with Chapter One giving an overall introduction of the research background and objectives of the research.

Chapter Two contains a comprehensive literature review on nanoscale features, development of surface nanotechnology, and electrochemical nanofabrication for surface engineering, including methodology, mechanisms, characterization techniques, and applications, as well as the functions possessed by nanostructured metals.

Chapter Three investigates the early-stage corrosion behavior of a pipeline steel in a simulated soil solution through an *in-situ* electrochemical atomic force microscopy, and
determines the effects of nanoscale surface finishing on active corrosion and topographic evolution of the steel.

Chapter Four investigates the topographic evolution of active corrosion and passivation of a pipeline steel in bicarbonate solutions with various concentrations. The corrosion and passivation mechanisms of the steel in the corrosive environments are studied based on a topographic profile analysis.

Chapter Five investigates the growth of passive films and the evolution of the nanoscale features on a pipeline steel at various passivating potentials.

Chapter Six develops nanostructured oxide coatings through anodization of pipeline steel in concentrated sodium hydroxide solutions for anti-bioadhesion. The morphology, composite, structure, wettability, anti-bioadhesion, and electrochemical stability of the coatings are characterized and measured, determining the mechanism of the coating formation and its anti-bacterial property.

Chapter Seven develops nanostructured oxide coatings through anodization of pipeline steel and characterizes the photocatalytic property of the coatings for anti-bioadhesion.

Chapter Eight modifies the anodized nanocoatings by doping Zn for enhanced electrochemical stability in corrosive environments, while maintaining high performance in anti-bioadhesion and self-cleaning of residual attached bacteria.

Chapter Nine summarizes the main conclusions of this research, and suggests recommendations for the further work.
Chapter Two: Literature Review

2.1 Recognition of surface nanostructures

On December 29, 1959, physicist Richard Feynman gave his famous lecture entitled “There's plenty of room at the bottom”, introducing the idea of direct manipulating matter over atomic scale [1]. This event is considered as the birth of nanoscience and nanotechnology. Classification of nanomaterials can be made based on the number of dimensions, which are not confined to the nanoscale range (i.e., the range less than 100 nm) [16]. The material with all dimensions confined at the nanoscale, such as nanoparticles, the material is defined as zero-dimensional (0-D) nanomaterial. Similarly, the 1-dimensional (1-D) and 2-D nanomaterials refer to the ones that have one and two dimensions out of nanoscale, respectively. The typical 1-D nanomaterial includes nanowires, nanorods, and nanotubes, while 2-D nanomaterial takes the nanofilms as representative. Some sort of complex nanomaterials have abundant substructures if the higher dimensional nanomaterial is composed of relative lower dimensional ones, i.e. the nanofilm fabricated by nanofibers [17, 18], the nanocoating containing arrayed nanotubes [19, 20], the nanowire decorated by nanoparticles [21, 22] and the nanoshell engaged with nanoparticles [23, 24]. Conventional bulk materials may bear nanostructures on their surface when nanomaterials are applied directly or treated by specific methods, leaving the internal structure and property unchanged.

Nanostructures can occur naturally, chemically, mechanically, physically or biologically. Generally, there are two strategies to prepare nanomaterials: i.e., the bottom-up and the top-down approaches [16]. In the bottom-up approach, the units are obtained at the atomic level and then integrated into the desired material. Examples include the
abundant synthesis methods, which built nanomaterials from atom until the integrated clusters reach nanometer scale. The top-down approach starts with a bulk material at the macroscopic level followed by trimming of the material to the desired scale. The nanoengineering methods, such as etching and ball milling, are the examples of top-down approaches [25]. The art of the bottom-up approach is manipulating the physical and chemical processes, i.e. nucleation, growth, and phase transition, through controlling the thermodynamic and kinetic parameters such as temperature, concentration, pH, potential, et al. [26]. At first, the pristine atoms are dispersed in specific media, producing gaseous or liquid solutions. The simplest way to trigger phase transition is to change the temperature. When the temperature drops to that below the solubility limit of a compound, precipitation occurs. This is the main principle of the hydrothermal method [27] and physical vapor deposition [28]. If chemical reactions occur during the temperature change, chemical method such as the chemical vapor deposition is a typical example. Moreover, changes in concentration and potential are associated with the sol-gel methods [29] and electrochemical methods [30]. Generally, the bottom-up approach is able to produce homogeneous nanomaterials with tunable structures, but it requires a large bulk of dispersive media (such as organic solvents, inert gases, etc.) to maintain a diluted precursor avoiding the nucleation-growth process out of control. For the top-down approach, the industrial nanoparticles are usually produced through ball-milling of bulk materials because of the zero waste and high efficiency [31]. Since the process is not so sophisticated, the nanomaterial has irregular shapes and bear with crystal defects. Contaminations from balls are unavoidable in final products.
Due to the unique structure, nanomaterials possess the distinctive physical, chemical, and biological properties compared to their bulks. The surface-to-volume ratio is highly improved by nanostructures, which benefits the properties relaying on the interfacial process since more absorption sites and shorter diffusion distance are available. As a result, nanostructured metal oxides (such as Al [32-35], Ti [13, 36-38], Zn [39-41] and Fe [19, 39, 42-44] oxides) and noble metals (i.e., Pt [45, 46], Au [47], Ag [48] and Ta [49]) have been paid much attention recently due to their extensive applications in catalysis, biosensors, photoelectrochemical electrodes, environmental remediation, etc. In addition to innovative applications offered by the nanomaterials, nanotechnology enables conventional bulk materials, including carbon steels [44, 50-54], stainless steels [55, 56], aluminum alloys [57] and copper alloys [58], to align with novel applications by creating nanostructures by either bottom-up or top-down approaches. The fabricated nanostructures on the material surface can be the same chemical composition as the internal of the bulk or not, highly depending on the methods adopted. Consequently, the preparation mechanism of nanostructured surface and achieved functions vary.

2.2 Electrochemical mechanisms for developing nanostructure on metals

Electrochemical methods can be used as either bottom-up or top-down approaches since the atomic behavior is able to be tuned by electrochemical controls, i.e., manipulating potential or current, to achieve directional assembling and selective dissolution at the nanoscale. Based on the direction of the controlled current getting through electrodes, the electrochemical methods for fabricating nanostructures are classified as anodization, electrodeposition, and corrosion. Anodization describes a process happening on the bulk
conductive material served as an anode, which connects to the positive terminal of a power supply and loses electrons during the reaction. Reversely, the electrodeposition refers to the accumulation of reduced matters on the cathode, which connects to the negative terminal of the power supply. Corrosion of bulk materials occurs at the corrosion potential in specific environments without any external electrochemical controls.

2.2.1 Corrosion

Metal corrosion occurs in an electrochemical cell. It involves two half-cell reactions, i.e., anodic reaction and cathodic reaction [2]. If the metal is immersed in an electrolyte, both reactions occur simultaneously on the same bulk material but different areas. The metals lose electrons at the anode when they are oxidized to produce ions in a corrosive environment (i.e., M → M^{n+} + ne\(^{-}\)). Species at the cathode consume electrons while they are reduced (e.g., O\(_2\) + 2H\(_2\)O + 4e\(^{-}\) → 4OH\(^{-}\), 2H\(^{+}\) + 2e\(^{-}\) → H\(_2\), or M\(^{n+}\) + ne\(^{-}\) → M). Therefore, metal corrosion can be defined as a destructive attack of a metal through electrochemical reactions with the environment, causing disintegration of the metal into its constituent ions or further forms oxides or salts [8]. The corrosion of metals is affected by many factors including oxygen content, ion concentration, pH, temperature, etc. since they determine the thermodynamic and kinetic processes of corrosion reactions. The naturally occurring metallic corrosion in an atmospheric or aqueous environment can result in nanostructures on the metal surface, but the process is very difficult to predict and control. Thus, the naturally occurring corrosion is less used for nanofabrication. In practice, controllable environments need to be designed and developed to corrode metals to achieve reproducible nanostructures. The rate of metal dissolution is at varied orders of magnitude, which
determine the way of corrosion used in fabricating nanostructures. When a metal is in the active state in a corrosive environment, the corrosion rate is higher in comparison to the passive condition.

2.2.1.1 Active dissolution

Active dissolution causes a continuing loss of metals in the environment. The easiest way to identify the corrosion phenomenon is through the topographic change on the surface. Since there is no electrochemical control applied, the corrosion process and resulted nanostructure are dependent on the properties of the metals and the environments.

Formamide (FAM) aqueous solutions have been successfully used for nanofabrication on metal substrates, e.g., copper [59], zinc [60, 61], and cadmium [62]. In the corrosive environment, metal ions are slowly released from the bulk surface through anodic reaction: $M \rightarrow M^{n+} + ne^-$. The generated electrons are consumed by cathodic reaction in aerated solution: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$. The oxygen involved in the cathodic reaction comes from the atmosphere since the solutions are usually open to air without control of oxygen content. Once released from anode, the metal ions are immediately captured via coordination with FAM to form metal complex ions $([M(FAM)_4]^{n+})$ [63], where formamide may easily be replaced by the cathodically reduced $OH^-$ resulting in hydroxide nanostructures on the substrates. Fig. 2.1 shows schematically the formation of copper hydroxide nanostructures on copper surface by corrosion of the copper substrate in a FAM solution. Like other bottom-top approaches, the corrosion-induced deposition process also enables the assembling of nanostructures on bulk surface through continues supply of metal ions and transport of metal complexes to substrates. The nanostructures are tuned through
the concentration of formamide, temperature and time. Since the precise control of surface nanostructures usually requires a homogeneous nucleation and constant diffusion rate of ions, the corrosion process is supposed to maintain at a low rate. Thus, this method is conducted at low temperatures (< 90 °C), and a prolonged time period (several days to weeks).

Fig. 2.1 Schematic diagram illustrating of the formation of copper hydroxide nanostructures on the copper surface through corrosion of copper substrate in a formamide aqueous solution [63].

Sometimes, other organic or inorganic matters, e.g., amino acid, glycine, ammonium persulfate, and ammonia, are added into formamide system, to obtain nanoflowers,
dendritic nanostructures, nanorods, and nanotubes [64, 65]. The atoms interact with the introduced particles when assembling on nuclei, allowing various growth rates along lattice orientations and ending up with different nanostructures. In addition, researchers have used metal corrosion in the presence of long-chain fatty acids for constructing a superhydrophobic surface, where the nanostructures composed of metal-organic complexes can reduce surface energy significantly, complementary to traditional approaches using hydrophobic polymers or self-assembled monolayers [66]. The dissolved salts of noble metals are usually corrosive to active metals, resulting in the dissolution of active metals. With the cathodic reaction of $M^{n+} + ne^- \rightarrow M$, the noble metal ions are cathodically reduced and deposited on the active metal substrate. For example, hierarchical gold nanostructures with a three-fold symmetry have been prepared on zinc substrates via the reaction of metal zinc with HAuCl$_4$ in ionic liquids [67].

In addition to designing solution ingredients, the preferential dissolution of metallic substrate over nanoscale can be used to fabricate nanostructures as well. The porous nanostructure can be achieved through dealloying of designed alloy systems in aggressive environments, where the less noble component of alloys is selectively dissolved, leaving the nanostructured noble metal [68]. For the dealloying process to occur, there must be a significant difference between the corrosion potentials of the two metal components in the alloys. Meanwhile, the concentration of the less noble component must also exceed a critical atomic percentage, known as a parting limit, to allow the formation of two phases. The Raney nickel used for organic syntheses are produced through the alkali treatment of bulk nickel-aluminum alloys, from which most of aluminum is dissolved out [69]. The porous nanostructure left behind has a large surface area, resulting in a high catalytic
activity. Furthermore, the alloy systems such as Cu/Pt, Mg/Cd, Ag/Au and Zn/Cu have been used to prepare a variety of nanoporous metals [70, 71].

Many alloys contain a variety of intermetallic compounds which usually show a higher corrosion resistance than other metal components. The inhomogeneity of the metal substrate over nanometer scale promotes the development of nanostructures during corrosion. For example, the widely used carbon steel is actually composed of iron and cementite (Fe₃C). The latter is hard and brittle, and normally classified as a ceramic. When exposing eutectoid steels to aerated NaCl solution, the iron contained in the steel is preferentially dissolved, with the cementite left. The resulted morphology is paralleling cementite laminae arrayed with intervals of nanometers [72], as shown in Fig. 2.2.

![AFM images of the eutectoid steel surface exposed to aerated 0.05 M NaCl solution for 2 h](image)

Fig. 2.2 AFM images of the eutectoid steel surface exposed to aerated 0.05 M NaCl solution for 2 h [72].
In addition to the chemical inhomogeneity involved in metallic substrate, the metallurgical and mechanical defects that usually perform high activity can cause the preferential dissolution of substrate at nanoscale. Grain boundary is a 2-D metallurgical defect with a thickness of several atomic layers, where the disorder of lattice structure and element impurities enhances the electrochemical activity. Generally, the corrosion thermodynamic process of steels in aqueous media is enhanced with the increase of boundary density, but the kinetic aspect depends on corrosion environments. The carbon steels with nano-grained surface has a higher general corrosion rate, but the localized corrosion is reduced [73]. It has been demonstrated [74] that the grain and grain boundary of the steel are anodic and cathodic, respectively, in a near-neutral pH solution. The increase of boundary density raises the area ratio between cathode and anode, and thus enhances the dissolution rate of grains in a corrosive environment. Mechanical deformation caused by surface finish is usually within nanometer scale. The residual stress concentration increases the localized concentration of dislocation on substrate, and thus the preferential dissolution is expected to occur at the nanofeatures left by mechanical surface treatment. In a previous study simulating the pipeline corrosion in underground water, it was found that the early stage corrosion rate increases with the surface roughness caused by finishing [75].

2.2.1.2 Passivation

Passivation is defined as the reduction in chemical or electrochemical activity of a metal due to the reaction of the metal with surrounding environments to form a protective film on the metal surface [76]. The protective film is known as passive film. The passive film
could be composed of any metallic ores, but usually oxides in most environments. Oxide films on metals are often (but not always) very thin within the scope of 2-D nanomaterials. The transitional metals (e.g., Fe, Cr, Co, Ni, Mo, Ti) and their alloys (e.g., Fe-Cr stainless steels, Ni-based alloys, etc.) tend to have thin passive films, which are several to tens of nanometers in thickness. The non-transitional metals (e.g., Al, Cd, Mg, Pb) can have much thicker passive films, which are hundreds to thousands of nanometers in thickness. The noble metals (e.g., Pt, Au) have much thinner oxide layers, which can be an atomic monolayer with a thickness less than 1 nanometer.

The electrochemical properties of passive films depend on the compositional oxides. According to Pourbaix diagram [77], which maps possible equilibrium phases of an aqueous electrochemical system in respect with pH, ion concentration and potential, one can manipulate passive films. For examples, iron or carbon steels show a stable region of iron oxide over high pH and high potential ranges. Oxidizing inhibitors such as chromate solutions (of neutral or basic pH) can confer passivation on carbon steel substrate, forming an iron oxide film [78]. The chromate inhibitor passivates the iron surface through raising the corrosion potential into the stable region of iron oxides.

Passive film is a nanocomposition of layers of oxides containing a large amount nanoscale defects, rather than an intact homogeneous film. Based on the extensive investigations [79, 80], the passive film on steels consists of an inner layer of Fe$_3$O$_4$ adjacent to the metal and an outer layer of $\gamma$-Fe$_2$O$_3$. In addition, there is an outermost thin layer of hydroxyl groups ($\gamma$-FeOOH) which is resulted from the aqueous surrounding and usually consists of only one to several monolayers much thinner than the oxide film itself [81]. It was found that there is no exact interface between inner and outer layers. The nature
of passive film is a single phase with a gradient distribution of ion and oxygen atoms from
the inside out. The inhomogeneous passive film contains a substantial number of 0-D
nanostructures, e.g., vacancies and interstitials, and higher dimensional nanostructures, e.g.,
finito crystallite size and stacking faults [82]. Moreover, the ingredient of a passive film
highly depends on the elements and their concentrations in the metal substrate. Chromium
is naturally passive when exposed to the outdoor atmosphere and remains bright for years.
It is well known that the introduction of 13 at. % Cr into iron passivates the binary iron-
chromium alloy, as known as stainless steel, with a film mixed with Cr2O3 and iron oxides.
The designing of corrosion resistant alloys is to adjust beneficial elements, e.g., Ni, Mo,
Ti, etc., in the substrate to improve the chemical and structural stability of the resulted
passive film [83].

The inhomogeneity of metallic substrate affects the kinetic of passive film forming on
it through improving metallic oxidation and mass migration [82, 84]. The high density of
grain boundary is demonstrated to enhance passive film stability and re-passivation kinetics
in electrolytes that promote passivity [85]. Take the metallic nanocrystal as an example,
the resulted in passive film tends to have finer grains and more compact substructures
comparing to the passive film forming on an annealing metal that usually is composed of
micron-sized grains [86]. When the passive film is destabilized by chloride ions, the
corrosion behavior is similar to the scenario of active dissolution [87]. The mechanical
surface treatment can transform metallic surface into a thin layer of the nanocrystal, and
thus improve the corrosion resistance based on the mechanism mentioned above [88].

In addition to corrosion control, passive films are designed to achieve more functions.
It has been demonstrated that refining crystal grains to nanoscale is able to form a stronger
passive film, and thus improves the corrosion resistance [89]. Development of nanostructures and metallic oxides on the substrate can reduce the adhesion of inorganic and biologic contaminations [90]. Moreover, the passive films usually grow up with the coordination of the pristine lattice structure of metallic substrate, ensuring good adhesion to the surface[84]. Therefore, the passive film can be utilized for further surface engineering.

2.2.2 Anodization

Anodization is an electrolytic passivation process used to increase the thickness of the natural oxide layer on the surface of metal parts [91]. The part to be treated forms the anode of an electrical circuit by connecting to the positive terminal of an external power supply. Both the anode and cathode (e.g., an inert carbon rod connecting to the negative terminal of the power supply) are submerged in an aqueous electrolyte bath. The power supply is used to apply appropriate potential differences between the two electrodes. Electrons are withdrawn from the anode, and the oxidized metal ions react with electrolyte to form an oxide layer on the surface. The electrons travel through the power supply to the cathode, where they typically reduce protons from the solution, forming H₂. In theory, all the oxide films naturally formed on transitional metals and non-transitional metals can increase thickness through this approach, by which the chemical composition and structures may vary with the electrochemical parameters (e.g., potential and current density) and electrolyte conditions (e.g., ingredient, temperature, viscosity, etc.).
2.2.2.1 Film growth models

Anodization is typically performed using either constant voltage (potentiostatic) or constant current (galvanostatic) conditions. Based on the point defect models (PDM) [92], the metal is potentiostatically anodized for enough time to allow the current pass to reach a steady-state, the oxide thickness obtained will be proportional to the potential applied during the anodization. Take the formation of oxide film on the iron surface as an example [93], the iron atoms are gradually oxidized into Fe(II) and Fe(III), and dissolved into electrolyte, as shown in Fig. 2.3. The Fe(II) and Fe(III) usually co-precipitate on the substrate in the form of Fe₃O₄ to form a passive film. The oxide film grows not by precipitation of soluble species, but by solid-state growth: (a) generation of Fe(II), and injection of electron holes (or Fe(III) sites) at the metal/oxide interface; (b) migration of electron holes (or Fe(III) sites via charge exchange between Fe(III) and Fe(II) through the Fe₃O₄ matrix) towards the γ-Fe₂O₃ oxide/aqueous interface; and (c) injection of O²⁻ into the oxide matrix or dissolution of Fe(III) species at the oxide/solution interface. The film is thus a mixed Fe₃O₄/γ-Fe₂O₃ oxide, closer to Fe₃O₄ in the inner regions and to γ-Fe₂O₃ in the outer regions. The average Fe valence (i.e., Fe(III) to Fe(II) ratio) in the Fe₃O₄/γ-Fe₂O₃ oxide phase increases with an increase in potential. Since the rates of processes (b) and (c) depend on potential and the electric field across the oxide film, the extent of conversion relies on the film growth potential. As the conversion approaches completion, the rate of (b) will slow down, as passivation limits further film growth. The growth reaches an equilibrium when the transport flux of charged species through the oxide layer is equal to the dissolution rate at the oxide/solution interface.
Fig. 2.3 Mechanism for surface oxide film formation/conversion processes on carbon steel at pH 10.6 and room temperature. The arrows indicate the directions of charge transfer and MV represents cation vacancies [93].

Fig. 2.4 Oxide morphologies that can be formed during electrochemical anodization of metals [94].

The anodized oxide films are usually porous rather than a compact structure of the naturally formed passive film, since there is a competition between oxide formation (Process (a)) and oxide dissolution (Process (b)) when a high potential getting through the
film. Porous oxides can be further subdivided into three specific cases: random porous oxides, oriented porous oxides, and oriented tubular oxides, as shown in Fig. 2.4.

2.2.2.2 Parameters affecting the nanostructures

Tuning nanostructures during anodization is to balance the film growth and film dissolution through controlling thermodynamic and kinetic parameters. Since parameters such as temperature, ion concentration, potential, and pH have a complex influence on both growth and dissolution of the oxide film, and they usually interact with each other resulting in unpredictable results, the design of nanostructures through anodization is mostly experiential.

The nanostructure of oxide films obtained through anodization is highly dependent on the electrolyte used in process. Since the fluorides are highly dissolvable in aqueous solutions, fluoride containing electrolytes have been widely used to develop nanostructures on transitional (e.g., Fe, Ti, Zr, Nb), non-transitional metals (e.g., Al), and alloys (e.g., TiAl, TiNi, stainless steels) [95]. The first generation of electrolyte is hydrofluoric acid solution or acidic mixture. The formed oxide layer is composed of arrayed nanotubes with various diameters, showing a limited thickness not more than 500-600 nm. By using buffered neutral electrolytes containing NaF or NH₄F and taking into account the importance of pH gradient within the tubes [96], self-organized nanotube layers with a thickness higher than 2 μm could be grown. The third generation refers to the organic solvent based electrolytes (water free or less). Nanotubes longer than 7 μm have been achieved in fluoride containing glycerol electrolytes, and the ones with a diameter less than 20 nm have been prepared in fluoride containing acetic electrolytes [97]. Recently, in aged
ethylene glycol electrolytes and by a further optimization of parameters, the nanotube length has reached 260 μm and the tubes have an almost ideal hexagonal arrangement [95].

Electrolytes containing perchlorate [55], halide ions [82], hydroxides [52, 53] were also reported to successfully prepare nanostructured oxide films on metals. However, the homogeneity of nanostructures is not comparable to that obtained in fluoride containing electrolytes, and the obtained structures are usually random porous or oriented channeled rather than oriented tubular. Those electrolytes usually require aggressive conditions (e.g., high ion concentration, high potential/current density, etc.) to grow films rapidly, which makes the nanostructures not easily tunable. Relevant reports are much fewer than those of fluoride-containing electrolytes, and thus the mechanism of developing nanostructures by those methods is yet to understand.

Nanostructures build up from the pristine topography of metal surface during anodization. The random topography results in disordered nanostructures at the initial stage of film growth. For the thicker film, the disordered portion is at the outermost surface of the nanostructured film, as nanostructures occur below the outer surface. Those disordered structures can be overcome by initiating film growth on an appropriate template. The ordered template can be imprinted into the metal substrate through surface finish or lithography, but is more commonly formed by a preparatory anodization step [56]. After the first anodization, disordered structures are removed from the surface, leaving a dimpled template, where the ordered structures are developed at the second anodization step.
2.2.3 Electrodeposition

Electrodeposition is normally conducted on cathode surface which is connected to the negative terminal of an external power supply. As same as other electrochemical approaches, both cathode and anode are submerged in electrolyte bath during electrodeposition. The power supply extracts electrons from anode and transfers them to cathode, reducing metallic ions or positively charged particles and depositing their reduced products on substrate surface, i.e., $M^{n+} + n e^{-} \rightarrow M$. The compositional particles of a coating, e.g., metallic ions, fillers, and pigments, are suspended or dissolved in electrolyte, in which complex compound precursors and organic solvents are widely used to maintain stability. The anode is made of inert material (e.g., carbon and noble metals) or the metal composing coating on cathode, where the reactions occur as $M \rightarrow n e^{-} + M^{n+}$ and $4OH^- \rightarrow 4e^- + O_2 + 2H_2O$, respectively. The nucleation and growth of nanostructures during electrodeposition are highly dependent on electrochemical parameters. Since the conventional direct current (DC) controlling approach is hard to tune deposition over nanoscales, the alternating current (AC) controlling approach or appropriate templates have been successfully applied in nanofabrication [98, 99]. Except for metallic coatings, depositing nanoporous metallic oxides on conductive substrates has been achieved through adding oxidative agents such as $H_2O_2$ into electrolyte [44].
2.3 Techniques for characterization of nanostructures

2.3.1 Morphology and topography

2.3.1.1 Atomic force microscopy (AFM) and electrochemical atomic force microscopy (ECAFM)

The AFM is able to image the 3-D topography of a specimen by scanning its surface with a sharp probe [100]. The probe is supported on a flexible cantilever and its tip gently touches the specimen surface. The controller system records the small force between the probe and the surface by measuring the deflection of the cantilever. For the popularly used contact mode, the force between the probe and the specimen remains unchanged during imaging by maintaining a constant deflection of the cantilever. If the AFM is configured as tapping mode, the cantilever makes intermittent contact with the specimen surface in a resonant frequency (normally hundreds of kHz). The interaction between the probe tip and the surface could reduce the amplitude of cantilever when the tip approaches the surface. The surface topography is measured by maintaining a constant cantilever amplitude during scanning.

The addition of an electrochemical cell and a potentiostat to AFM results in the development of the ECAFM that allows the simultaneous electrochemical control/measurements and topographical imaging. In ECAFM characterization, either the potential or current to/from the probe tip or substrate is controlled, but not both. In most cases, the potential of the substrate is manipulated, and the topography of the substrate is measured and analyzed. The generic ECAFM usually adopts a design shown in Fig. 2.5 to avoid hindering the free movement of the AFM scan head. A three electrodes cell is used to achieve a precise control of potential or current on the working electrode, i.e., the
substrate material under study, with respect to the reference electrode. Some quasi-reference electrodes, e.g., platinum wire, silver wire or silver wire coated with silver oxide [101], can also be used instead of the traditional reference electrodes (e.g., saturated calomel electrode, SCE) in specific aqueous environments, compromising with the limited space in the cell and potential contamination from salt bridge. The counter electrode is used as current source or sink. The ECAFM scanner controls the scanning of piezo to sense the substrate topography with respect to the position signal of laser reflected by the cantilever, which is similar to the principle of generic AFM. The scanner is sealed with rubber, with a glass laser window to avoid damage of electric circuits in aqueous environments. Moreover, both the contact and tapping modes are practicable for the in-situ ECAFM characterization in an aqueous environment. The versatile functions enable mechanistic studies of nanostructures and nanocomposition in a direct and quantitative way.

Fig. 2.5 Schematic model of an ECAFM cell with scanning stage [102].
Nanostructures can be analyzed through either visual or statistical approach. The topographic data measured by AFM are used to create 3-D images depicting the exact shape of nanostructures through software. The fluctuation of a surface can be evaluated through roughness, which has several definitions in respect to mathematic expression. The average roughness ($R_a$) is the most widely used one because it is a simple parameter to obtain when compared to others. The average roughness of a surface is described as [103]:

$$R_a = \frac{1}{mn} \sum_{l=1}^{m} \sum_{i=1}^{n} |y_{il}|$$

(2.1)

where $y_{il}$ are the height at the sampling position, and $n$ and $m$ are the numbers of sampling point on the x and y axis, respectively. Thus, the $R_a$ is the arithmetic mean of the absolute values of the height of the surface profile or area. The root mean square (RMS) of roughness ($R_q$) is a function that takes the square of the measures. The RMS roughness of a surface is similar to the roughness average, with the only difference being the mean squared absolute values of surface roughness profile [103]:

$$R_q = \sqrt{\frac{1}{mn} \sum_{l=1}^{m} \sum_{i=1}^{n} (y_{il})^2}$$

(2.2)

Unlike the above two methods that can only provide the information of surface height, the power spectral density (PSD) is a complementary analysis that gives information related to parameters of the height and spacing. The PSD links the Fourier Transform (FT) with the RMS roughness. Thus, it is able to perform a decomposition of the surface information.
into its spatial wavelengths, and allows comparison of the roughness at different frequency ranges. The mathematic description is shown [104]:

$$PSD(f_x, f_y) = \frac{\Delta L^2}{N^2} \left\{ \sum_{m=1}^{N} \sum_{n=1}^{N} h_{mn} e^{-2\pi i \Delta L (mf_x + nf_y)} \right\}^2$$  \hspace{1cm} (2.3)

where $N$ is the number of data points per line, $h_{mn}$ is the profile height at position $(m,n)$, $f_x, f_y$ are the spatial frequency in the x- and y-directions, and $\Delta L$ is the sampling distance.

2.3.1.2 Scanning electron microscopy (SEM)

SEM is employed for characterization of surface morphology. In SEM, electrons are generated by a field emission source and accelerated in a field gradient under a vacuum. The electron beam is focused by electromagnetic lenses onto the sample surface. Once the electron beam strikes the sample, electrons having various energies are emitted from varied sample depths. The electrons emitted from regions close to the surface (secondary electrons, 50 nm deep) can give morphological information, by comparing the intensity of these secondary electrons to the pristine electrons [105].

Unlike SEM, in which electrons emission is induced thermally, a field-induced emission is used in the case of field emission scanning electron microscopy (FESEM). For the thermal induced emission of electrons, electrical current heats the filament to a certain temperature (based on the filament material), at which the electrons are excited from filament. In the field emitter, the filament is placed in a huge electrical potential gradient ($> 10^7 \text{ V/cm}$) to emit electrons (cold emitter) [106]. Typically, the field emitters are capable
of producing a high primary electron brightness and a small spot size even at low accelerating potentials, and thus, achieving a relatively higher resolution than the conventional thermal emitters.

2.3.2 Composition and structure

2.3.2.1 X-ray photoelectron spectroscopy (XPS)

XPS is a powerful chemical analysis technique for nanofilms, because it collects information confined in the thickness less than 10 nm. When an X-ray photoelectron with a certain energy ($E_{\text{photon}}$) beams on the sample surface, the orbital electrons of the sample material will be excited and ejected. By measuring the kinetic energy ($E_k$) of the ejected photoelectrons, the binding energy ($E_b$) of orbital electrons is calculated (also using the work function ($\phi$) of the instrument) by [107]:

$$E_b = E_{\text{photon}} - E_k - \Phi \quad (2.4)$$

The $E_b$ values give information about the atomic concentration, oxidation state, and the environment around each element (e.g., the electronic properties).

The depth from which the X-ray photoelectron can be emitted depends on the mean free path of the electrons ($\lambda_e$) in the material and the emission angle ($\theta_E$) [107]:

$$\text{Depth} = \lambda_e \cos \theta_E \quad (2.5)$$
Changing the emission angle from $0^\circ$ to near $90^\circ$ results in a significant decrease in the analysis depth (i.e., the depth from which the photoelectrons emerge) and more chemical information confined at the surface is expected to be obtained. By analyzing a sample as a function of the emission angle, i.e., the depth into the sample, the material distribution within the sample and the relationship between surface and bulk chemistry is obtained.

2.3.2.2 Raman spectroscopy

Raman spectroscopy has been widely used to identify phases in either amorphous or crystalline materials. The principle of Raman spectroscopy was discovered by Sir Chandrasakara Raman in 1921. It was found that the diffusive radiation from the light source was "accompanied by a modified scattered radiation of degraded frequency" [108]. Nowadays, Monochromatic light sources, such as a laser, have been used in Raman spectroscopy to focus on a specimen, where electrons in the material are excited to a "virtual" state from the ground electronic state. As the electrons relax back to their ground state, a photon that is either Rayleigh or Raman scattered is emitted.

Rayleigh scattered light is the elastically scattered light that returns to its original ground state and releases a photon that has the same wavelength and energy as the incident beam. However, Raman scattered light is inelastically scattered, of which it either has a lower energy and longer wavelength (Stokes radiation) or it has a larger energy and a shorter wavelength (anti-Stokes radiation). The energy change is related to the vibrational energy levels in the ground state of the molecule, and as such, the observed Raman shift of the Stokes and anti-Stokes features are a direct measure of the vibrational energies of the molecule.
Scattered light that returns through the objective lens is filtered to remove Raleigh scattered light, which has a high intensity. The beam then passes through a monochromator and the Raman shifted radiation is detected with a charge coupled device (CCD). The Stokes and Anti-Stokes lines are equally displaced from the Raleigh line. The intensity of the anti-stokes lines is typically lower. Since the electrons would need to be induced into an excited state prior to measurement in order to produce a higher intensity at the Anti-Stokes lines, more intense Stokes spectra are used in Raman spectroscopy. The fingerprint peaks in spectra can be utilized to determine the chemical composition and quantify the constituents.

2.4 Properties of nanostructured surface on metals

Creating nanostructures on the metal surface usually alters the chemical composition and physical and chemical properties, improving pristine properties of the substrate or developing versatile functions that are not possessed by raw materials.

2.4.1 Photocatalysis

Photocatalysis refers to the chemical reaction induced or accelerated by a suitable photocatalyst through absorption of light energy. The photocatalysts are usually semiconductors, e.g., metallic oxides. Photocatalysts can promote reactions in the presence of light without being consumed in the overall reaction. Good photocatalysts should be photoactive, biologically and chemically inert, able to utilize ultraviolet (UV) and visible light, inexpensive and non-toxic. The schematic illustration of the principle of photocatalysis is shown in Fig. 2.6 [109]. Irradiation of a semiconductor structure with
light possessing an energy greater than or equal to the band gap of the material results in jumping of an electron from the valence band to the conduction band. The photoexcited electron leaves behind a positive hole in the valence band. The pair of the valence band hole and the conduction band electron is referred to as an electron-hole pair. Following formation, the charge carriers may recombine in the bulk material, dissipating the energy as heat or light. They may also move (via diffusion or migration) to the solid-liquid interface, reacting with absorbed species directly or indirectly. If oxygen is dissolved in water, it can capture the electron in the conduction band to form the superoxide radical ion (O$_2^{-}$), while the valence band hole can react with surface-bound water molecules or hydroxide ions to produce hydroxyl radicals (·OH). The radicals may induce secondary reactions with other matters in the electrolyte. Normally, the nanostructured photocatalysts would reduce the recombination of electron-hole pairs and favor the adhesion of charge scavengers, resulting in improvement of photocatalytic activity.
Hematite, or $\alpha$-Fe$_2$O$_3$, is a promising candidate for photocatalytic applications due to its narrow band gap of about 2.0-2.2 eV. Further, hematite absorbs light up to 600 nm, collects up to 40% of the solar spectrum energy, is stable in most aqueous solutions (pH > 3), and is one of the cheapest semiconductor materials available [110]. However, $\alpha$-Fe$_2$O$_3$ materials have a poor electron mobility, generally in the range of 0.01 cm$^2$/(V·s$^4$) to 0.1 cm$^2$/(V·s$^4$), which results in a high electron-hole recombination rate, and a short hole diffusion length (2-4 nm) [111]. Recently, nanostructuring techniques have been proven useful in improving the performance of $\alpha$-Fe$_2$O$_3$ for photocatalysis. Hematite nanotubes prepared through anodizing an iron sheet in fluoride solution show 200 times higher photoactivity than a nanoparticle form [19]. The nanotubes ensure better light harvest than
randomly distributed particles, and further, the extremely thin wall (6 nm) of nanotubes reduces the recombination of electron-hole pairs.

In order to further improve the photocatalytic activity, multiple approaches may combine to achieve hierarchical nanostructures or nanocomposites. A two-step electrochemical anodization method created well-oriented α-Fe$_2$O$_3$ nanotubes, which showed 2 times higher than TiO$_2$ nanotubes in the degradation rate of organic dyes under sunlight irradiation [112]. Electrochemical deposition of transitional metal (e.g., Co, Ni, Pt) on nanostructured semiconductors promotes the migration of charges to absorbed water molecules, improving the yield rate of hydrogen gas or oxygen gas splitting from water [113]. Annealing nanostructured semiconductors with pre-treatment of metal salts (e.g., Zinc acetic, SnCl$_4$, TiCl$_4$) introduces quantum defects or create oxide complexes, which are capable of expanding absorbing spectrum and reducing electron-hole pairs [39, 110].

2.4.2 Hydrophobicity and super-hydrophobicity

The wettability of a surface is indicated by contact angle, which is measured through the outline of liquid phase, where a liquid-vapor interface meets a solid surface. The solid surface is considered hydrophobic when the water contact angle is larger than 90°. The super-hydrophobic surfaces normally have a water contact angle larger than 150°. A high contact angle means the interaction between liquid and solid phase is small, and thus the attachments of extraneous matters can be removed with less effort, which would benefit the structures with reduced routine cleaning and energy consumption for fluid transportation in pipelines [114]. Super-hydrophobic surfaces have been produced mainly in two ways. One is to create a rough structure on a hydrophobic surface, and the other is
to modify a rough surface by materials with a low surface free energy [115]. Some electrochemical methods can achieve super-hydrophobicity on metal surfaces through altering chemical compositions and creating rougher structures, or the both at the same time.

Directly immersing copper plate into fatty acids at ambient temperature results in nanoflowers composed of Cu(CH₃(CH₂)₁₂COO)₂ on the plate surface, which has a high water contact angle of 162° [66]. The nanostructured surface would not compromise the super-hydrophobic property even after treated by several organic solvents due to the chemical stability of the product. In some specific systems, other properties of nanostructures may degrade the super-hydrophobicity of a surface.

2.4.3 Corrosion resistance

Corrosion is an electrochemical reaction. It only occurs when anode, cathode, and electrolyte contact with each other [2]. The conventional strategies of preventing corrosion, e.g., inhibitor injection, cathodic protection, coating, etc., are to isolate those three conditions. For example, inhibitor molecules are able to adsorb on a metallic surface and result in a dielectric layer that blocks the substrate from electrolyte, or reduce the anodic and cathodic reaction rates. Cathodic protection uses an external electron supplier to cathodically polarize the metal structure, where the anodic reaction that causes the dissolution of metals is inhibited. Coatings designed for corrosion protection are usually made of dielectric materials, which prevent anode and cathode from contacting electrolytes. Generally, the nanomaterials have a relative lower chemical stability than the bulk forms due to a high surface area exposed to media. However, the nanostructured
surface has a complex interaction with the three conditions of corrosion and thus can be carefully designed to prevent corrosion.

Passive film as a naturally formed nanomaterial on metals surface makes metals and alloys resistant to corrosive environments. The main composition of oxides that are usually semiconductors performs like a coating and usually slows down corrosion rate for several orders of magnitude. The passive films formed on some corrosion resistant alloys (CRA) that containing a high portion of chrome, nickel, and molybdenum are “smarter” than a coating since they are able to self-repair in most service environments. Once the integrity of the passive film is damaged by mechanical or chemical factors, the exposed substrate reacts with electrolyte to generate metallic oxides, ending with a new passive film covering the surface [116].

Electrochemical methods improve the stability of the oxide film on metals through fabricating hierarchical nanostructures, which are capable of preventing corrosion in various mechanisms. The nanotubes and nanochannels created on aluminum and titanium by anodization have been successfully used as capsules to store inhibitors and biocides (to kill the bacteria resulting corrosion), where the loaded inhibitor and biocide molecules are slowly released into electrolyte, ensuring effective to corrosion protection [117, 118]. The anodized carbon steel in a fluoride solution bearing nanoporous iron oxides can generate electrons when irradiated by sunlight, which enables the cathodic protection of stainless steels [119]. The nanostructures created on anodized aluminum make the surface to be super-hydrophobic, causing the surface to barely contact with the electrolyte and decreasing the corrosion tendency [120].
2.4.4 Anti-bioadhesion and self-cleaning

Biological fouling is defined as the accumulation of algae, plants, fungi, sludge, yeast, or microorganisms on the wet surface of materials, resulting in the formation of a slimy biofilm [121]. Bacterial attachment and biofilm formation on structural materials have been a common problem in various industry sectors, such as oil pipelines, water treatment equipment, heat exchangers, marine structures, etc., causing structural failure, reduction of operating efficiency and increased maintaining cost [122, 123]. Corrosion may also occur under the biofilm, resulting in the so-called microbiologically influenced corrosion (MIC) [124]. Generally, high-performance coatings capable of anti-attachment of bacteria and biofouling have been used due to their high efficiency and easy application. However, biocides and heavy metal ions contained in the coatings serving as the functional matters are usually toxic, impacting adversely the sustainability of environments and the ecosystem [10].

Recently, surface nanotechnologies have been used for effective control of bacterial attachment and biofilm formation on metals. Two main strategies are used for the purpose, i.e., reducing the adhesive force between bacteria and the metal surface [11, 125], and deactivating (killing) the attached bacteria by environment-friendly ways [13]. The nature of the adhesion forces plays a crucial role in mediating interactions in bacterial adhesion to surfaces [126]. It has been confirmed that a nanostructured surface is effective to reduce the adhesive force between bacteria and the surface [127]. The pillar-patterned poly(ethylene glycol) hydrogels decreased notably the adhesion of staphylococci when the spacing between the structures was 1.5 μm or less. This phenomenon suggests the critical length scale of surface features for effective preventing bacterial adhesion should be
nanoscale (i.e., smaller than the size of a bacterium) [128]. The synergetic effects of surface nanostructures and hydrophobicity were also demonstrated using well-regulated nanostructures on softwood fiber, polyurethane, and titanium, making the surface superhydrophobic with the enclosure of air in the textured structures, which enhances the reduction of bacterial contact with the material and thus prevents bioadhesion [129].

However, the nanostructured surface film usually loses its function after a certain time period of service as bacteria can always find preferential sites to attach and grow. Deactivation (killing) of the attached bacteria based on photocatalysis provides a potential alternative for surface engineering due to its mechanism for anti-microbial activity under light illumination [13-15, 23]. Generally, electrons and holes generated on the surface of photocatalysts under light illumination exhibit high reducing and oxidizing activities, respectively. Reactive oxygen species, i.e., O$_2^-$ and 'OH, are generated during photocatalysis, contributing to degradation and deactivation of biological systems through their biological toxicity or strong oxidizability [130]. For example, O$_2^-$ is toxic to organisms, and 'OH can degrade all types of organic biomolecules [131]. Considering that biofilm assembling and bacterial attachment are mainly attributed by glycoprotein on the membrane, which could be degraded by 'OH. It was reported that the nanostructured surface combined with a high photocatalytic activity resulted in a promising anti-formation of biofilm on TiO$_2$ nanotubes [132], ZnO nanofilm [41], and Fe$_2$O$_3$ nanosheets [14]. Since the anti-bioadhesive property of semiconductors is mainly depending on the concentration of 'OH, adding electron scavengers (e.g., H$_2$O$_2$) into electrolyte or adopting high power light source would improve the production of 'OH. Therefore, the biomacromolecule with
long chains is decomposed into dissolvable molecules, achieving the self-clean from biofouling.

2.5 Summary

The complete literature review on nanostructures and nano-engineering of metals shows that, while major accomplishments have been made in the area, there still are many mechanistic and practical issues that have remained unknown. These include, but not limited to,

1) Electrochemical activity of nanoscale features on pipeline steel surface in corrosive aqueous environments.
2) Dependence of the stability of nanostructured passive films on pipeline steel on the passivating potential.
3) Anodization of pipeline steel and the resulting nanostructured oxides for anti-bioadhesion.
4) Light-enhanced anti-bioadhesion and deactivation of bacteria on nanostructured oxides.

Thus, it is expected that further efforts will be made to fabricate novel nanostructured materials and surface coatings for improved properties to meet newly emerging requirements.
Chapter Three: Effect of surface finishing on early-stage corrosion of a pipeline steel studied by electrochemical and atomic force microscope characterizations

3.1 Introduction

Investigations of the early-stage features of metallic corrosion are essential to understand the mechanistic aspect of corrosion initiation [63, 72, 133]. By virtue of AFM, the corrosion process can be characterized in-situ by topographic imaging. And thus, it makes possible to detect non-equilibrium features associated with corrosion initiation, which was yet understood by previous studies. Frequently, corrosion of metals, including steels, starts from a preferential dissolution at finer metallurgical features and surface irregularities with a nano-meter dimension [82, 134, 135]. These irregularities can be induced by surface finishing, which is widely applied as the final procedure of manufacturing metal parts and pretreatment for nano-fabrication [56].

This work attempted to understand the effect of surface finishing on the early stage corrosion of an X100 pipeline steel in a simulated soil solution by various electrochemical measurements and AFM imaging and analysis. The effect of surface roughness on the steel corrosion was analyzed, and the correlation between the early-stage feature of corrosion and the surface roughness was established.

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* This work has been published as: Yuan Li, Y. Frank Cheng. Effect of surface finishing on early-stage corrosion of a carbon steel studied by electrochemical and atomic force microscope characterizations, Applied Surface Science.366 (2016) 95-103.
3.2 Research Methods

3.2.1 Electrode and solution

Specimens used in this work were cut from an X100 steel plate, with a chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016, Mo 0.2, V 0.005, Cu 0.243, Al 0.027, S 0.005, P 0.018, and Fe balance. The microstructure of the steel consists of ferrite and bainite. The size of the polygonal ferrite ranges from 3 to 10 μm [136]. There is no obvious orientated structure induced by manufacturing processes.

The specimens used for in-situ AFM observation were machined into a rectangular shape with a dimension of 22 mm × 22 mm × 1 mm. To create various surface roughness, the specimens were ground using #400, #800 and #1200 emery papers, as well as polished by 1 μm diamond paste, respectively. The specimens were then degreased in ethanol via an ultrasonic bath, rinsed with deionized water and dried by highly purified nitrogen. The specimens used for electrochemical measurements were sealed in an epoxy resin, leaving a working face with a dimension of 10 mm × 10 mm. The surface finishes of the specimen were identical to those for AFM observations.

The test solution, simulating a soil-extracted solution, contained 0.483 g/L NaHCO₃, 0.122 g/L KCl, 0.181 g/L CaCl₂·H₂O and 0.131 g/L MgSO₄·7H₂O, with a pH of 7.8 [137]. All solutions were prepared using analytical grade chemicals and deionized water. All tests were performed at ambient temperature (~20°C) and open to air.

3.2.2 Electrochemical measurements

Electrochemical measurements were performed through a Solatron 1280 C electrochemical system on a conventional three-electrode cell, where the steel specimen
was used as working electrode, a carbon rod as the counter electrode (CE), and a saturated calomel electrode (SCE) as the reference electrode (RE). Upon immersion in the solution, the open circuit potential (OCP) of the working electrode was measured for 1 h until a steady-state value was reached. The electrochemical impedance spectroscopy (EIS) was measured in the frequency range from $2 \times 10^4$ Hz to $10^{-2}$ Hz, with an applied disturb signal of 10 mV. The EIS measurements were carried out at OCP. Potentiodynamic polarization curves were measured at a potential sweep rate of 0.167 mV/s. The potential scanning direction was from cathodic to anodic. The start and end potentials were -850 mV and -450 mV, respectively.

3.2.3 In-situ AFM characterization

An AFM (Keysight 5500 scanning probe microscope system) was used for topographic characterization of the steel specimen. A scanner carrying a long rectangular cantilever with a spring constant of 0.2 N/m (apex radius <10 nm) was placed above the specimen. The scanning mode was configured as contact, with a scanning rate of 1 Hz and a resolution of $512 \times 512$ pixels. The specimen was installed in a perforated plastic cell, which served as the solution container. The size of the cell was sufficiently large for easy installation of the specimen, and an environmental chamber was used to avoid evaporation of the solution.

Prior to the addition of solution in the plastic cell, the topography of the steel specimen was imaged within an area of $50 \mu m \times 50 \mu m$. A region with the size of $10 \mu m \times 10 \mu m$ was selected for further AFM characterization of the specimen as corrosion proceeds. The obtained images were processed to remove background signals, and to extract results such as surface roughness and topographic profiles. The topographic profiles were extracted and
illustrated in respect to time, the 1st profile was extracted at the beginning of AFM imaging, and the 2nd profile was extracted after 256 s, i.e., the middle position of the 1st image. The following profiles were extracted at the interval of 512 s, i.e., the middle position of the following images. The surface roughness is defined as the height difference between the peak and the valley in the imaging region.

3.3 Results

3.3.1 AFM characterization of surface topography of steel specimens prior to corrosion

Fig. 3.1 shows the AFM images of the surface topography of steel specimens prepared with various surface finishing methods, i.e., #400, #800 and #1200 emery papers grounding, and 1 μm diamond paste polishing, respectively. It is seen that there are obvious parallel scratches induced by surface finishing. With the finishing condition becoming finer, the steel surface is smoother, and the surface scratches are less obvious.

In order to quantify the surface roughness of the specimen under various finishing conditions in Fig. 3.1, a total of 13 points is selected on each specimen, as shown in Fig. 3.2a, to measure the roughness by AFM, where the sampling scale is 50 μm. The statistical results and standard deviations are shown in Fig. 3.2b. It is seen that both the surface roughness and the standard deviation of the measured results decrease when the surface finishing condition becomes finer. For example, the specimen grounding with #400 paper has a surface roughness of 223 nm, while the surface roughness of the specimen polished by 1 μm diamond paste is about 4 nm only.
Fig. 3.1 AFM views of the surface topography of the steel specimen grounding with (a) 
#400, (b) #800, and (c) #1200 emery paper, and (d) polishing by 1 μm diamond paste,
respectively.

It is noted that a measurement of the average roughness of each sample is more accurate
than scattered measurements at individual points. However, the AFM is not capable of
measuring the average surface roughness due to its limited scanning range. In order to
improve the measuring accuracy, measurements of the surface roughness were conducted
at many points on the specimen, as indicated in Fig. 3.2a.
Fig. 3.2 (a) The schematic diagram of the sampling positions on the steel specimen to measure the surface roughness by AFM. (b) Dependence of the surface roughness on the surface finishing.

### 3.3.2 Electrochemical measurements

Fig. 3.3a shows the time dependence of OCP of the steel electrode with various surface roughness in the test solution. It is seen that, upon immersion in the solution, the OCP drops rapidly until a knee point is reached. Then the OCP gradually becomes constant. Moreover, a longer time is required to achieve the approximately stable OCP for the specimen with a finer surface finish. The steady-state OCP is more negative when the surface polishing of the specimen is coarser.
Fig. 3.3 (a) Time dependence of OCP and (b) potentiodynamic polarization curves measured on steel specimens with different surface roughness in the test solution. (c) Comparison of the corrosion potentials derived from the equilibrium value of OCP and the fitting results of polarization curves, each data point is attributed to at least three measurements.

Fig. 3.3b shows the potentiodynamic polarization curves measured on steel specimens with different surface roughness in the solution. It is seen that all the measured curves exhibit a similar characteristic, i.e., an active dissolution featured anodic branch and a mass transfer controlled cathodic branch. Moreover, the polarization curves almost copy each other.
The corrosion potentials obtained from Figs. 3.3a and 3.3b are summarized in Fig. 3.3c. It is seen that the corrosion potential is shifted less negatively with the decrease of the surface roughness. Generally, the corrosion potential of a metal electrode indicates the corrosion tendency of the metal in corrosive environments, where a more negative potential is associated with the increased corrosion activity of the metal in the environment. It is thus seen that, as the surface roughness decreases, the corrosion activity of the steel reduces. Moreover, the corrosion potentials derived from the polarization curves in Fig. 3.3b are more negative than those obtained from Fig. 3.3a. This is attributed to the cathodic pre-polarization applied on the steel electrode during potentiodynamic polarization measurements.

Fig. 3.4 Nyquist diagrams measured on the steel electrode with various surface roughness at OCP in the test solution.
Fig. 3.4 shows the Nyquist diagrams measured on the steel electrode with various surface roughness at OCP in the test solution. It is seen that the impedance plots are featured with one depressed semicircle at high frequency and a small loop tail at low frequency. The size of the high-frequency semicircle increases slightly with the decreasing surface roughness. As the size of the semicircle is proportional to the charge-transfer resistance of corrosion reaction occurring at the steel/solution interface, the corrosion rate of the steel thus reduces when the surface finish of the electrode becomes finer, which is consistent with the corrosion potential measurements in Fig. 3.3.

3.3.3 AFM characterization of surface topography of corroded steel

Fig. 3.5 shows the AFM topographic images of the steel specimen finished by #400 emery paper before and during corrosion in the test solution, as well as the derived surface roughness profile. For the AFM image taken on the freshly prepared steel specimen, as shown in Fig. 3.5a, a square is marked to identify the region for further in-situ AFM observation during the steel corrosion. Surface irregularities such as scratches and grooves, which are generated by the emery paper, are obvious. Upon immediate immersion of the specimen in the solution, there is no obvious difference of the surface topography compared to that prior to immersion in the solution. At this stage, the surface topography is dominated by dissolution of the air-formed oxide film. Corrosion of the steel is yet to occur. The dissolution of the substrate steel is followed, and particularly, the scratch marked as 1 and the edge of a deep groove marked as 2 suffer from preferential attack, as shown in Fig. 3.5b. After 70 min of testing, corrosion occurs on the whole imaged area in Fig. 3.5c, and the surface roughness increases due to the newly formed pits, such as the
one marked as 3. The topographic profile derived from ten consecutive images is shown in Fig. 3.5d, where the 1st profile exhibits the surface topography almost free of corrosion, and the others show the topographic profiles of corroded steel. It is seen clearly that, during corrosion, the initial round, shallow scratches such as that at position 1 become narrower and deeper with time. The grooves with a sharp edge, such as the one marked as 2, becomes deeper. Moreover, new pits are generated, such as the one marked 3, which is 60 nm in depth.
Fig. 3.5 AFM topographic images of the steel specimen finished by #400 emery paper (a) before, (b) just after and (c) after 70 min of the immersion in the solution, as well as (d) the derived surface roughness profiles.

The AFM topographic evolution on the steel specimen finished by #800 emery paper in the test solution is shown in Fig. 3.6. Identical to Fig. 3.5a, the square marked in Fig.
3.6a shows the region for in-situ AFM imaging during corrosion of the steel. Upon immersion of the specimen in the solution, the topographic image is dominated by the surface oxide film, as shown in the 1st profile in Fig. 3.6b. An immediate dissolution of the steel is followed, causing removal (dissolution) of an average of 100 nm of surface layer in about 100 s, as seen in the 2nd profile in Fig. 3.6b. Moreover, the surface roughness of the steel increases, resulting in deepening of scratches, such as those marked as 1 and 2. After 70 min of corrosion, there are no new pits observed on the specimen, but the original features become deeper due to corrosion, which can be seen in Fig. 3.6c. The topographic profiles derived from all consecutive images are shown in Fig. 3.6d. The scratches marked as 1 and 2 are deeper than their original depths. Compared to the corrosion-induced deepening of the features marked in Fig. 3.5, the increase in depth of the local features is smaller for the specimen ground with #800 emery paper in Fig. 3.6.

The AFM topographic evolution for the specimen finished by #1200 emery paper during corrosion is shown in Fig. 3.7. Again, Fig. 3.7a shows the surface finish of the specimen prior to immersion in the solution, and the square indicates the selected region for further in-situ AFM imaging on the corroded specimen. Upon immersion in the solution, the 1st image is from the surface oxide formed in air. Corrosion occurs rapidly to remove a surface layer of about 60 nm in thickness, resulting in an increase of the surface roughness, as seen in Fig. 3.7b. The local features marked as 1 and 2 are selected for further characterization by AFM. Fig. 3.7c shows the topographic view of the specimen after about 70 min of corrosion. The surface becomes rougher and the local features are deeper. The topographic profiles extracted from the AFM images are shown in Fig. 3.7d, where the
arrow marked 1 indicates that the local feature is deeper after 70 min of corrosion, and its width is almost doubled, increasing from 1.5 μm to 3 μm.

Fig. 3.7 AFM topographic images of the steel specimen finished by #1200 emery paper (a) before, (b) just after and (c) after 70 min of the immersion in the solution, as well as (d) the derived surface roughness profiles.
Fig. 3.8 AFM topographic images of the steel specimen finished by 1 μm diamond paste (a) before, (b) just after and (c) after 70 min of the immersion in the solution, as well as (d) the derived surface roughness profiles.

Fig. 3.8 shows the AFM topographic views of the steel specimen polished by 1 μm diamond paste during corrosion in the solution. It is seen from Fig. 3.8a that the surface of the specimen is much finer than those ground by emery papers. The square marked is the region selected for further in-situ AFM imaging. Identical to Figs. 3.5, 3.6 and 3.7, the 1st AFM image is dominated by the air-formed oxide. Corrosion is followed immediately, as seen in the 2nd shot. An estimated 50 nm of surface layer is removed during corrosion.
between the two shots. After 70 min of corrosion, the scratches deepen and the surface roughness of the specimen further increases, as seen in Fig. 3.8c. The topographic profiles extracted from AFM images in Fig. 3.8d show that there is a slight increase in the depth of the features.

The time dependence of the surface roughness of the steel specimen with various surface finishing is shown in Fig. 3.9, where the data points are derived from the AFM topographic profiles in Figs. 3.5, 3.6, 3.7 and 3.8. The dependence of the normalized corrosion depth of the specimen on time is listed in Fig. 3.10. It is seen that, initially, both the surface roughness and the corrosion depth increase rapidly, and then reaches gradually towards a relative constant value. This tendency is consistent with the corrosion behavior of the steel. Generally, corrosion occurs quickly in the beginning when the steel is immersed in corrosive solutions. The corrosion would then achieve an equilibrium state with time. Moreover, the rougher the surface finish of the specimen, the faster the steady surface roughness is reached. As shown in electrochemical corrosion measurements, the corrosion activity of the steel increases with its surface roughness. Moreover, the EIS measurements show that the charge-transfer resistance reduces with the increasing roughness. Thus, the steel specimen with a bigger surface roughness corrodes more rapidly than that with a finer surface condition. As a result, less time is required to reach the equilibrium state with an approximately constant surface roughness.
Fig. 3.9 Time dependence of the surface roughness of the steel specimen with various surface finishing.

Fig. 3.10 Time dependence of corrosion depth (nm) of steel specimens with various surface finishing.
3.3.4 Determination of corrosion rate by changes of AFM topography of the steel

The corrosion rate of a steel, i.e., corrosion current density, can be calculated from the change of the surface topography of the steel characterized by AFM imaging during the early-stage corrosion. From the time dependence of the surface roughness shown in Fig. 3.9, two stages are included in the early-stage corrosion of the steel. While stage I involves a rapid dissolution of the steel, stage II is in an equilibrium state to have an approximately stable corrosion rate.

The corrosion rate of the steel is calculated by:

\[ i_{corr} = \frac{\Delta H \rho n F}{\Delta t M} \]  \hspace{1cm} (3.1)

where \( i_{corr} \) is corrosion current density, \( \Delta H \) is the average change of the height of the substrate steel during corrosion, \( \rho \) is density (7.86 g/cm³), \( n \) is the numbers of charges (\( n = 2 \) for steel), \( F \) is Faraday's constant (96485 C/mol), \( \Delta t \) is time, and \( M \) is the molar mass of steel (56 g/mol). Fig. 3.11 shows the corrosion current densities at stages I and II as a function of surface roughness of the steel specimen. It is seen that the corrosion rate at stage I, i.e., the rapid dissolution stage, is almost two orders of magnitude larger than that of stage II. Obviously, corrosion occurs primarily at the early stage of the immersion in the solution. Moreover, with the decrease of the surface roughness of the steel specimen, i.e., the surface of the specimen becomes finer, the corrosion rate decreases.
3.4 Discussion

It is accepted [138] that the anodic and cathodic reactions during corrosion of pipeline steels in aerated soil solutions contain the oxidation of steel and the reduction of dissolved oxygen.

Anodic reaction: \( \text{Fe} \rightarrow \text{Fe}^{2+} + 2e \) \hspace{1cm} (3.2)
Cathodic reaction: \( 2\text{H}_2\text{O} + \text{O}_2 + 4e \rightarrow 4\text{OH}^- \) \hspace{1cm} (3.3)

The present work shows that the steel corrosion at its early stage is featured with a high corrosion rate. The corrosion process is dominated by the active dissolution of the steel. As corrosion proceeds, the corrosion process achieves a dynamic equilibrium, and reaches a relative constant corrosion rate, especially when corrosion products form and deposit on
the steel surface [139]. In the present system, the main corrosion product is Fe(OH)$_2$, which is deposited on the specimen surface until the solubility product constant, $K_{sp}=1.64 \times 10^{-14}$, is exceeded. The pH of the solution is near-neutral of about 7.2. The corrosion products may generate and deposit after a certain amount of time when corrosion starts.

During mechanical grounding or polishing, local micro-deformations will be generated with the topographical irregularities on the specimen surface, affecting the electrochemical activity by [140]:

$$\Delta \varphi^0 = -\frac{\Delta P V_m}{nF}$$  \hspace{1cm} (3.4)

where $\Delta \varphi^0$ is change of electrode potential, $\Delta P$ is excessive pressure resulted from local stress concentration, and $V_m$ is the molar volume of the substrate steel. With the generation of local stress concentrations, the electrode potential of the steel is shifted negatively. It is expected that the surface irregularities generated by a coarser finishing result in a larger stress concentration, and thus a larger $\Delta P$. With the increase in the coarse degree of the surface finishing, the potential of the steel is shifted more negatively, as demonstrated in Fig. 3.3. Furthermore, the corrosion rate also decreases with the reduced surface roughness (Fig. 3.11), as the local stress concentration decreases. The enhanced corrosion activity of carbon steels by introducing surface irregularities was also confirmed in previous work [141]. Moreover, the measurement of electron work function [134, 135, 142] was related to corrosion potential of copper alloys in others’ work [143], confirming that an increased
surface roughness can enhance the thermodynamic activity of the metal and raise its corrosion rate.

It is noted that, as the corrosion rate decreases with the finer surface finish of the steel specimen, the difference of the corrosion rate is slight. This is also confirmed by the steady-state electrochemical measurements in Figs. 3.3 and 3.4b. The stress enhancement due to mechanical surface finishing occurs locally only, and the average stress level of the macroscopic steel electrode does not have an obvious change. Thus, attention should be paid to local preferential corrosion at the sites with irregular patterns and a high local stress concentration. Those features can be distinguished by advanced nano-electrochemical and surface techniques only [142, 144, 145].

Furthermore, from a microscopic viewpoint, the scratches induced by grounding or polishing are associated with a higher stress concentration than the adjacent areas [134, 135, 142]. According to the relationship between the electrode potential of a metal and the stress condition shown in Eq. (3.4), the local corrosion activity would be increased upon introduction of scratches. As corrosion proceeds, a preferential dissolution would occur at the scratches, making them deeper than the open areas. It is thus seen that the local features marked in AFM images become deeper and wider compared to their original images, as seen in Figs. 3.5, 3.6, 3.7 and 3.8. The reported work on electron work function measurements on copper alloys also demonstrated that electrons are easier to escape from the vicinity of geometrical peaks than other regions, resulting in a preferential attack locally [134, 142]. This is well consistent with the observations in this work.
3.5 Conclusions

Various surface roughness are created by grounding and polishing the steel specimen with different levels of surface finishing. The generated surface roughness is at nanometer scale.

As the surface roughness of the specimen increases, the corrosion activity of the steel increases, as indicated by the negative shift of electrode potential and increasing corrosion current density.

The early-stage corrosion of the steel is featured with two stages of anodic dissolution. While the first stage involves a rapid dissolution and increasing surface roughness, stage two is in an equilibrium state to have an approximately constant corrosion rate and surface roughness. A longer time is required to achieve stage two for the specimen with a finer surface finish.

Although the corrosion rate decreases with the finer surface finish of the steel, the difference of the corrosion rates is slight only. However, local preferential corrosion occurs at irregular patterns such as scratches with a high local stress concentration, resulting in corrosion deepening.
Chapter Four: In-situ characterization of the early stage of pipeline steel corrosion in bicarbonate solutions by electrochemical atomic force microscopy*

4.1 Introduction

In the previous chapter, the early stage of active corrosion of pipeline steel in a simulated soil solution was characterized by ECAFM. The relationship between surface roughness of the steel specimen and corrosion features was established. In addition to active corrosion, pipeline steels can be passivated to form passive film on the steel substrate when concentrated carbonate- bicarbonate ions are contained in electrolytes [2]. There has been rare work to characterize the two types of corrosion phenomena (i.e., active corrosion and passivation) of pipeline steels at a nanoscale topographic evolution.

The RMS roughness is one of the most popular statistical parameters to quantitatively evaluate surface fluctuations [103], but it is not able to identify the lateral distribution of surface features. A more advanced topographic treatment by PSD can derive the surface information in terms of its spatial wavelength and thus, allow comparison of the roughness at different frequency ranges [104]. It is noted that the PSD analysis has not been applied for in-situ imaging of corrosion features as large deviations induced by the different background of ex-situ images compromise the virtue of this approach.

In this work, the ECAFM was used to in-situ characterize the early stage of corrosion of an X100 pipeline steel in bicarbonate solutions with varying concentrations, which resulted in the steel in either an active corrosion state or passivation, by synchronous

* This work has been published as: Yuan Li, Y. Frank Cheng, In-situ characterization of the early stage of pipeline steel corrosion in bicarbonate solutions by electrochemical atomic force microscopy, Surface and Interface Analysis 49 (2017) 133-139.
measurements of electrochemical potential of the steel and its topographic evolution with time. The correlation of the surface roughness of the steel electrode with corrosion processes was established. The PSD of the electrode surface at various corrosion stages was calculated. The effect of bicarbonate concentration on corrosion mechanisms and topographic evolutions of the steel at the early stage was discussed.

4.2 Experimental

4.2.1 Material and solution

Specimens used in this work were cut from an X100 steel plate, with a chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016, Mo 0.2, V 0.005, Cu 0.243, Al 0.027, S 0.005, P 0.018, and Fe balance. The specimens were machined into a cubic shape with a dimension of 1 mm × 1 mm × 10 mm, which were embedded into epoxy to prepare work electrodes, with an exposed area of 1 mm$^2$. The work surface of the electrode was subsequently ground up to 1500 grits SiC abrasive paper, followed by ethanol degreasing in an ultrasonic bath, rinsing with deionized water, and drying by high purity (99.999%) nitrogen.

The test solutions used in this work contained 0.01 M, 0.1 M, and 0.5 M NaHCO$_3$ solution, respectively, simulating the primary compositions of electrolytes trapped under disbonded coating on pipelines [2]. They were prepared using analytical grade chemicals and deionized water.
4.2.2 In-situ ECAFM measurements

An ECAFM (Keysight 5500 scanning probe microscope) was used in this work, where a scanner carrying a long rectangular cantilever (450 µm × 50 µm × 2 µm) with a spring constant of 0.2 N/m (apex radius < 10 nm) was placed above the steel electrode. The scanning mode was configured as contact, with a scanning rate of 1 Hz, scanning range of 10 µm × 10 µm, and resolution of 512 × 512 pixels. To avoid contamination of the probe by aqueous environments, new probes were used for each test. The working electrode was installed at the bottom of a homemade solution container, with 30 mm in diameter, 10 mm in height, and 7 mL in volume. An environmental chamber was used to avoid evaporation of the test solution during testing. The topography of the steel electrode was visualized consecutively during corrosion.

Electrochemical measurements were performed through a Gamry Reference 600 system on a conventional three-electrode cell installed in the ECAFM solution container, where the steel electrode was used as WE, a platinum wire as CE, and an SCE as RE. Upon immersion of the steel electrode in the solution, the OCP was measured continuously for 5200 s until a steady-state value was reached. The corrosion potential of the steel was then recorded at a sampling rate of 5 points per second.

All tests were performed at ambient temperature (~20 °C) and open to air.

4.3 Results

In order to examine the surface heterogeneity of the steel electrode and quantify the surface roughness of the specimen, a total of 9 points are selected on the electrode surface, as shown in Fig. 4.1, to measure the surface roughness by AFM. The square indicates the
exposed area of the steel electrode, and the numbers mark the sampling points with an area of 40 µm × 40 µm for measurements of the roughness. The RMS roughness of the sampling positions is 13.10 ± 2.07 nm, showing the nanoscale heterogeneity of the electrode surface. For the following characterizations, the point marked as 5 is the area where the *in-situ* AFM imaging is conducted in the solution.

![Fig. 4.1 Schematic diagram of the sampling positions on the steel specimen to measure the surface roughness by AFM.](image)

Fig. 4.2 shows the time dependence of OCP of the steel in the test solutions, where the individual times to conduct AFM topographic imaging are marked with arrows. It is seen that, in 0.01 M NaHCO₃ solution, the OCP drops rapidly in the beginning upon immersion of the steel electrode in the solution, and then gradually reaches a relatively stable value of −765 mV (SCE). A knee point of OCP is observed at 500 s. On the contrast, in the solutions containing higher bicarbonate concentrations, i.e. 0.1 M and 0.5 M NaHCO₃ solutions, the OCP increases with time, and tends to reach relatively stable values of −238 mV (SCE)
and −195 mV (SCE) respectively. Moreover, as the bicarbonate concentration increases, the stable value of OCP is higher.

![Graph showing the time dependence of OCP of the steel in test solutions](image)

**Fig. 4.2** Time dependence of OCP of the steel in the test solutions, where the individual times to conduct AFM topographic imaging are marked with arrows.

Fig. 4.3 shows the AFM topographic images recorded prior to and after 100 s and 4500 s of immersion (as marked in Fig. 4.2) of the steel electrode in 0.01 M NaHCO₃ solution, respectively. It is seen from Fig. 4.3a that, prior to immersion in the solution, the electrode in the square contains topographic features of about 30 nm in depth relative to the surface. The observed irregularities (scratching lines) are introduced mainly by surface
finishing and are parallel with each other. After about 100 s of immersion in the solution (Fig. 4.3b), a layer of about 50 nm in thickness on the electrode surface starts to be dissolved away after 100 s of imaging. The topographic fluctuations are obvious. A feature, as marked by a white arrow, becomes deeper and wider because of local dissolution. The AFM image taken after 4500 s of immersion in the solution shows increased topographic fluctuations and rougher surface compared to that in Fig. 4.3b because of further corrosion and deposit of corrosion products. Moreover, the local features such as the one marked in Fig. 4.3b experience further preferential dissolution, and become linked together, forming deep and long grooves on the electrode surface, as seen in Fig. 4.3c.

Fig. 4.4 shows the AFM topographic images recorded prior to and after 100 s and 4500 s of immersion of the steel electrode in 0.1 M NaHCO₃ solution, respectively. It is seen in Fig. 4.4a that the square area for further in-situ AFM imaging contains topographic features of about 35 nm in depth. After 100 s and 4500 s of immersion in the solution, the topography of the steel electrode shows no obvious change in both surface pattern and roughness, but the electrode is smoother than that immersed in 0.01 M NaHCO₃ solution, as indicated by the height scale bars (Zrange) in Figs 4.3b and 4.3c, which are 300 nm, compared to those in Figs 4.4b and 4.4c of 100 nm. Furthermore, some dots as marked are formed on the steel surface after 4500 s in Fig. 4.4c but they are not observed in Fig. 4.4b. The dots have diameters in 0.7 to 4.2 μm, and heights in 1 to 5 nm.
Fig. 4.3 AFM topographic images recorded (a) prior to and (b) after 100 s and (c) 4500 s of immersion of the steel electrode in 0.01 M NaHCO$_3$ solution, respectively. The square in (a) indicates the area for further \textit{in-situ} AFM imaging, i.e. the views in (b) and (c). The black arrows in (b) and (c) indicate the locations where the topographic profiles are extracted, and the white arrows indicate a valley for further profile analysis.

Fig. 4.5 shows the AFM topographic images recorded prior to and after 100 s and 4500 s of immersion of the steel electrode in 0.5 M NaHCO$_3$ solution, respectively. The square area for further \textit{in-situ} imaging contains features of about 35 nm in depth in Fig.
4.5a. The topography shows no apparent change in both surface pattern and roughness. Some small particles, as marked by vertical arrows in Fig. 4.5c, are found, with diameters ranging from 0.1 to 0.6 µm and heights from 1 to 4 nm. Compared to Fig. 4.4c, the particles formed become smaller, but with the similar height. The steel electrode becomes smoother compared to that in Fig. 4.4 because of smaller particles present on the electrode surface.

Fig. 4.4 AFM topographic images recorded (a) prior to and (b) after 100 s and (c) 4500 s of immersion of the steel electrode in 0.1 M NaHCO₃ solution, respectively. The square in (a) indicates the area for further in-situ AFM imaging, i.e. the views for (b) and (c).
The black arrows in (b) and (c) indicate the locations where the topographic profiles are extracted. The white arrows in (c) indicate the dots formed on the steel surface.

Fig. 4.5 AFM topographic images recorded (a) prior to and (b) after 100 s and (b) 4500 s of immersion of the steel electrode in 0.5 M NaHCO₃ solution, respectively. The square in (a) indicates the area for further in-situ AFM imaging, i.e. the views for (b) and (c). The black arrows in (b) and (c) indicate the locations where the topographic profiles are extracted. The white arrows in (c) indicate the formed particles on the steel surface.
Fig 4.6 shows the topographic profiles of the steel electrode during corrosion in the various test solutions. This allows a clear view of the topographic evolution with time during corrosion of the steel. Each profile is extracted at the same location as marked with black arrows in Figs 4.3-4.5. In Fig. 4.6a where the profiles reflect the topographies of the steel in 0.01 M NaHCO₃ solution, the first profile, which is obtained immediately after immersion of the steel electrode in the individual solution, is featured a smooth curve containing a few fluctuations. With the increased time, the topographic fluctuations increase obviously. Both the average height and the roughness between peaks and valleys increase with time, indicating that corrosion results in increasing surface roughness of the steel. However, the topographic profiles obtained in 0.1 M and 0.5 M NaHCO₃ solutions do not show obvious changes from the beginning to the end of the test, as shown in Figs 4.6b and 4.6c.
Fig. 4.6 Topographic profiles of the steel electrode during corrosion in the various test solutions (a) 0.01 M, (b) 0.1 M, and (c) 0.5 M NaHCO₃ solutions.

Fig. 4.7 shows the time dependence of the surface roughness of the steel electrode in the three solutions. The data dots represent the roughness calculated from the topographic profiles in Fig. 4.6. They are plotted as a function of time to show the evolution of surface roughness of the electrode in the solutions. In 0.01 M NaHCO₃ solution, three stages are identified in terms of the evolution of surface roughness and topographic profiles. After
immersion of the electrode in the solution, the corrosion of air-formed oxide layer causes less change of surface roughness, as seen in Fig. 4.3b and the first profile in 4.6a. This is the feature in stage I, which lasts for about 200 seconds since immersed electrode in the solution. With further corrosion, steel exposes to solution resulting into a rapid dissolution, bring with the sudden increase of the surface roughness of the steel, which is marked as stage II in the figure. The generation of corrosion products on the steel surface slows down the corrosion process. When the corrosion process reaches a steady state, a dynamic equilibrium between the steel dissolution and the formation of corrosion products is achieved. The change of the surface roughness of the steel keeps almost constant with time, which is marked as stage III. However, for the steel electrodes immersed in 0.1 M and 0.5 M NaHCO₃ solutions, the surface roughness of the steel does not show an obvious change in the whole testing period, and there is no visible evolution stage, as shown in the 0.01 M NaHCO₃ solution, observed. Moreover, the surface roughness reduces slightly with time, indicating the steel surface becomes smoother after 4500 s of immersion in the solutions.
The 3-D AFM images can be processed to obtain PSD plots in the frequency domain in order to characterize the intensity of topographic fluctuations of features with varied sizes. In this work, the PSD is computed from the topographic profile of individual image, and then averaged over y-coordinate by [104]:

\[
PSD(f_x) = \frac{L}{N^3} \sum_{n=1}^{N} \left[ \sum_{m=1}^{N} Z_{mn} \exp \left( -\frac{j2\pi f_x m L}{N} \right) \right]^2
\]  

(4.1)

where \( L \) is the scanning scale of the AFM probe, \( N \) is the number of data per profile, \( f_x \) is the spectral frequency over x-coordinate, taking discrete values \( (1/L, \cdots, N/L) \), and \( Z_{mn} \) is the height of the data point. Fig. 4.8 shows the PSD of the steel electrode after 100 s and
4,500 s of immersion in 0.01 M, 0.1 M and 0.5 M bicarbonate solutions, respectively. It is seen that all PSD plots contain the same feature, i.e., a plateau in the low spatial frequency end and a roll-off with frequency towards the upper part of the spectrum. Since the spatial frequency is inversely proportional to the size of a feature, the PSD at the lower frequency spectrum is related to the fluctuation intensity of larger scaled features, e.g., the scratches induced by surface finishing, while the PSD at higher frequency part is attributed to smaller features such as nano-scaled metallurgical features. As seen in Fig. 4.8a, the PSD obtained on the steel electrode immersed in 0.01 M NaHCO$_3$ solution increases over the whole spatial frequency spectrum after 4,500 s of immersion compared to that after 100 s of testing, indicating that all the surface features increase their topographic fluctuations during corrosion. However, in the solutions containing higher concentrations of bicarbonate, the high-frequency PSD decreases after 4,500 s of immersion compared to that obtained after 100 s of immersion, as seen in Fig. 4.8b and 4.8c. The threshold spatial frequencies for the reduced PSD in 0.1 M and 0.5 M NaHCO$_3$ solutions are approximately 50 Hz and 20 Hz, respectively. This means that the surface features within 20 nanometers become eliminated after 4500 s of immersion in 0.1 M NaHCO$_3$ solution, while larger features within 50 nanometers in size are eliminated in 0.5 M NaHCO$_3$ solution in the same time period.
Fig. 4.8 PSD of the steel electrode after 100 s and 4,500 s of immersion in (a) 0.01 M, (b) 0.1 M and (c) 0.5 M bicarbonate solutions, respectively.

4.4 Discussion

Upon immersion of the steel in 0.01 M NaHCO₃ solution, the initial high potential in Fig. 4.2 is associated with the air-formed oxide film on the steel surface. The steel corrosion is yet to occur, and the topographic profile shows a smooth curve in Fig 4.6a. It is acknowledged [146, 147] that pipeline steels, including X100 steel, follows an active corrosion mechanism in diluted bicarbonate solutions. When corrosion starts, the OCP of the steel drops rapidly. The corrosion products, primarily ferrous ions (Fe²⁺), are released into the solution. Generally, the local features on the steel surface suffer from preferential,
large dissolution [135, 141], resulting in a rapidly increasing surface roughness, which can be seen in Fig. 4.3b and stage II in Fig. 4.7. At the same time, the cathodic reduction of dissolved oxygen generates hydroxyl ions (OH\(^-\)). The generation of corrosion products FeCO\(_3\) would slow down the kinetics of corrosion [148]. When the steel corrosion achieves a relatively steady state, as indicated by the knee point in Fig. 4.2, the formation of corrosion products is in a dynamic equilibrium, causing the increase of surface roughness to maintain at an approximately constant value, where stage III in Fig. 4.7 is reached. Obviously, the topographic evolution characterized by AFM is consistent well with the time dependence of the OCP at the early stage of the steel corrosion. Furthermore, compared with the PSD plots after 100 s and 4500 s of immersion in Fig. 4.8a, the surface roughness increases at the whole spatial frequency range. This indicates that both the surface scratches initially induced by surface finishing and metallurgical features, which can be nanometer scaled, contained in the steel become deeper and wider during active corrosion of the steel in the solution.

In the concentrated bicarbonate solutions such as 0.1 M and 0.5 M, the steel can be passivated [149], and a layer of passive film forms on the steel surface for corrosion protection [150]. The passivated steel shows a smooth, continuously increasing OCP, as shown in Fig. 4.2. During the growth of passive films on the steel surface, the surface features including polishing scratches and metallurgical defects where usually come with residual stress concentration that enhances the localized film growth can potentially be covered and eliminated, causing a decreasing surface roughness [135, 141]. In this work, both topographic profiles and surface roughness, as seen in Figs. 4.6 and 4.7, demonstrate
the effect of the steel passivation on the surface features, and the effect becomes obvious at the early stage of the steel corrosion in the solutions.

Furthermore, the eliminating effect of the formed passive film on the surface features depends on the size of the features. The AFM topographic views in Figs. 4.4 and 4.5 show that, generally, large and deep features such as surface scratches cannot be eliminated, and small and shallow features such as the nanoscale features can become smoothed with the growth of passive film at the early stage. The analysis of the PSD plots can give further quantitative information. A comparison of the PSD plots obtained after 100 s and 4,500 s of immersion in 0.1 M and 0.5 M NaHCO₃ solutions in Figs. 4.8b and 4.8c, shows that the reduced PSD value after 4500 s of testing is well consistent with the conclusion that the growing passive film can improve the surface roughness of the steel. Moreover, the threshold frequency where the PSD decreases in 0.5 M NaHCO₃ solution, i.e., 20 Hz, is lower than that measured in 0.1 M NaHCO₃ solution, i.e., 50 Hz. Thus, the passive film formed in the more concentrated solution (0.5 M) is able to eliminate larger scaled topographic features than that formed in less concentrated solution (0.1 M). This is understandable because the capability of a bicarbonate solution to passivate pipeline steel is increased with its concentration [146, 149]. Indeed, in 0.1 M NaHCO₃ solution, many big dots are formed on the film and result in a bit rougher surface compared to the film formed in 0.5 M NaHCO₃ solution, as seen in Figs. 4.4c and 4.5c. The rough surface of passive film caused by the presence of irregular nanostructures usually indicates a relative low electrochemical stability of passive film or the low passivating capability of an environment to metallic substrate [86, 151].
4.5 Conclusions

The synchronous measurements by ECAFM of the topographic evolution and electrochemical potential are able to characterize the early stage of corrosion of the steel in the solutions. The steel corrodes actively in 0.01 M NaHCO$_3$ solution, and the early stage of the steel corrosion includes three stages. Upon immersion of the steel in the solution, both electrochemical potential and topographic profile are associated with the dissolution of air-formed oxides present on the steel surface. The dissolution of air-formed oxides induces OCP drops and negligible roughness increases, which are the key features at stage I. When corrosion of the steel occurs, the OCP further drops, and the surface roughness of the steel increases rapidly, which is stage II. As the steel corrosion achieves a steady state, the generation of corrosion products reaches a dynamic equilibrium state. Both the surface roughness and the OCP maintain an approximately stable value in stage III.

In solutions containing increased bicarbonate concentrations, such as 0.1 M and 0.5 M NaHCO$_3$, the steel can be passivated, resulting in a positive shift of the OCP. The formed passive film can eliminate some surface features and improves the surface roughness. Since the passivating ability of the bicarbonate solution is increased with its concentration, the topographic profile of the steel surface in 0.5 M NaHCO$_3$ solution is smoother than that in 0.1 M solution. This result is consistent with the PSD analysis, which shows that the passive film formed in the more concentrated solution (0.5 M) is able to eliminate larger scaled topographic features than that formed in less concentrated solution (0.1 M). The surface features within 20 nanometers become eliminated after 4500 s of immersion in 0.1
M NaHCO$_3$ solution, while larger features within 50 nanometers in size are eliminated in 0.5 M NaHCO$_3$ solution in the same time period.
Chapter Five: Passive film growth on pipeline steel and its nanoscale features at various passivating potentials*

5.1 Introduction

Passive films formed on the metal surface have been recognized as natural nanomaterials. Understanding the topographic and structural evolution of the passive film formed under various conditions is vital for nanofabrication based on electrochemical methods [52-54]. By the virtue of ECAFM, the in-situ characterization of passive films at nanoscale is expected to provide mechanistic information on the film formation and growth at an early stage [82, 86]. To date, there has been limited work conducted in this area.

In this work, the passivation of an X100 pipeline steel was investigated in a concentrated carbonate/bicarbonate solution by EIS and potentiodynamic polarization curve measurements. The topography of the passive films formed at different film-forming potentials over various time periods was in-situ characterized by ECAFM, and the chemical composition of the films was determined by XPS. The surface roughness and sub-micron structure of passive films formed at the early stage were analyzed. It is expected that this work improves our understanding of the pipeline steel passivation and the film formation at its early stage.

* This work has been published as: Yuan Li, Y. Frank Cheng, Passive film growth on carbon steel and its nanoscale features at various passivating potentials, Applied Surface Science 396 (2017) 144-153.
5.2 Experimental

5.2.1 Material and solution

Specimens used in this work were cut from an X100 steel plate, with a chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016, Mo 0.2, V 0.005, Cu 0.243, Al 0.027, S 0.005, P 0.018, and Fe balance. The microstructure of the steel contains ferrite and bainite. The specimens used for ECAFM imaging were machined into a quadrangular shape with a dimension of 1 mm × 1 mm × 10 mm, and were then painted with a primer and embedded into epoxy resin, leaving an exposed area of 1 mm². The specimens were sequentially ground by emery papers up to 1200 grit, polished by 0.5 µm diamond paste, and degreased in ethanol using an ultrasonic bath, rinsed with deionized water, and dried by highly purified nitrogen. The specimens used for electrochemical measurements and XPS analysis were painted with a primer and then sealed in epoxy resin, leaving a work face of 10 mm × 10 mm. The test solution contained 0.05 M Na₂CO₃ + 0.1 M NaHCO₃, with a pH of 9.5. All solutions were prepared using analytical grade chemicals and deionized water. All tests were performed at ambient temperature (~20 °C) and open to air.

5.2.2 Electrochemical measurements

Electrochemical measurements were performed through a Solatron 1280C electrochemical system on a three-electrode cell, where the steel specimen was used as WE, a carbon rod as CE, and an SCE as RE. A salt bridge was used to avoid the chloride contamination from the RE to the solution. Prior to measurements, the freshly prepared specimen was cathodically polarized at -1.0 V (SCE) for 5 min to remove air-formed oxide.
The potentiodynamic polarization curve was measured from -1.0 V (SCE) to 1.0 V (SCE) at a potential scanning rate of 0.167 mV/s. Prior to EIS measurements, the specimen was pre-polarized at various film-forming potentials (i.e., passive potentials), which were chosen from the measured polarization curve, for 1 h. The EIS measurements were conducted, with a disturbance signal of 10 mV and the frequency range from $2 \times 10^4$ Hz to $10^2$ Hz. Each test was repeated at least three times to ensure the reproducibility of the results.

### 5.2.3 In-situ ECAFM characterization

An ECAFM (Keysight 5500 scanning probe microscope system) was used for topographic characterization on the steel specimens which were passivated at film-forming potentials of -0.1 V (SCE), 0.5 V (SCE) and 0.7 V (SCE), respectively, for 60 min. A scanner carrying a long rectangular cantilever with a spring constant of 0.2 N/m (apex radius<10 nm) was placed above the specimen. The scanning mode was configured to contacting, with a scanning rate of 4 Hz and resolution of 512 × 512 pixels. The working electrode was installed at the bottom of a homemade solution container, with 30 mm in diameter, 10 mm in height and 7 mL in volume. The size of the cell was sufficient to avoid evaporation of the solution during testing. Electrochemical controls were applied with a three-electrode setup, where the steel WE was installed at the bottom of the cell and a platinum wire was used as the RE, which was calibrated versus SCE in the same solution. The potential of the WE was controlled by a built-in potentiostat assembled with an AFM controller. The obtained images were processed by supplied software to remove tilt, and
the topographic profile of the specimen was derived along the surface-finishing direction to diminish the effect of original topographic fluctuations.

5.2.4 XPS characterization

The XPS characterization was performed using a PHI VersaProbe 5000 spectrometer operating at a fixed pass energy of 23.5 eV and working in vacuum ($<10^{-7}$ Pa). The spectra were taken using a monochromatic Al source (1486.6 eV) at 50 W and a beam diameter of 200 μm with a take-off angle of 45°. The specimen was fixed with a double-sided tape, and spectra were taken with double neutralization. The binding energies were reported relative to C 1s at 284.8 eV. After 60 min of polarization at various film-forming potentials in the test solution, the specimen was rinsed with deionized water, dried by highly purified nitrogen, and was ready for XPS characterization.

5.3 Results

5.3.1 Measurements of potentiodynamic polarization curve

Fig. 5.1 shows the potentiodynamic polarization curve measured on the steel specimen at a potential scan rate of 0.167 mV/s in the test solution. It is seen that the steel can be passivated over a wide passive potential range from nearly -0.1 V (SCE) to 0.8 V (SCE). The corrosion potential of the steel is about -0.76 V (SCE). A complex active-passive transition occurs in the potential range of -0.64 V (SCE) to -0.10 V (SCE), where at least two null-current peaks at about -0.59 V (SCE) and -0.40 V (SCE) are observed.
Fig. 5.1 Potentiodynamic polarization curve measured on the steel specimen at a potential scan rate of 0.167 mV/s in the test solution.

5.3.2 EIS measurements at various film-forming potentials

Fig. 5.2 shows the Nyquist diagrams and Bode modulus plots measured on the steel specimen at various film-forming potentials, which are determined from the polarization curve in Fig. 5.1, after 1 h of immersion in the test solution. It is seen that all Nyquist diagrams have a common feature, i.e., one incomplete semicircle in the whole frequency range. The size of the semicircle increases with the film-forming potential until 0.5 V (SCE) and then decreases. The measured Bode plots show a linear slope in the measuring frequency range. Moreover, the magnitude of the low-frequency impedance also increases.
with the film-forming potential. After 0.5 V (SCE), the impedance magnitude decreases with positive shift of the film-forming potential.

Fig. 5.2 Nyquist diagrams (a) and Bode modulus plots (b) measured on the steel specimen at various film-forming potentials, which are determined from the polarization curve in Fig. 5.1, after 1 h of immersion in the test solution.

The impedance data are fitted with an equivalent circuit shown in Fig. 5.3a, where a film resistance \( R_f \) is parallel connected with a constant phase element (CPE), which is then connected in series with a solution resistance \( R_s \). The reason that the double layer capacitance is negligible in impedance fitting is that the measured film resistance ranges from 0.5 to 1 MΩ cm\(^2\), which is much higher than charge-transfer resistance. Moreover, the double layer capacitance is usually about 200 ~ 500 µF/cm\(^2\), which is much larger than the measured film capacitance. Therefore, the electrochemical impedance is dominated by the behavior of the film, while the contribution of the double layer is ignorable during impedance fitting. Actually, this method has been used in the fitting of measured impedance data for filmed metal electrodes [93, 152].
Fig. 5.3 (a) The equivalent circuit for the fitting of the measured impedance data, and
dependence of the fitting electrochemical impedance parameters, i.e., (b) passive film
resistance and (c) passive film capacitance, on film-forming potential.

The CPE is governed by an exponent, \( n \), which indicates the capacitive idealness. The
impedance of the CPE, \( Z_{CPE} \), is expressed as [153]:

\[
Z_{CPE} = \frac{1}{Q(j\omega)^n}
\]  

(5.1)
where $Q$ is the passive film capacitance, and $\omega$ is angular frequency. The fitting parameter, including the resistance and capacitance of the passive film, are shown in Figs. 5.3b and 5.3c. The chi-square of the data fitting, which represents the coincidence of the fitted results with the measured data and is calculated by the software supplied with the EIS measurement system, is $1.5 \times 10^{-4}$. It indicates that the fitting quality is sufficient, and the fitting results are reliable. Furthermore, it is seen that the passive film resistance increases with the film-forming potential, with the largest value observed at about 0.4 - 0.5 V (SCE). After that, the film resistance decreases with the potential. Moreover, the capacitance of the passive film decreases with the film-forming potential. As the exponent $n$ is close to 1, the formed passive film can be described as a parallel-plate capacitor. The capacitance is inversely proportional to the distance between the two plates. It increases with the decrease of $Q$, implying that the passive film thickens and behaves like an ideal capacitor as the film-forming potential increases.

### 5.3.3 Potentiostatic current density at different film-forming potentials

The film-forming potential of -0.1 V (SCE) is selected from the measured polarization curve in Fig. 5.1 as it refers to the potential where the passivity of the steel starts after the active-passive transition. The potentials of 0.5 V (SCE) and 0.7 V (SCE) are also selected as the ones where stable passivation is achieved and the film resistance reduces (Fig. 5.3b), respectively. Fig. 5.4 shows the time dependence of potentiostatic current densities of the steel electrode at film-forming potentials of -0.1 V (SCE), 0.5 V (SCE) and 0.7 V (SCE), respectively. It is seen that, in the first 100 s of passivation, the current density increases with the positive shift of the film-forming potential, indicating that the steel passivation is
increased with the increasing film-forming potential. After 100 s, at the film-forming potential of 0.5 V (SCE), there is the smallest current density, compared to those measured at -0.1 V (SCE) and 0.7 (SCE). As the potentiostatic current density measured at various film-forming potentials is inversely proportional to the film resistance, the smallest current density at the potential of 0.5 V (SCE) after 100 s indicates that there is the largest resistance for the film formed at 0.5 V (SCE). The result is consistent with the film resistance derived from impedance measurements in Fig. 5.3b.

---

**Fig. 5.4** Time dependence of potentiostatic current densities of the steel electrode at film-forming potentials of -0.1 V (SCE), 0.5 V (SCE) and 0.7 V (SCE) in the solution, respectively. The inset shows the time dependence of the current densities in a linear scale after 100 s of film formation.
5.3.4 AFM characterization of the passive film growth at various film-forming potentials

Fig. 5.5 shows the AFM image of the steel specimen after 5 min of cathodic polarization at -1 V (SCE) in the solution, and the derived topographic profile. It is seen in Fig. 5.5a that some grooves that are parallel to surface finishing. The maximum topographic fluctuation is 32.4 nm over the imaging area of 2 µm × 2 µm. Along the finishing direction as marked in Fig. 5.5a, the maximum topographic fluctuation is 3.3 nm, and the RMS roughness is 0.50 nm only, as shown in the topographic profile in Fig. 5.5b.

Fig. 5.5 (a) AFM topography of the steel specimen after 5 min of cathodic polarization at -1.0 V (SCE) in the solution, and (b) the derived topographic profile along the marked dash line.
Fig. 5.6 The AFM images of the steel specimen polarized at −0.1 V (SCE) for (a) 0.5 min, (b) 5 min, (c) 30 min and (d) 60 min, respectively, in the solution.

Fig. 5.6 shows the AFM topographic images of the steel specimen polarized at -0.1 V (SCE) for 0.5 min, 5 min, 30 min and 60 min, respectively, in the solution. The grooves generated by surface finishing on the specimen are obvious in the image taken after 0.5 min of passivation. This indicates that a complete film is yet formed on the electrode.
surface. After 5 min of passivation, the film is more obvious. Numerous nanoscale features, which refer to the scale-like spots with a diameter of about 110 nm on the specimen surface, as marked in Fig. 5.6b, where the diameter is defined as that of the circumscribed circle for noncircular nano-features. As the passivating time increases to 30 min and 60 min, the diameter of the features increase (Figs. 5.6c and 5.6d), indicating the growth of passive film on the steel surface. In particular, the grooves due to surface finishing are not observed in the AFM image taken after 60 min of passivation as a thick passive film is formed.

Fig. 5.7 shows the AFM topographic images of the steel specimen polarized at 0.5 V (SCE) for 0.5 min, 5 min, 30 min and 60 min, respectively, in the solution. It is seen in Fig. 5.7a that the electrode surface features the polishing induced grooves after 0.5 min of polarization, showing the similar topographic feature in Fig. 5.6a. As the polarization time increases to 5 min, nanoscale features varying from several to tens of nanometers in diameter are formed on the surface, as seen in Fig. 5.7b. After 30 min and 60 min of passivation, the diameter of the features increases up to about 80 nm, as seen in Figs. 5.7c and 5.7d.
Fig. 5.7 The AFM images of the steel specimen polarized at 0.5 V (SCE) for (a) 0.5 min, (b) 5 min, (c) 30 min and (d) 60 min, respectively, in the solution.
Fig. 5.8 The AFM images of the steel specimen polarized at 0.7 V (SCE) for (a) 0.5 min, (b) 5 min, (c) 30 min and (d) 60 min, respectively, in the solution.

Fig. 5.8 shows the AFM images of the steel specimen polarized at 0.7 V (SCE) for 0.5 min, 5 min, 30 min and 60 min, respectively, in the solution. After 0.5 min of polarization, the specimen surface features apparent polishing induced grooves. As the polarization time increases to 5 min, spots that have a diameter ranging from 100 to 400 nm are formed, as
seen in Fig. 5.8b. After 30 min and 60 min of passivation, the size of the spots further increases with time, generating some particles up to 100 to 500 nm (Figs. 5.8c and 5.8d).

Fig. 5.9 shows the time dependence of RMS roughness of the specimens that are polarized at different film-forming potentials. The RMS roughness is calculated from the topographic profiles in Figs. 5.6-5.8, with each data point obtained from at least three profiles. It is seen that the surface roughness increases from the fresh electrode to the 0.5 min of polarization for all specimens. This is mainly caused by the initial dissolution of the steel in the solution. The surface roughness then decreases slightly after 5 min of polarization, which is attributed to the growth of passive film on the steel surface. For the specimens polarized at -0.1 V (SCE) and 0.5 V (SCE), the surface roughness increases slightly with time up to 60 min, which is attributed to the uniform growth of passive film on the whole surface of the steel. Moreover, the surface roughness of the specimen polarized at 0.5 V (SCE) is smaller than that at -0.1 V (SCE). This is due to finer features formed at 0.5 V (SCE) than those formed at -0.1 V (SCE). However, when the specimen is polarized at 0.7 V (SCE), the surface roughness increases greatly with time, and has a much larger value than others. The result analysis shows that the passivation at 0.5 V (SCE) would achieve the most stable passivity and generate a homogeneous passive film, while at 0.7 V (SCE), the passive film is the most non-uniform. The results are consistent with the electrochemical impedance results in Fig. 5.3.
Fig. 5.9 Time dependence of the RMS roughness of the specimens that are polarized at different film-forming potentials. Each data point was attributed to at least 5 measurements.

5.3.5 XPS characterization of the passive films

Fig. 5.10 shows the high-resolution XPS spectra and their decompositions for (a) Fe 2p peaks, and (b) O 1s peaks obtained on the specimens that are polarized for 60 min at -0.1 V (SCE), 0.5 V (SCE) and 0.7 V (SCE), respectively. A Shirley-type background subtraction is performed prior to fitting of the spectra. For passive films formed at all potentials, the Fe 2p peaks generally contain four components, i.e., Fe-1, Fe-2, Fe-3 and Fe-4, which are metallic associated with Fe, Fe₃C, Fe₃O₄/FeO (Fe(II) cation) and Fe₂O₃/iron hydroxides (Fe(III) cation), respectively. An additional component, Fe-5, is associated
with Fe$_2$O$_3$-satellite and is used in curve analysis, as reported in others' studies [154-156]. The O 1s spectra are generally composed of three components, i.e., O-1, O-2 and O-3, associating with iron oxide (lattice O$^{2-}$), FeOOH (lattice OH$^-$), and H$_2$O, respectively. The XPS parameters for Fe 2p and O 1s spectra are shown in Table 5.1. Although identical peaks are included in the passive films formed at various potentials, the intensity of individual peak is different when the film-forming potential changes, as seen in Fig. 5.10.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compounds</th>
<th>Peak position 2P3/2 (eV)</th>
<th>Full width at half maximum (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-1</td>
<td>Fe metal</td>
<td>707</td>
<td>1.5</td>
</tr>
<tr>
<td>Fe-2</td>
<td>Fe$_3$C</td>
<td>708.2</td>
<td>2</td>
</tr>
<tr>
<td>Fe-3</td>
<td>Fe$_3$O$_4$/FeO</td>
<td>709.6</td>
<td>2</td>
</tr>
<tr>
<td>Fe-4</td>
<td>Fe$_2$O$_3$/Iron hydroxides</td>
<td>711</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fe$_2$O$_3$/Iron hydroxides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe-5</td>
<td>hydroxides - Satellite</td>
<td>712.7</td>
<td>2.5</td>
</tr>
<tr>
<td>O-1</td>
<td>Iron oxide</td>
<td>529.8</td>
<td>1.1</td>
</tr>
<tr>
<td>O-2</td>
<td>Iron hydroxides</td>
<td>531.5</td>
<td>2</td>
</tr>
<tr>
<td>O-3</td>
<td>H$_2$O</td>
<td>533.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>
Fig. 5.10 High-resolution XPS spectra and their decompositions for (a) Fe 2p peaks, and (b) O 1s peaks obtained on the steel specimen polarized for 60 min at −0.1 V (SCE), 0.5 V (SCE) and 0.7 V (SCE), respectively.

Fig. 5.11 shows the area ratios derived from the XPS spectra in Fig. 5.10 of metallic iron to total iron oxides, Fe(II) to Fe(III), and lattice O$^2-$ to lattice OH$^-$, respectively, as a function of film-forming potential. It is seen in Fig. 5.11a that the area ratio of metallic iron to iron oxides decreases with the film-forming potential, indicating that the passive film thickens with the increasing potential. The sampling depth, $d$, of XPS characterization can be calculated by [157, 158]:

$$d = \frac{k}{N}$$
\[ d = 0.288 \sqrt{h \nu - E_b} \cos \theta \]  \hspace{1cm} (5.2)

where \( h \nu \) is the characteristic energy of X-ray photons (1486.6 eV), \( E_b \) is core level binding energy of Fe 2p (~710 eV), and \( \theta \) is the take-off angle relative to the specimen surface (in this work, 45°). The average sampling depth of XPS is 5.68 nm. As the metallic iron beneath the passive film formed at 0.5 V (SCE) is just detected by XPS, the average thickness of the passive film is approximately equal to the XPS sampling depth. The films forming at -0.1 V (SCE) and at 0.7 V (SCE) are thinner and thicker than 5.68 nm, respectively. Furthermore, the area ratios of Fe(II) to Fe(III) and lattice O\(^{2-}\) to the lattice OH\(^-\) decrease with the film-forming potential, as shown in Figs. 5.11b and 5.11c, suggesting that the positive shift of the film-forming potential favors the formation of Fe\(_2\)O\(_3\) and iron hydroxides. The dramatic decrease of the area ratios at 0.7 V (SCE) implies that a high concentration of iron hydroxides is formed in the passive film.
Fig. 5.11 Area ratios, derived from the XPS spectra in Fig. 5.10, of (a) metallic iron to iron oxides, (b) Fe(II) to Fe(III), and (c) lattice $O^{2-}$ to lattice $OH^-$, respectively, as a function of film-forming potential.

5.4 Discussion

5.4.1 Passive film growth on steel at different film-forming potentials

It has been accepted [159] that the anodic and cathodic reactions during corrosion of pipeline steels in aerated, concentrated carbonate/bicarbonate solutions are the oxidation of iron and the reduction of dissolved oxygen, respectively:
Anodic reaction: \( \text{Fe} \rightarrow \text{Fe}^{2+} + 2e \) \hspace{1cm} (5.3)

Cathodic reaction: \( \text{O}_2 + 2\text{H}_2\text{O} + 4e \rightarrow 4\text{OH}^- \) \hspace{1cm} (5.4)

The oxidative current density increases with potential when the steel is anodically polarized until the formation of a layer of \( \text{FeCO}_3 \) scale on the specimen surface, as indicated by the current peak at about -0.65 V (SCE) in Fig. 5.1 [160]:

\[ \text{Fe} + \text{HCO}_3^- \rightarrow \text{FeCO}_3 + \text{H}^+ + 2e \] \hspace{1cm} (5.5)

With further anodic polarization, the ferrous ions are oxidized into ferric ions, i.e., \( \text{Fe}_3\text{O}_4, \text{Fe}_2\text{O}_3 \), and iron hydroxides in aerated solutions [149], which causes the presence of a current peak at about -0.2 V (SCE) in Fig. 5.1.

\[
\begin{align*}
4\text{FeCO}_3 + \text{O}_2 + 4\text{H}_2\text{O} & \rightarrow 2\text{Fe}_2\text{O}_3 + 4\text{HCO}_3^- + 4\text{H}^+ \hspace{1cm} (5.6) \\
6\text{FeCO}_3 + \text{O}_2 + 6\text{H}_2\text{O} & \rightarrow 2\text{Fe}_3\text{O}_4 + 6\text{HCO}_3^- + 6\text{H}^+ \hspace{1cm} (5.7) \\
4\text{FeCO}_3 + \text{O}_2 + 10\text{H}_2\text{O} & \rightarrow 4\text{Fe}(`\text{OH})_3 + 4\text{CO}_3^{2-} + 8\text{H}^+ \hspace{1cm} (5.8) \\
4\text{FeCO}_3 + \text{O}_2 + 6\text{H}_2\text{O} & \rightarrow 4\text{FeOOH} + 4\text{CO}_3^{2-} + 8\text{H}^+ \hspace{1cm} (5.9)
\end{align*}
\]

The passive film formed at -0.1 V (SCE) in this system is a mixture of \( \text{Fe}_3\text{O}_4, \text{Fe}_2\text{O}_3 \), and iron hydroxides, which is consistent with the XPS result in Fig. 5.10.

Previous studies [93, 161] have confirmed that passive films formed at a stationary state are comprised of a \( \text{Fe}_3\text{O}_4 \) inner layer and a \( \text{Fe}_2\text{O}_3/ \text{iron hydroxides} \) outer layer. According to the point defect model (PDM) [92], the oxidation of Fe(II) to Fe(III) takes
place at the interface between the inner and outer layers, following the mechanism as illustrated in Fig. 5.12. In process (a), electrons and oxygen vacancies are generated by converting Fe(II) to Fe(III) at the steel/oxide interface. Process (b) includes the migration of the oxygen vacancies towards the outer layer/solution interface through exchanging the lattice position with O(II). In process (c), O(II) is injected into the outer layer or the dissolution of Fe(III). The rates of the processes (a) and (b) are proportional to the intensity of electric field through the oxide film. The thickness of the outer layer containing Fe(III) increases with the positive shift of film-forming potential and passivating time, as indicated by the decreased ratio of Fe(II)/Fe(III) in Fig. 5.11b. During the film growth, the lattice structure is maintained by creating cation vacancies [92, 93]. At the same time, the inner layer becomes thicker when the film-forming potential shifts from -0.1 V (SCE) to 0.5 V (SCE), causing the increasing thickness of the oxide film, as indicated by the decrease of the ratio of metallic iron/iron oxides shown in Fig. 5.11a.

![Diagram](image)

**Fig. 5.12** Schematic diagram illustrating the growth of passive film on the steel surface, where Fe(II), Fe(III), V_o(II), O(II) and OH(I) represent ferrous cation, ferric cation, oxygen vacancy, oxygen anion and hydroxyl anion, respectively.

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Furthermore, the potential drop at the outer layer/solution interface, which determines the rate of process (c), is proportional to the applied film-forming potential. A high anodic potential of 0.7 V (SCE) may cause the over-injection of OH\(^-\) and induce a compositional transformation in the outer layer [93], i.e., the formation of iron hydroxides. The iron hydroxides has an irregular structure [162], as shown in the \textit{in-situ} observation in Fig. 5.8, which is confirmed by the dramatic decrease of the ratio of lattice O\(^2\)/lattice OH\(^-\) in Fig. 5.11c.

It is noted that the potential for oxygen evolution, i.e., \(\text{O}_2 + 2\text{H}_2\text{O} + 4e \rightarrow 4\text{OH}^-\), can be calculated by:

\[
E = E_{\text{O}_2/\text{OH}^-}^0 - \frac{0.0592}{4} \log \frac{p_{\text{O}_2}}{[\text{OH}^-]^4} \tag{5.9}
\]

where \(E_{\text{O}_2/\text{OH}^-}^0\) is the standard equilibrium potential for oxygen evolution (1.229 V, standard hydrogen electrode, SHE), \(p_{\text{O}_2}\) is partial pressure of oxygen, and \([\text{OH}^-]\) is the concentration of OH\(^-\) ions in the solution (pH 9.5). The oxygen evolution potential in this system is 0.669 V (SHE), or 0.425 V (SCE). Thus, the potentials selected for passivation of the steel, i.e., 0.5 V (SCE) and 0.7 V (SCE), exceeds the oxygen evolution potential. However, the effect of oxygen evolution on steel passivation and the passive film is ignorable. The presence of passive film would block the access of oxygen molecules to the steel surface. According to PDM stated above, the ionic species diffusing in the passive film is oxygen anion, i.e., O\(^2-\), rather than O\(_2\).
5.4.2 Effect of topographic features on corrosion resistance of passive films

This work shows that the passive films formed at passive potentials of -0.1 V (SCE), 0.5V (SCE) and 0.7 V (SCE) possess different corrosion resistance. As shown in electrochemical impedance measurements in Figs. 5.2 and 5.3, along with the positive shift of the passive potential from -0.1 V (SCE) to 0.5 V (SCE), the film becomes more protective, as indicated by the increasing film resistance and decreasing film capacitance. This is mainly attributed to the improved compactness and increased thickness of the passive film. The chemical composition of the passive film does not change. For the passive film formed at a potential near the active-passive transitional range, such as -0.1 V (SCE), the initial passive film is not homogeneous on the steel surface. The presence of exposed steel causes a high anodic current density at the initial stage of the film formation, as shown in Fig. 5.4. After 5 min of passivation, the passive film covers the specimen surface. The AFM observation shows that the passive film formed at -0.1 V (SCE) has a topographic fluctuation of 4.5 nm, which is approximately equal to the average thickness estimated by XPS. When the steel specimen is polarized at a more positive potential, such as 0.5 V (SCE), the formed passive film is thicker and more compact at individual passivating time compared to the passivation at -0.1 V (SCE). As the composition of passive films does not change in this potential range, the increased corrosion resistance is mainly attributed to improved sub-structure of the film during the positive shift of film-forming potential. Even though the passive film formed at 0.7 V (SCE) can be thicker than that formed at -0.1 V (SCE) and 0.5 V (SCE), the passive film shows significant changes
in the chemical composition and presents an irregular structure, reducing the corrosion resistance of the film.

5.5 Conclusions

The X100 pipeline steel can be passivated in the concentrated carbonate-bicarbonate solution over a wide passive potential range from -0.1 V (SCE) to 0.8 V (SCE). As the passive potential shifts from -0.1 V (SCE) to 0.5 V (SCE), the passive film becomes more protective, as indicated by the increasing film resistance and decreasing capacitance. However, when the passive potential exceeds positively 0.5 V (SCE), the passivation degrades as indicated by the decreasing film resistance and the increased capacitance of the passive film.

The improved corrosion resistance of the passive film with the increasing passive potential from -0.1 V (SCE) to 0.5 V (SCE) is attributed to the increased thickness of the film and improved sub-structure of the passive film. For the passive film formed at a potential near the active-passive transitional range, such as -0.1 V (SCE), the film contains numerous scale-like spots that are composed of a mixture of Fe$_3$O$_4$, Fe$_2$O$_3$, and iron hydroxides. As the steel specimen is passivated at a more positive potential, such as 0.5 V (SCE), a thicker passive film containing finer features is formed on the steel surface, while the composition of the film keeps almost unchanged. When the film-forming potential is up to 0.7 V (SCE), the content of iron hydroxides in the film increases, resulting in an irregular structure of the passive film following with the reduction of the corrosion resistance.
Chapter Six: Nanopatterning of steel by one-step anodization for anti-adhesion of bacteria

6.1 Introduction

Steels have been the most widely used engineering materials in current civilization due to their availability, economic benefits, and unreplaceable mechanical property. However, the steels usually suffer from degradation by various mechanisms when exposed to aqueous environments. Particularly, MIC and biofouling are two primary mechanisms resulting in detrimental effects on steel structures [3]. Of various microorganisms that can adhere to metals (steels), SRB, one type of anaerobic bacteria using sulfate as a terminal electron acceptor to degrade organic compounds, are widely spread in environments and easily form a biofilm on the steel surface [4]. It was estimated that corrosion loss induced by SRB accounts for over 50 % of all MIC [163].

Nanoscale surface topography enables controlling or even elimination of bacterial adhesion to metals by affecting the bacteria-substrate interaction [164, 165]. This provides an environment-friendly alternative that avoids introduction of chemicals such as biocides or inhibitors to the environment to potentially result in toxicity and low durability of the system. Electrochemical approaches, such as anodization and electrodeposition, have been demonstrated as efficient and convenient methods to generate nanostructures on metals [7]. They enable coherent growth of coating on the substrate and ensure well binding between the layers. Primarily, electrochemical fabrication of nanostructure is conducted on metals

* This work has been published as: Shiqiang Chen, Yuan Li, Y. Frank Cheng, Nanopatterning of steel by one-step anodization for anti-adhesion of bacteria, Scientific Reports 7 (2017) 5326-5334. Shiqiang Chen and Yuan Li contributed equally to this work.
such as aluminum, titanium and their alloys, as well as stainless steels, which are easily passivated to form a uniform passive film on the metal surface [55, 56]. Some works used fluorinated chemicals, which are environmental hazards and difficult to treat, in order to achieve the nano-patterning [51]. To date, nanopatterning by “green” electrochemical methods on pipeline steels, the most commonly used engineering materials, has been rarely reported. This is attributed to the facts that the pipeline steels are usually electrochemically active in most environments. Moreover, they are chemically and structurally non-uniform at the nanometer scale. The anisotropy of the steels affects the growth of the surface film, usually resulting in an inhomogeneous coating.

In this work, we use a one-step anodization technique to develop nanostructured coatings on the surface of an X100 pipeline steel in 50 wt. % NaOH solution, for controlling bacterial adhesion to steel. The morphology, structure, and composition of the coatings are characterized by SEM, AFM and Raman spectroscopy. We measure the contact angle of the nanopatterned surface of the steel to determine its hydrophobicity. Moreover, we quantify the SRB adhesion to the steel as a function of anodizing potential by fluorescent analysis. The mechanistic aspects of the formation of nanocoating on the steel during anodization and the effective anti-adhesion to SRB on the nanopatterned steel are discussed. We demonstrate that the technique reported in this work provides a promising alternative for surface nanopatterning of pipeline steels, effectively controlling bacterial adhesion and prevention of MIC and biofouling to maintain the integrity of facilities.
6.2 Experimental

6.2.1 Material and specimen preparation for anodization

Specimens used in this work were cut from an X100 steel plate, with a chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016, Mo 0.2, V 0.005, Cu 0.243, Al 0.027, S 0.005, P 0.018, and Fe balance. The specimens used for anodization were machined into rectangular shape, with a dimension of 10 mm × 10 mm × 1 mm. They were embedded into epoxy resin, leaving an exposed area of 100 mm². The exposed surface was sequentially ground by emery papers up to 1,200 grit, then polished by 0.5 µm diamond paste, and degreased in ethanol using an ultrasonic bath, rinsed with deionized water, and dried by highly purified nitrogen.

The prepared steel specimen, which was used as an anode, and an X100 steel strip (cathode, with a dimension of 100 mm × 10 mm × 1 mm) were immersed into a thermostatic beaker containing 50 wt. % NaOH solution, and connected to the positive and negative terminals of a direct current power supply, respectively. The experimental setup is shown in Fig. 6.1a. The distance between the anode and the cathode was 50 mm. The solution was stirred by a magnetic bar at 600 rad/min during anodization. The temperature of the solution was monitored by a thermometer and maintained at 30 °C through a water bath. The steel specimen was anodized for 10 min at various potentials (Fig. 6.1b). After anodization, the specimen was removed and washed with deionized water and ethanol, and dried by high-purity (99.999 %) nitrogen.
Fig. 6.1 (a) Schematic setup for anodization of X100 pipeline steel. (b) Potentiodynamic polarization curve of X100 steel in 50 wt. % NaOH solution. The anodic curve includes two sections. When the potential is between 0.08 V and 1.5 V vs. SCE, the steel is in passivity. When the potential is from 1.5 to 5 V vs. SCE, the steel is in transpassive range. In this work, the potentials of 1.0 V, 1.5V, 2.0V, 3.0V and 4.0 V are selected as the anodizing potentials, as marked with red stars.

Prior to anti-bacterial testing, the samples were sterilized by exposure to ultraviolet radiation for 30 min.

6.2.2 Surface characterization

The morphology of the anodized steel specimens was characterized using a field emission scanning electron microscope (FEI Quanta 250 FEG). When using the SEM to observe the surface morphology of the anodized steel after 18 h of immersion in SRB medium, the specimen was washed with phosphate buffered saline (PBS, 0.05M, pH=7.4) solution for three times, immersed in 2.5 % glutaraldehyde solution for 2 h, and then
washed with PBS solution for three times. After that, the specimen was dehydrated with different concentrations of ethanol (30 %, 50 %, 70 %, 90 % and 100 % for 15 min each), fully dried in high-purity (99.999 %) nitrogen.

An atomic force microscope (Keysight 5500 scanning probe microscope system) was used for topographic characterization on the anodized steels. A scanner carrying a rectangular cantilever with a spring constant of 48N/m (resonant frequency 150 kHz, apex radius<10 nm) was placed above the specimen. The scanning mode was configured as tapping, with a scanning rate of 0.5 Hz and a resolution of 512 × 512 pixels. The supplied software was used to create 3-D topographic images and calculate the surface roughness.

The composition of the coating formed on the anodized steels was characterized by Raman spectra, which were recorded through a Witec alpha 300 R Confocal Raman Microscope (WITec GmbH, Germany) using a 532 nm laser source. Integration time was 60 seconds with 3 accumulations.

Bacterial attachment on the anodized steel specimens was observed by a confocal laser scanning microscope (CLSM, Olympus FV-1000). After 18 h of immersion in the SRB-containing PBS solution, the steel specimens were washed with a sterile PBS solution, and then stained with a fluorescent dye (Molecular Probes™ FilmTracer™ LIVE/DEAD® Biofilm Viability Kit) in darkness according to the manufacturer's procedure. The Kit utilized the mixture of SYTO™ 9 green fluorescent nucleic acid stain and red-fluorescent nucleic acid stain, i.e., propidium iodide (PI). The SYTO 9 stain could generally label all bacteria in a population, and the PI penetrated those bacteria with damaged membranes, causing a reduction in the SYTO 9 stain fluorescence while both dyes were present. Therefore, in this work, the SRB with intact cell membranes stained fluorescent green,
whereas the SRB with damaged membranes stained fluorescent red. The tests were conducted in an anaerobic glove box.

Water contact angles were measured on the steel surface using a contact angle meter (100-26-TH, Ramé-hart Instrument Co.), which was combined with a video camera and software for image capture and analysis. A sessile water droplet of ~0.75 μL was placed on the steel surface using a microsyringe. The image was captured within 5 s of the water drop placement on the specimen.

6.2.3 Bacterium culturing and anti-adhering test

The SRB (Desulfovibrio desulfuricans subsp. desulfuricans (Beijerinck) Kluyver and van Niel) used in this work were purchased from American Type Culture Collection (ATCC). The following procedure was used to prepare the culture solution. Chemicals including 2.0 g MgSO\(_4\), 1.0 g CaSO\(_4\), 0.5 g K\(_2\)HPO\(_4\), 0.5 g (NH\(_4\))\(_2\)SO\(_4\), 5 g sodium citrate, 3.5 g sodium lactate and 1.0 g yeast extract were added to 1 L of deionized water. The sealed mixture was autoclaved at 121 °C for 20 min. After cooling in air to an ambient temperature, the culture medium was purged with N\(_2)/CO_2\) (9:1) gas for 20 min to remove oxygen until dissolved oxygen is lower than 0.4 mg/L, which is measured using a dissolved oxygen meter (ExStik DO600). The pH of the prepared culture medium was adjusted to 7.5 using 1 M NaOH solution. The SRB were then added in the medium for growth at 30 °C. The bacterial growth curves in the culture medium and PBS solution were measured by the most probable number (MPN) method.

After 4 days of SRB culturing, the concentration of SRB cells is increased to 9.26×10\(^7\) CFU/mL, as seen in Fig. 6.2a. The 200 mL culture medium were washed for 3 times using
PBS to eliminate the sulfide and metabolic products in the culture medium. The SRB cells were then inoculated to 200 mL PBS solution, and the concentration of SRB cells is about $9.07 \times 10^7$ CFU/mL (Fig. 6.2b). The steel specimens were immersed in the SRB-containing PBS solution in a 30 °C incubator in darkness for 18 h for anti-adhering testing. The testing was performed in duplicate, with each one using three parallel steel specimens which were under various anodizing treatments. All the tests were conducted in an anaerobic glove box.

Fig. 6.2 The growth curve of SRB in (a) culture medium and (b) PBS solution. From the third day to the tenth day, the SRB growth maintains a stable metabolic activity in the culture medium, which is called stable phase. The SRB in this stage are used for anti-adhering testing. After 8 h of culturing, the bacterial concentration keeps stable around $7.1 \times 10^7$ CFU/mL, which indicates that SRB are alive and maintain metabolic activity in the oligotrophic PBS solution.
6.2.4 Electrochemical measurements

Electrochemical measurements were conducted on the anodized steel specimens in the anaerobic PBS solution in a three-electrode electrochemical cell, where the anodized steel specimen was used as WE, a carbon rod as CE, and an SCE as RE. The OCP of the steel specimens was monitored using an electrochemical workstation (Gamry reference 600) in a water bath of 30 ℃ for 18 h.

The potentiodynamic polarization curve of X100 steel in 50 wt. % NaOH solution was measured at a potential scanning rate of 0.33 mV/s after the OCP of the steel reached a steady state value.

6.3 Results

6.3.1 Characteristics of the nanostructured coating formed on the anodized steel

We prepare nanostructured coatings on the surface of X100 pipeline steel in 50 wt. % NaOH solution by one-step anodization at various potentials. Fig. 6.3 shows the SEM view of the morphology of the coatings formed at various anodizing potentials at 30 ℃ for 10 min, where photos taken at two magnifications (i.e., 25,000 and 100,000 times) are given for each anodizing condition. It is noted that the SEM photos represent the ones with the best quality of numerous images taken by the equipment. The anodized coatings do not have a proper conductivity. Moreover, the presence of magnetic components, such as Fe₃O₄, in the coating makes it very difficult to obtain a better picture at the high magnitude of 100,000 times.
Fig. 6.3 SEM views of the morphology of the nanostructured coatings. (a) Bare steel, (b-f) Coatings formed at the anodizing potentials of 1.0 V, 1.5 V, 2.0 V, 3.0 V and 4.0 V, respectively. For each anodizing condition, photos taken at magnifications of 25,000 times and 100,000 times are given.

For compositional characterization of the coatings, the Raman spectroscopy is used and the results are shown in Fig. 6.4. Obviously, the morphological feature and composition of the nanostructured coatings depend heavily on the anodizing potential. For bare steel, the surface is flat and smooth (Fig. 6.3a). There is no iron oxide formed, where the broad peak from 700 to 900 cm$^{-1}$ is from the environment [166], as seen in Fig. 6.4b. When the anodizing potential is 1.0 V, a uniform, compact coating containing fine nanoparticles with the average diameter of about 37 nm is formed on the steel surface (Fig. 6.3b). The coating is composed of magnetite (Fe$_3$O$_4$), as indicated by a broadband peak around 670 cm$^{-1}$ and
another two weak broad peaks around 538 cm\(^{-1}\) and 306 cm\(^{-1}\) in the Raman spectrum (Fig. 6.4b) [167]. The open circuit potential (OCP) of the steel anodized at 1.0 V in phosphate buffered solution (PBS) drops rapidly from the initial -74 mV vs. saturated calomel electrode (SCE) to the steady value of -712 mV vs. SCE after 18 h of immersion, as shown in Fig. 6.5. This indicates that the coating formed at the anodizing potential of 1.0 V is active and cannot maintain stable in the solution.

Fig. 6.4 Compositional characterization of the coatings by Raman spectroscopy. (a) Bare steel and coatings formed at various anodizing potentials. (b) Enlarged spectra measured on the bare steel and the steels anodizing at 1.0 V and 1.5 V.
Fig. 6.5 Evolution of open circuit potential of bare steel and the anodized steels at various potentials in PBS solution as a function of time. With the increasing time, the potential of bare steel keeps unchanged, while those of the anodized steels decrease and then reach steady-state values after 18 h of immersion.

When the steel is anodized at 1.5 V, a uniform coating containing larger nanoparticles is formed, as seen in Fig. 6.3c. The Raman result shows that the main component of the coating is also Fe$_3$O$_4$, but the intensity of the peaks becomes stronger (Fig. 6.4b), indicating that the coating contains more Fe$_3$O$_4$ at the anodizing potential of 1.5 V than at 1.0 V. The OCP measurement on the steel anodizing at 1.5 V gives the steady state value of -136 mV vs. SCE (Fig. 6.5), which is much less negative than the steady OCP of -712 mV vs. SCE.
when the steel is anodized at 1.0 V, indicating the increased stability of the coating in the solution. At the anodizing potential of 2.0 V, the size of nanoparticles and the feature of the coating are different from that anodized at 1.5 V. The nanoparticles with an average diameter of around 60 nm distributes uniformly on the coating. Some irregular nanopores with the diameter less than 150 nm and nanocracks with a width about 15 nm can be observed in Fig. 6.3d. The Raman spectrum indicates that, in addition to Fe₃O₄, maghemite (γ-Fe₂O₃) is also formed on the steel that is anodized at 2.0 V, as indicated by the new peaks at 360 cm⁻¹, 500 cm⁻¹ and 704 cm⁻¹ (Fig. 6.4a) [167]. The intensity of the Fe₃O₄ and γ-Fe₂O₃ peaks are stronger, which means that there are more iron oxides produced in the coating when formed at 2.0 V. The steady-state OCP of the steel anodizing at 2.0 V is -121 mV vs. SCE, which becomes further less negative, demonstrating the improved stability of the coating anodizing at 2.0 V, as seen in Fig. 6.5.

When the anodizing potential is increased to 3.0 V and 4.0 V, while the size of nanoparticles remains unchanged compared to that obtained at 2.0 V, the nanopores become bigger. The integrity of the coating is worse along with the presence of broken areas in the coatings, as seen in Figs. 6.3e and 6.3f. In addition to Fe₃O₄ and γ-Fe₂O₃, hematite (α-Fe₂O₃) is formed on the coating, which is indicative of the new peaks around 220 cm⁻¹, 282 cm⁻¹, 397 cm⁻¹, 487 cm⁻¹ and 604 cm⁻¹ in Fig. 6.4a [167]. The intensity of the peaks for α-Fe₂O₃ at 3.0 V is smaller than that at 4.0 V, showing that the amount of α-Fe₂O₃ in the coating increases with the anodizing potential. The stability of the coating increases with the anodizing potentials, as indicated by the more positive OCP in Fig. 6.5. The OCP values of the coated steel are -121 mV vs. SCE, -70 mV vs. SCE, and 90 mV vs. SCE at the polarizing potentials of 2.0 V, 3.0 V, and 4.0 V, respectively. This behavior is
related to the increase of the coating thickness as the anodizing potential increases. As shown in Fig. 6.6, the thickness of the coating increases with the anodizing potential. For example, the thicknesses of the coating at potentials of 1.0 V, 1.5 V, 2.0 V, 3.0 V and 4.0 V are about 1 µm, 2 µm, 9 µm, 11 µm and 15 µm, respectively.

![SEM views and Fe element maps of the cross-section of anodizing coatings formed at potentials of (a) 1.0 V, (b) 1.5 V, (c) 2.0 V, (d) 3.0 V and (e) 4.0 V. The thickness of the coating increases with the potential.](image)

Fig. 6.6 SEM views and Fe element maps of the cross-section of anodizing coatings formed at potentials of (a) 1.0 V, (b) 1.5 V, (c) 2.0 V, (d) 3.0 V and (e) 4.0 V. The thickness of the coating increases with the potential.

The AFM topographic images of the steel anodizing at various potentials are shown in Figs. 6.7a-6.7f. The results are well consistent with the SEM views in Fig. 6.3. It is seen that, with the anodizing potential increasing from 1.0 V to 1.5 V and 2.0 V, the nanostructured coating is more uniform, along with the increasing size of nanoparticles.
(Figs. 6.7b-6.7d). When the potential is up to 3.0 V and 4.0 V, holes and broken areas are present on the coating (Figs. 6.7e and 6.7f). The surface roughness of the anodized steels at various potentials is shown in Fig. 6.7g, where the RMS roughness is derived from the AFM topographic profile of the surface coatings. It is seen that the coating formation reduces the surface roughness of the bare steel from 2.62 nm to 1.87 nm. This is attributed to the fact that the nanoscale coating can eliminate topographic irregularities present on the steel substrate [168]. With the increases in the anodizing potential, the surface roughness increases, which is due to the growing nanoparticles and the presence of holes/broken areas on the coating, especially at high anodizing potentials. The hydrophobicity of the nanostructured coating is characterized by contact angle measurements, and the result is shown in Fig. 6.7h. The water contact angle of the bare steel is about 65.08°, indicating that the steel is hydrophilic. Upon anodization, the contact angle increases. At the anodizing potentials of 1.0 V, 1.5 V, and 2.0 V, the contact angles are 81.98°, 112.90°, and 118.53°, respectively. However, with the further increase of the potential to 3.0 V and 4.0 V, the contact angle decreases to 104.43° and 91.16°, respectively. Thus, the anodization treatment is able to improve the hydrophobicity of the steel. The maximum hydrophobicity is achieved when anodizing the steel at 2.0 V, as indicated by the largest contact angle.
Fig. 6.7 (a-f) AFM images of bare steel and the steel anodizing at 1.0 V, 1.5 V, 2.0 V, 3.0 V and 4.0 V, respectively, in 50 wt. % NaOH solution at 30 °C for 10 min. (g) The surface roughness derived from AFM images. (h) The contact angles of bare steel and the steel anodizing at various potentials, each data point was attributed to at least three measurements.
6.3.2 Anti-adhesion to SRB on the nanopatterned steel

To characterize the anti-adhesion to SRB by the nanopatterned steel, we conduct the fluorescent accounting of vital bacteria on the surface of anodized steel specimens. Fig. 6.8 shows the fluorescent images of bare steel and the steel anodizing at various potentials after 18 h of immersion in SRB-containing PBS solution and the statistic results of the quantity of adhered SRB cells on the steel. Obviously, SRB cells adhere extensively on the bare steel surface (Fig. 6.8a), and the quantity of the adhered SRB cells is about 4.7×10⁴ CFU/mm². In addition to SRB cells, there are abundant of extracellular polymeric substances (EPS) and corrosion products present on the steel surface as well, as seen in Fig. 6.9a. Thus, the X100 pipeline steel is vulnerable to SRB cell adhesion and corrosion. Upon anodization to form a layer of nanostructured coating, the SRB adhesion is reduced remarkably. When the steel is anodized at 1.0 V and 1.5 V, the density of SRB cells adhered on the steel surface decreases obviously (Figs. 6.8b and 6.8c). From the statistic analysis, the density of adhered SRB cells on the steel anodized at 1.0 V and 1.5 V are 2.09×10⁴ and 1.0×10⁴ CFU/mm², respectively. Moreover, the EPS and corrosion products decrease with the increased anodizing potential, as shown in Figs. 6.9b and 6.9c. When the anodizing potential is increased to 2.0 V, there is a further decrease of the density of adhered SRB cells (Fig. 6.8g), and the amount is 2.0×10³ CFU/mm² only. However, when the anodizing potential is up to 3.0 V and 4.0 V, the amount of SRB cells on the steel surface increases slightly (Fig. 6.8g), and the density of adhered SRB cells are 5.6×10³ and 8.1×10³ CFU/mm², respectively. There is no EPS adhered on the steel anodized at 2.0 V, 3.0 V and 4.0 V (Figs. 6.9d-6.9f). Thus, an optimal anodizing potential exists, where the
nanostructured coating formed on the steel possesses the best performance against SRB adhesion. In this work, the optimal anodizing potential is 2.0 V for X100 steel. Compared to bare steel, there is a 23.5 time of reduction of the quantity of adhered SRB cells to the nanopatterned steel anodizing at 2.0 V.

Fig. 6.8 Fluorescent images of bare steel and the nanopatterned steels and the statistical quantity of adhered SRB cells. (a-f) The fluorescent images of bare steel and the anodized steels at 1.0 V, 1.5 V, 2.0 V, 3.0 V and 4.0 V, respectively, taken after 18 h of immersion in SRB-containing PSB solution. (g) Statistic results of the quantity of SRB cells adhered on the steels anodizing at various potentials.
Fig. 6.9 SEM views of (a) bare steel, and (b-f) the steels anodizing at (b) 1.0 V, (c) 1.5 V, (d) 2.0 V, (e) 3.0 V and (f) 4.0 V after 18 h of immersion in SRB-containing PBS solution.

6.4 Discussion

6.4.1 Formation of nanostructured coatings by anodization of pipeline steel

The anodic reaction of pipeline steels in an alkaline solution is oxidation of iron to Fe$^{2+}$, Fe$^{3+}$, and Fe$^{6+}$ along with the increasing potential, where Fe$^{6+}$ is present in the form of FeO$_4^{2-}$ in alkaline solutions. Analysis of electrolyte after anodization at various potentials shows that FeO$_4^{2-}$ is not formed under low potentials (i.e., 1.0 V and 1.5 V), as shown in Figs. 6.10a and 6.10b. When the anodizing potential exceeds 2.0 V, the FeO$_4^{2-}$ concentration increases with the potential (Figs. 6.10c-6.10e). This work finds that, at the potentials of 1.0 V and 1.5 V, the formed iron oxides are mainly Fe$_3$O$_4$. According to the
proposed mechanism illustrated in Fig. 6.11a, the oxidation of Fe to \( \text{Fe}^{2+} \) and \( \text{Fe}^{3+} \) takes place on the steel surface by:

\[
\begin{align*}
\text{Fe} & \rightarrow \text{Fe}^{2+} + 2e \quad (6.1) \\
\text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} + e \quad (6.2)
\end{align*}
\]

According to PDM [92], electrons and oxygen vacancies (\( \text{V}_{\text{O}}^{2-} \)) are generated by converting \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \). \( \text{V}_{\text{O}}^{2-} \) migrate towards the solid/solution interface, where \( \text{O}^{2-} \) is injected into the outer layer, causing dissolution of Fe oxide to generate \( \text{Fe}^{3+} \). Anodization of the steel at 1.0 V and 1.5 V results in the formation of iron oxide with an average valence equivalent to \( \text{Fe}_3\text{O}_4 \), and the coating grows until the transport flux of charged species through the oxide becomes equivalent to the dissolution rate of \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \) at the solid/solution interface, as shown in Fig. 6.11a.

Fig. 6.10 Digital photos of the electrolyte used for anodization at (a) 1.0 V, (b) 1.5 V, (c) 2.0 V, (d) 3.0 V and (e) 4.0 V. The concentrations of \( \text{FeO}_4^{2-} \) in each electrolyte are given.
Fig. 6.11 Mechanisms for the formation of nanostructured coating during anodization of pipeline steel. (a) Formation of Fe$_3$O$_4$ at 1.0 V and 1.5 V. (b) Formation of Fe$_3$O$_4$ and γ-Fe$_2$O$_3$ at 2.0 V. (c) Formation of Fe$_3$O$_4$, γ-Fe$_2$O$_3$ and α-Fe$_2$O$_3$ at the anodizing potentials of 3.0 V and 4.0 V. Yellow balls refer to Fe$^{3+}$ ions, green balls for Fe$^{2+}$ ions, blue balls for O$^{2-}$ ions, and mazarine balls for Fe$^{6+}$ ions.

When the anodization potential is increased to 2.0 V, in addition to the formation and growth of Fe$_3$O$_4$, the conversion of Fe$_3$O$_4$ to γ-Fe$_2$O$_3$ becomes feasible since both oxides have a similar crystallographic structure [169]. It has been confirmed that oxide coatings can be composed of a Fe$_3$O$_4$ inner layer and a Fe$_2$O$_3$ outer layer [93]. As illustrated in Fig. 6.11b, after formation of Fe$_3$O$_4$, it is converted to γ-Fe$_2$O$_3$ through the migration of V$_{O^2-}$ towards the γ-Fe$_2$O$_3$/solution interface, followed by injection of O$^{2-}$ into the oxide matrix. During this process, some Fe$^{3+}$ lose electrons to form Fe$^{6+}$, which reacts with OH$^{-}$ to form FeO$_4^{2-}$:
Fe³⁺ + 8OH⁻ → FeO₄²⁻ + 4H₂O + 3e

(6.3)

This is confirmed by the low concentration of FeO₄²⁻ (0.023 mmol/L) measured in the electrolyte, as seen in Fig. 6.10c. Thus, the coating formed at 2.0 V is a mixed Fe₃O₄/γ-Fe₂O₃ oxide, with Fe₃O₄ in the inner region and γ-Fe₂O₃ in the outer region of the coating.

When the anodizing potential increases to 3.0 V and 4.0 V, as shown in Fig. 6.11c, the ferric oxides loss electrons at the oxide/solution interface to produce FeO₄²⁻, which reacts to form α-Fe₂O₃ and O₂, as indicated by the presence of bubbles during anodization at 3.0 V and 4.0 V [52]:

4FeO₄²⁻ → 2Fe₂O₃ + 5O₂ + 8e

(6.4)

The α-Fe₂O₃ deposits on the steel surface, as shown by the high intensity of characteristic peaks of α-Fe₂O₃ in Fig. 6.4a. The increased surface roughness of the nanostructured coating and the reduced electrochemical stability, compared to the coating formed at the anodizing potential of 2.0 V, are associated with the dissolution of ferric oxides.

6.4.2 Anti-adhesion to SRB on the nanopatterned steel

Bacterial adhesion and the biofilm formation are essential to MIC occurrence and biofouling of metals. The adhesion of a bacterial cell to the metal is governed by several factors, including the physicochemical properties of the bacteria and the metal, and the
environmental conditions [170, 171]. In this work, the physicochemical property of the X100 pipeline steel is the dominant factor for SRB adhesion under the given testing condition. Both SEM and OCP results show that the bare steel and the anodized steel at 1.0 V and 1.5 V are not sufficiently stable in SRB medium, as seen in Fig. 6.5 and Figs. 6.9a-6.9c. The Fe^{2+} ions formed during steel corrosion can react with EPS [172], which are produced through SRB metabolism and have a high complexation activity, facilitating the SRB adhering to the steel [173]. Thus, extensive SRB cells are observed on the surface of bare steel and the steel anodizing at 1.0 V and 1.5 V (Fig. 6.8).

The steel anodizing at 2.0 V, 3.0 V, and 4.0 V is stable in the SRB-containing medium. Generally, the surface roughness and hydrophobicity are two important properties affecting the bacterial adhesion to metals [174]. Extensive studies have shown that bacterial attachment is directly related to the surface roughness at nanoscales [175]. The steel anodizing at 2.0 V shows the best performance for anti-adhesion by SRB due to the smallest surface roughness compared to those anodizing at other potentials. As shown in Fig. 6.9, the adhesion of SRB (i.e., gram-negative bacteria) on the steel specimens anodized at 2.0 V, 3.0 V, and 4.0 V shows a similar characteristic, i.e., a hair-like nanofiber is present between the SRB cell and the coating (see the insert figures). This is called pilus and fimbria, the most well-known proteinoids adhesion for gram-negative bacteria. The adhesion of this protein on nanoscale surfaces depends closely on the surface roughness [176]. With an increase of the surface roughness, the fraction of proteins orienting perpendicularly to the surface increases since a protein needs a small area to adsorb perpendicularly to the surface. The perpendicular orientation is usually energetically favorable because of the possibility for the protein to form additional bonds to the surface.
Moreover, hydrophobic bacteria prefer to adhere to hydrophobic surfaces, and hydrophilic bacteria adhere well to hydrophilic surfaces [178]. The SRB used in our study is hydrophilic [179]. The maximum contact angle measured on the steel anodizing at 2.0 V proves the best hydrophobicity for the nanopatterned steel at this condition, which thus possesses the best property against SRB adhesion. Furthermore, it is noted that, from Fig. 6.9, although the cellular morphology of SRB on the surface of anodized samples (2.0 V, 3.0 V, and 4.0 V) are not as dense as that on bare steel, they are still integral. This indicates that SRB are not killed on the specimen surface. The decreased bacterial number is attributed to the anti-adhesion effect of the nanostructured anodizing coating.

6.5 Conclusions

The one-step anodization technique reported in this work has the potential to replace the conventional methods, e.g., biocides and anti-biofouling coatings, for anti-adhesion of bacteria such as SRB on pipeline steels. We find that, at the anodizing potential of 2.0 V, a homogeneous, compact nanostructured coating is formed, which possesses the best hydrophobicity (with a water contact angle of 118.53°) and anti-adhesion performance to SRB (a 23.5 time of reduction of SRB adhesion compared to bare steel). The main components of the nanostructured coating contain Fe$_2$O$_3$, have a good mechanical strength and chemical stability in aqueous environments. Our technique to form a nanostructured coating on pipeline steel is simple, economic and environment-friendly, providing a promising approach to for industry-area fabrication. Furthermore, the Fe$_3$O$_4$ and α-Fe$_2$O$_3$ nanoparticles formed during anodization on pipeline steel are multifunctional, such as
magnetism and photocatalytic activity, offering bright perspectives in applications in a wide variety of areas.
Chapter Seven: Visible light enhanced anti-adhesion and deactivation of bacteria on nanostructured iron oxide coatings

7.1 Introduction

Bacterial attachment and biofilm formation on structural materials have been a common problem in various industry sectors [122]. Generally, high-performance coatings capable of anti-attachment of bacteria and biofouling have been used due to their high efficiency and easy application [9]. However, biocides and heavy metal ions contained in the coatings serving as the functional matters are usually toxic, impacting adversely the sustainability of environments and the ecosystem [10].

Recently, novel surface engineering and nanotechnology have been developed for effective control of bacterial attachment and biofilm formation on metals. Two main strategies are used for the purpose, i.e., reducing the adhesive force between bacteria and metal, and deactivating (killing) the attached bacteria by environment-friendly ways [11, 12]. Deactivation (killing) of the attached bacteria based on photocatalysis provides a potential alternative for surface engineering due to its environment-friendly mechanism for anti-microbial activity under light illumination [13-15].

As demonstrated in Chapter six, the nanostructured iron oxide coating presents the photocatalytic content of Fe₂O₃ in some specific anodizing conditions. In this work, we were going to fabricate nanostructured iron oxide coatings on a pipeline steel surface by the refined method described in chapter six, and study the visible light enhanced anti-adhesion and deactivation of bacteria on them. The morphology and composition of the

* This work has been submitted to Electrochimica Acta. It is under review.
prepared nanocoatings were characterized by AFM and Raman spectroscopy, respectively. The photoelectrochemical property of the coatings was measured by UV-visible diffuse reflectance spectroscopy. *P. aeruginosa*, one type of bacteria commonly found in natural water and a model strain in biofilm study [180], was used for anti-adhesion and deactivation of the bacteria on the nanostructured oxide coatings under visible light illumination through a CLSM. The morphology of the bacteria adhered to the steel was observed by SEM. The mechanistic aspect for the light-enhanced anti-adhesion and deactivation of the bacteria of the nanostructured iron oxide coatings was discussed.

7.2 Experimental

7.2.1 Materials

Steel specimens used for preparation of iron oxide coatings were cut from an X100 carbon steel pipe, with the chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016, Mo 0.2, V 0.005, Cu 0.243, Al 0.027, S 0.005, P 0.018, and Fe balance. Sodium hydroxide (≥ 98.5 % purity), sodium chloride (≥ 99.5 % purity), methylene blue (MB), 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT, ≥98 % purity), monopotassium phosphate, disodium hydrogen phosphate, tryptone and yeast extract were purchased from Sigma-Aldrich. High-purity nitrogen (99.999 %) was purchased from PRAXAIR. All chemicals were of analytic grade agents, and all solutions were prepared with ultrapure water.
7.2.2 Preparation of nanostructured iron oxide coatings

The steel specimens were machined into two dimensions, i.e., 50 mm × 10 mm × 1 mm and 50 mm × 30 mm × 1 mm, which were used as anode and cathode, respectively, during anodization. After sealing with epoxy, the exposed face of the specimen was sequentially ground by emery papers up to 1,200 grit, then polished by 0.5 µm diamond paste, degreased in ethanol using an ultrasonic bath, rinsed with deionized water, and dried by high-purity nitrogen. The experimental setup for anodization of the steel is shown in Fig. 7.1a. The solution, i.e., 13 M NaOH, was stirred by a magnetic bar at 600 rad/min. The temperature of the solution was maintained at 22 °C, which was monitored by a thermometer. The anodization was conducted at 3.0 V for varied times, i.e., 2 (A-2), 10 (A-10), 30 (A-30) and 60 min (A-60). The steel specimen without anodization was used as the blank specimen (BS). After anodization, the specimens were removed from the solution, washed with deionized water and ethanol, and dried by high-purity nitrogen.

![Experimental setup for anodization of the steel](image)
Fig. 7.1 (a) Schematic diagram of the experimental setup for anodization of the steel specimen. (b) Time dependence of the electrochemical potential of the steel specimen (vs. Hg/HgO) when anodizing at 3.0 V in 13 M NaOH solution. (c) Digital photos of the electrolyte before and after 1 h of anodization.

7.2.3 Characterization of the prepared iron oxide nanocoatings

An AFM (Keysight 5500 scanning probe microscope system) was used for topographic characterization of the prepared iron oxide nanocoatings. The scanner carrying a rectangular cantilever with a spring constant of 48 N/m (resonant frequency 150 kHz, apex radius <10 nm) was operated at a tapping mode. The supplied software enabled the creation of 3-D topographic profiles of the coating surface and calculation of the surface roughness. The composition of the coating was characterized by Raman spectroscopy through a Witec alpha 300 R Confocal Raman Microscope (WITec GmbH, Germany) using a 532 nm laser source. The photoelectrochemical property of the nanocoatings was investigated by a UV-visible diffuse reflectance spectroscopy over a wavelength ranging from 300 to 800 nm using a Cary 5000 spectrophotometer (Agilent, USA).
7.2.4 Bacterium culturing

Fig. 7.2 The growth curve of *P. aeruginosa* in (a) LB medium and (b) PBS solution. From the 18th h to the 70th h, the bacterial growth maintains a stable metabolic activity in the LB medium, which is called the stable phase. The bacteria harvested at the 40th h (as marked by the red arrow) with a concentration of $7.5 \times 10^9$ CFU/mL were used for testing in this work. As the bacteria were re-suspended from LB into the PBS, the bacterial concentration drops gradually until an equilibrium state of $1.6 \times 10^7$ CFU/mL was reached, indicating that bacteria can keep live in the oligotrophic PBS.

*P. aeruginosa* bacteria used in this work were provided by the University of Calgary’s Department of Biology Science. The culturing medium, called lysogeny broth (LB), was prepared by dissolving 10 g tryptone, 5 g yeast extract and 10 g sodium chloride in 1 L of deionized water. The medium was autoclaved at 121 °C for 20 min. After air-cooling down to the room temperature of $22 \pm 1$ °C, the bacteria were inoculated in the medium and
incubated at a rotating rate of 150 rpm at 37 °C. After cultivation for 40 h, the bacteria reached a stable growth phase, as shown in Fig. 7.2a. The culturing medium with a cell concentration of $7.5 \times 10^9$ CFU/mL was centrifuged for 5 min, and re-suspended in 0.05 M PBS (pH=7.4) for 3 times to eliminate the metabolic products in the medium. The bacterial pellets were suspended in the PBS solution to achieve a final concentration of $2.0 \times 10^7$ CFU/mL prior to the further test, as shown in Fig. 7.2b. The bacterial growth curves in both the LB medium and the PBS solution were measured by the MPN method.

7.2.5 Photoelectrochemical measurements

Photoelectrochemical measurements were conducted on the anodized steel specimens in a three-electrode cell, where the anodized steel covered with the prepared nanocoating, a platinum sheet, and an SCE were used as WE, CE and RE, respectively. The light source was a Xenon arc lamp (Ushio, Japan, 150 W). A UV cut-off filter ($\lambda < 400$ nm) and an IR absorbed water filter were used to ensure visible light illumination. The light intensity, which was measured by a light meter (LI-COR, USA), was fixed at 100 mW/cm$^2$ to simulate the natural sunlight. The On-Off photocurrent was measured by an electrochemical workstation (Gamry Reference 600) at 0.0 V bias in either sterilized or bacterium-containing ($2.0 \times 10^7$ CFU/mL of $P. aeruginosa$) 0.05 M PBS solution. Prior to measurements, the coated steel electrode was immersed in the test solution for at least 1 h until a steady-state OCP was reached.
7.2.6 Testing for anti-adhesion of bacteria

The nanostructured steel specimens were immersed in the bacterium-containing PBS solution at room temperature. The simulated visible light ($\lambda > 400$ nm) was applied to investigate photo-enhanced anti-adhesion of bacteria to the nanocoating. The testing was performed in duplicate, with each one using three parallel specimens. After testing, the specimens were washed with sterile PBS, and then stained with a fluorescent dye (Molecular Probes™ FilmTracer™ LIVE/DEAD® Biofilm Viability Kit) in darkness according to the manufacturer's procedure. Bacterial attachment on the coated steel specimen was observed by a CLSM (Olympus FV-1000), where the live and dead cells were identified as green and red colors, respectively. For comparison, the testing for bacterial attachment was also performed on bare steel. At least three regions were selected randomly on each specimen for imaging. A processing software (Image J, National Institutes of Health, USA) was used to measure the coverage ratio of biofilm on the specimens.

The morphology of bacteria attached on the specimens was characterized using a field emission SEM (FEI Quanta 250 FEG). Prior to SEM characterization, the specimens were washed with PBS, immersed in a 2.5 % glutaraldehyde solution for 2 h, and then washed with PBS. After that, the specimens were dehydrated with various concentrations of ethanol (30 %, 50 %, 70 %, 90 % and 100 %) for 15 min each, and fully dried in high-purity (99.999 %) nitrogen environment.
7.2.7 Quantitative measurements of reactive oxygen species

The XTT and MB were used as indicators for reductive oxygen species (O$_2^-$) and oxidative oxygen species ('OH), respectively [15]. The coated steel specimens with an exposed area of 4 cm$^2$ were immersed in a 5 mL PBS solution containing 100 μM XTT or 5 ppm MB. After visible light illumination for various time periods, the concentrations of the reacted XTT and MB were measured by a UV-vis spectrophotometer (UV1800, SHIMADZU) at 470 nm and 664 nm, respectively [181].

7.2.8 Data analysis

The data points present in this work were expressed as mean values with a standard deviation (SD). Analysis of variance was conducted through OriginPro with $p = 0.05$.

7.3 Results and discussion

7.3.1 Characterization of prepared nanostructured iron oxide coatings

Fig. 7.3 shows the AFM images of the blank and coated steel specimens with various anodizing time at 3.0 V in 13 M NaOH solution, as well as the surface roughness of the specimens derived from the AFM images. It is seen that some scratches induced from surface finishing are present on the blank specimen (Fig. 7.3a). When anodized for 2 min, some nanoscale features form on the specimen surface (Fig. 7.3b), and the surface roughness is similar to that of the blank specimen, with an average of about 2.2 nm (Fig. 7.3f). With the increase of the anodizing time, nodular nanofeatures develop on the specimen surface. When the anodizing time is 10 min, the nano-nodules have an average size of around 150 nm and a depth of 60 nm (Fig. 7.3c). The thickness of the wall between
adjacent hollow nano-nodules is about 50 nm. There is the largest surface roughness with the RMS value up to 12.1 nm (Fig. 7.3f). For the specimens anodized for 30 and 60 min, the wall between nano-nodules is not observed, and a compact nanostructured coating is formed (Figs. 7.3d and 7.3e). The RMS roughness of the coated specimens reduces to 10.2 nm and 8.3 nm, respectively (Fig. 7.3f).

Fig. 7.3 AFM images of (a) blank steel specimen and the specimens anodized at 3.0 V in 13 M NaOH solution for (b) 2, (c) 10, (d) 30 and (e) 60 min, respectively. The measured area is 1.0 μm × 1.0 μm and the range of the topographic height is normalized as 100 nm.

(f) The surface roughness of the specimens derived from the AFM images.

The prepared nanostructured specimens are characterized by Raman spectroscopy, and the results are shown in Fig. 7.4. After 2 min of anodization, the coating mainly consists
of $\text{Fe}_3\text{O}_4$, as indicated by the Raman peaks at 306, 538 and 670 cm$^{-1}$ [166]. The broad peaks at 360, 500, 704 and 1360 cm$^{-1}$ indicate that a relatively low amount of $\gamma$-$\text{Fe}_2\text{O}_3$ is formed [182]. When the anodizing time is increased to 10 min, the $\alpha$-$\text{Fe}_2\text{O}_3$ is associated with the Raman shifts at 220, 282, 397, 487, 604, 658 and 1310 cm$^{-1}$ [166]. With the further increase of the anodizing time, the amount of $\alpha$-$\text{Fe}_2\text{O}_3$ increases, while the sum of $\text{Fe}_3\text{O}_4$ and $\gamma$-$\text{Fe}_2\text{O}_3$ reduces, as indicated by the relative intensity of the characteristic peaks.

![Fig. 7.4 Raman spectra of the blank specimen and the steel specimens anodizing at 3.0 V in 13 M NaOH solution for 2, 10, 30 and 60 min, respectively. Offset is applied on the curves for clarity.](image)

During anodization, the electrochemical potential of the steel specimen is maintained constant at 1.13 V (Hg/HgO), i.e., 1.23 V (SHE), as seen in Fig. 7.1b. The pH of the solution is about 15. According to Pourbaix diagram, the equilibrium product is $\text{FeO}_4^{2-}$, which results in a purple color of the electrolyte as seen in Fig. 7.1c [183]. Iron is oxidized, following the sequence of Fe(0) $\rightarrow$ Fe(II) $\rightarrow$ Fe(III) $\rightarrow$ Fe(VI). According to point defects
model [92], Fe(II) is attributed to Fe$_3$O$_4$, and Fe(III) is attributed to both Fe$_3$O$_4$ and Fe$_2$O$_3$. The oxidation of Fe(II) into Fe(III) transforms Fe$_3$O$_4$ into Fe$_2$O$_3$. Thus, more Fe$_2$O$_3$ presents in the prepared oxide coating to replace Fe$_3$O$_4$. The compositional characterization in Fig. 7.4 is well consistent with the analysis.

The photoabsorption spectra of the blank specimen and the nanostructured specimens prepared after various times of anodization at 3.0 V in 13 M NaOH solution are shown in Fig. 7.5. It is seen that, compared to the spectrum recorded on the blank specimen, the specimen anodizing for 2 min shows a higher absorption over the testing wavelength range without any preference, which is resulted from the formation of conductive Fe$_3$O$_4$ on the specimen surface [12]. When the anodizing time increases, the specimens exhibit preferential absorption at the wavelength range below 590 nm. Since the main component of Fe$_2$O$_3$ in the formed iron oxides is a direct band gap material, the band gaps of the prepared specimens with nanostructured oxide coatings are evaluated by extrapolation based on Tauc’s relationship, i.e., $(\alpha h\nu) \propto (h\nu - E_g)^{1/2}$, where $\alpha$ is absorption coefficient, $h\nu$ is the energy of the light, and $E_g$ is band gap [184]. The derived value of 2.1 eV agrees well with the reported direct band gap of Fe$_2$O$_3$ in the literature [42]. There is a similar electronic structure between $\gamma$-Fe$_2$O$_3$ and $\alpha$-Fe$_2$O$_3$, which show the approximately identical absorption spectra [185]. Thus, the results from the optical absorption spectra measured on the nanostructured specimens are consistent with the results identified by the Raman spectra in Fig. 7.4.
Fig. 7.5 (a) UV-Vis diffuse reflectance spectra, and (b) the corresponding Tauc plots of the blank specimen and the specimens anodizing at 3.0 V in 13 M NaOH solution for 2, 10, 30 and 60 min, respectively.
7.3.2 Anti-adhesion of bacteria on the nanostructured iron oxide coatings

Fig. 7.6 shows the CLSM images of the attachment of live and dead *P. aeruginosa* bacteria on blank specimen and the specimens anodizing at 3.0 V for various times in 13 M NaOH solution under various conditions of visible light illumination, i.e., Light-Off 3 h, Light-On 3 h and Light-Off 3 h + Light-On 3 h, respectively. Obviously, there is an extensive bacterial attachment on the blank specimen no matter if the light is illuminated. When the specimen is anodized for 2 min, the attached bacteria reduces in quantity, but slightly only. Bacteria attach extensively to the specimen in both the absence and presence of light illumination. For the specimens anodizing over 10 min, i.e., the specimens A-10, A-30 and A-60, the bacterial attachment is inhibited remarkably, especially upon the light illumination. The OCP measurements of the anodized specimens in PBS solution (Fig. 7.7) show that the iron oxide nanocoating formed for 2 min of anodization may not be sufficiently intact. The solution would penetrate into the pores of the coating to cause a drop of the OCP. Bacteria would attach to the specimen as well. For the nanocoatings formed for 10, 30 and 60 min, they are sufficiently stable and intact, as indicated by the constant OCP over time in the solution, preventing attachment of the bacteria to the specimen effectively.
Fig. 7.6 CLSM images of the live and dead *P. aeruginosa* bacteria (2.0 × 10^7 CFU/mL in PBS) on blank specimen and the specimens anodizing at 3.0 V for various times in 13 M NaOH solution upon varied conditions of visible light illumination, i.e., Light-Off 3 h, Light-On 3 h and Light-Off 3 h + Light-On 3 h, respectively. The green and red colors indicate live and dead cells, respectively. The yellow color is caused by the overlay of live and dead cells. The scale bar is 40 μm.
Fig. 7.7 Time dependence of OCP of the blank steel specimen and anodized specimens in PBS solution. Over the test period, the OCP of BS, A-10, A-30 and A-60 specimens keeps unchanged, while that of the A-2 decreases and then reaches a steady-state value identical to that of the BS. This suggests that the iron oxide coating formed on A-2 specimen is not sufficiently intact. Upon immersion in the PBS solution, the solution penetrates through the coating, exposing the steel substrate directly to the solution and causing the drop of OCP.

It is also seen from Fig. 7.6 that, quantitatively, the A-10, A-30 and A-60 specimens under Light-Off 3 h contain most bacteria on each specimen compared to the illuminating conditions of Light-On 3 h and Light-Off 3 h + Light-On 3 h. Particularly, there are the least bacteria on each specimen under Light-On 3h. These results suggest that, while the
nanostructured iron oxide coating is able to resist bacterial attachment, the visible light illumination can further enhance the anti-adhesion of bacteria on the specimen. Moreover, there are more dead cells on the specimens under Light-Off 3 h + Light-On 3 h condition, indicating that the photocatalytic effect of the iron oxide nanocoating can kill bacteria present on the specimen surface. It is noted that the specimen A-10, i.e., the steel anodizing for 10 min, has the least bacteria under all three illuminating conditions, suggesting the best anti-bioadhesion performance among all the prepared specimens.

To further quantify the bacterial coverage and the ratio of the quantities between dead and live cells on the specimens under various illuminating conditions, statistic results are obtained from fluorescent images and shown in Fig. 7.8. It is seen that the bacterial coverage on the blank specimen and the specimen A-2 are more than 50 % and 40 % under various light conditions, respectively. In particular, the coverages of bacteria on blank specimen are 52.64 %, 53.89 % and 73.39 % under Light-Off 3 h, Light-On 3 h, and Light-Off 3 h + Light-On 3 h, respectively, while those on A-2 are 42.38 %, 43.53 % and 50.63 % under individual light illuminating conditions, respectively, as seen in Figs. 7.8a-7.8c. These indicate that P. aeruginosa bacteria easily adhere to the steel surface (i.e., both bare steel and the steel anodizing for 2 min only) to form a biofilm. The visible light illumination does not influence the bioadhesion. Additionally, the coverage of bacteria on both specimens under Light-Off 3 h + Light-On 3 h is higher than that under Light-Off 3 h and Light-On 3 h. This is mainly due to the increased immersion time of the specimens in the bacterium-containing solution.
Fig. 7.8 Bacterial coverage on the blank specimen and the specimens anodizing for 2, 10, 30 and 60 min under various illuminating conditions (a) Light-Off 3 h, (b) Light-On 3 h, (c) Light-Off 3 h + Light-On 3 h. Asterisks indicate the statistical significance ($p < 0.05$). (d) The ratio of the coverage between dead and live cells on the specimens under various illuminating conditions, where asterisks indicate the statistical significance ($p < 0.05$) against the control condition, i.e., Light-Off 3h. The insets are the zoom-in of each diagram.

For the specimens anodized to form stable nanostructured iron oxide coatings, i.e., specimens A-10, A-30 and A-60, the bacterial coverage remarkably decreases to less than
10 % for all illuminating conditions. Under the Light-Off 3 h, the bacterial coverage increases with the anodizing time (Fig. 7.8a), which is due to the fact that a high surface roughness can reduce the effective contact area for bacterial adhesion [127, 186]. This is well consistent with the results in Fig. 7.3, where the surface roughness of the nanostructured specimens decreases with the extended anodizing time. For the condition of Light-On 3 h, the biofilm coverage on the specimens is dramatically smaller than that under Light-Off 3 h (Figs. 7.8a and 7.8b). Moreover, under the condition of Light-Off 3 h + Light-On 3 h, i.e., the bacteria adhere to the specimen in darkness for 3 h and then under the visible light for 3 h, the bacterial coverage is more than that under Light-On 3 h, but less than that under Light-Off 3 h. Particularly, for specimen A-10 under Light-On 3 h, the bacterial coverage decreases from 0.24 % (Light-Off 3 h) to 0.036 % only, which demonstrates that, compared to bare steel, a 99.9 % anti-bioadhesion is achieved by the formed iron oxide nanocoating under visible light illumination. Obviously, the light illumination further enhances the anti-adhesion of bacteria to the nanostructured coatings.

The effect of the nanostructure of the coating on bacterial adhesion is also demonstrated by the bacterial coverage obtained under the condition of Light-Off 3 h + Light-On 3 h, where the bacterial coverages of specimens A-10, A-30 and A-60 are 0.127 %, 0.701 %, and 5.95 %, respectively. This indicates that the highest surface roughness of specimen A-10 (anodizing at 3.0 V for 10 min) results in the strongest ability against bacterial adhesion, compared to specimens A-30 and A-60 which have smaller surface roughness.

Furthermore, the ratio between the quantities of dead and live cells attached on the specimens after various illuminating conditions is shown in Fig. 7.8d. For Light-Off 3 h, the ratio is nearly 1, which is determined by the life cycle of this strain in PBS, i.e., stable
growth stage (Fig. 7.2b). However, upon visible light illumination, i.e., Light-On 3 h and Light-Off 3 h + Light-On 3 h, the ratio increases apparently, especially for the specimen A-10 under Light-Off 3 h + Light-On 3 h condition, where the ratio of the dead/live cells is around 11. These results suggest that the visible light enhanced anti-adhesion of bacteria is attributed to the bacterial deactivation. The ratio decreases with the anodizing time, confirming again that specimen A-10 possesses the best performance in anti-adhesion by bacteria.

7.3.3 Characterization of bacteria attached on the nanostructured coatings

As seen in Figs. 7.6 and 7.8, the specimen A-10, i.e., the iron oxide nanocoating formed after 10 min of anodization, under Light-On 3 h illuminating condition possesses the best performance for anti-adhesion of bacteria, and is thus selected for the further morphological characterization. Fig. 7.9 shows the SEM images of the bacteria attached to bare steel and the specimen A-10 under various illuminating conditions. It is seen that the shape of *P. aeruginosa* bacteria attached to the bare steel under light illumination (Fig. 7.9a), and that on specimen A-10 without the light illumination (Fig. 7.9b) keep intact, and the bacteria are still alive. However, the bacteria present on the specimen A-10 under visible light illumination are completely damaged (Fig. 7.9c). The comparison indicates that the photocatalytic effect of the iron oxide nanocoating is able to deactivate the bacteria and prevent the biofilm formation.
Fig. 7.9 SEM images of *P. aeruginosa* bacteria attached on (a) bare steel under Light-On 3 h, (b) specimen A-10 under Light-Off 3 h, and (c) specimen A-10 under Light-On 3 h.

The insets photos show the morphology of the bacteria under a high magnification.

7.3.4 Effect of light illumination on bacterial concentration in PBS solution

To confirm if the light illumination affects the population of bacteria in the aqueous medium, the concentration of *P. aeruginosa* bacteria in PBS solution, where the coated steel specimens are immersed, under light illumination is measured, and the results are shown in Fig. 7.10. It is seen that the statistical deviation is not distinguishable between the Control conditions (i.e., no light illumination and no iron oxide nanocoating formed) and other nanostructured specimens under illumination. Thus, the nanostructured iron oxide coating under visible light illumination does not affect the bacterial concentration in the bulk solution, although it can kill the bacteria attached to the specimen surface.
Fig. 7.10 Concentrations of bacteria in the PBS solution where immersed the bare steel and the specimens anodized for 2, 10, 30 and 60 min as a function of the light illuminating time. The data points of Control specimen are measured in darkness.

7.3.5 Quantification of reactive oxygen species produced under light illumination and photocurrent measurements

The yield rates of reductive $O_2^-$ and oxidative radicals are estimated by XTT and MB indicators, respectively, and the results are shown in Fig. 7.11. While an approximate zero of the absorbance intensity of XTT indicator is observed for bare steel and the specimen A-2, a linear increase in the XTT absorbance intensity vs. light illuminating time is
obtained for specimens A-10, A-30, and A-60, as shown in Fig. 7.11a. The results demonstrate a constant yield rate of O$_2^-$ for the specimens A-10, A-30 and A-60. The yield rate of XTT indicator, $r$, can be calculated by:

$$ r = \frac{An}{l\alpha St} $$ (7.1)

where $A$ is the absorbance of XTT indicator measured at 470 nm, $n$ is the reaction coefficient [187], $l$ is the light path of cuvette, i.e., 5 cm, $\alpha$ is the absorbance coefficient of XTT-formazan, i.e., 21600 M$^{-1}$ cm$^{-1}$ [188], $S$ is the surface area of the specimen exposed to solution, i.e., 4 cm$^2$, and $t$ is the illuminating time.

The absorbance of MB under light illumination follows a first-order exponential decay relationship vs. time, and the decay constant, $k$, can be calculated by:

$$ k = \frac{\ln \left( \frac{A_0}{A(t)} \right)}{t} $$ (7.2)

where $A_0$ is the initial MB absorbance intensity, $A(t)$ is the absorbance intensity after a period of illumination, and $t$ is the illumination time. The fitting results of the yield rate of O$_2^-$ and the decay constant of MB are listed in Table 7.1.
Fig. 7.11 (a) The absorbance intensity of XTT-formazan, and (b) normalized concentration of MB as a function of the light illuminating time. The solid lines are the fitting results of the data points.
Table 7.1 The fitting results of the O$_2^-$ yield rate and the decay rate of MB for the bare steel and the specimens anodizing for 2, 10, 30 and 60 min, respectively.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>BS</th>
<th>A-2</th>
<th>A-10</th>
<th>A-30</th>
<th>A-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield rate of O$_2^-$ (μM/cm$^2$ h)</td>
<td>-</td>
<td>-</td>
<td>0.310±0.011</td>
<td>0.248±0.007</td>
<td>0.212±0.010</td>
</tr>
<tr>
<td>Decay rate of MB (1/h)</td>
<td>0.003±0.000</td>
<td>0.004±0.000</td>
<td>0.045±0.004</td>
<td>0.039±0.002</td>
<td>0.035±0.003</td>
</tr>
</tbody>
</table>

Compared to the results obtained on bare steel and the specimen A-2, the concentrations of XTT and MB decrease with time for the specimens A-10, A-30, and A-60 under light illumination, indicating that the prepared iron oxide nanocoatings possess a photocatalytic activity to produce reactive oxygen species under visible light illumination. For the specimen A-10 with the nodular iron oxide nanostructure, there are the highest $r$ and $k$ values, indicating the best photocatalytic activity for killing bacteria attached to the specimen. The reactive oxygen species, i.e., O$_2^-$, and •OH, enable deactivation of bacteria by destroying their physiological functions. In this work, the maximum yield of O$_2^-$ and oxidative radicals on specimen A-10 are about 0.92 μM and 0.18 μM, respectively. Generally, bacteria produce O$_2^-$ in their respiration process, and remove it by relevant enzymes to protect themselves [189]. The amount of O$_2^-$ produced by *P. aeruginosa* bacteria in this work is about 11.74 μM, as seen in Fig. 7.12, which is much more than that produced on specimen A-10 under light illumination. These results mean that the bacteria can survive in the presence of photo-generated O$_2^-$. Therefore, the limited concentration of reactive oxygen species produced by the nanostructured iron oxide coating under visible light illumination cannot fully deactivate the bacteria in the aqueous medium, as
demonstrated by the results in Fig. 7.10. This is also consistent with the previous publication [15].

Fig. 7.12 The absorbance intensity ($\lambda = 470 \text{ nm}$) of XTT-formazan in $P. \text{ aeruginosa}$ culturing medium with the addition of 100 $\mu$M XTT as a function of time, where $A$ is the absorbance of XTT indicator measured at 470 nm. Based on equation (7.1), the estimated yield rate of $O_2^{-}$ by the bacteria is 3.74 $\mu$M/h.

Fig. 7.13 shows the photocurrent measured on the bare steel and nanostructured specimens in bacterium containing PBS solution. It is seen that there is no photo response on the bare steel and A-2 specimens, i.e., the measured current shows a constant value near zero under both Light-On and Light-Off (Figs. 7.13a and 7.13b). There are obvious current responses for the specimens A-10, A-30, and A-60, as seen in Figs. 7.13c-7.13e, indicating
that the nanostructured oxides coatings formed under these anodizing times have the photocatalytic activity. Particularly, the photocurrent response decreases with the increased anodizing time, and there is the highest photocurrent measured on specimen A-10. Specifically, the average photocurrent response of specimens A-10, A-30 and A-60 are 0.107 µA/cm², 0.021 µA/cm² and 0.005 µA/cm², respectively (Fig. 7.13f).
Fig. 7.13 Photocurrent measured on (a) bare steel specimen and the nanostructured specimens of (b) A-2, (c) A-10, (d) A-30 and (e) A-60 in bacterium containing PBS solution (the bacterial concentration is $2.0 \times 10^7$ CFU/mL). (f) Enlarged diagram of the $8^{th}$ Light On/Off cycle for all specimens.

According to the results obtained from AFM, nodular nanostructure coatings develop on the steel during anodization, facilitating the transfer of photo-excited electrons produced by $\text{Fe}_2\text{O}_3$ to improve the hole/electron separation [12, 119]. The reduction of the nodule nanostructure and $\text{Fe}_3\text{O}_4$ in the oxide coatings results in degradation of the photocatalytic activity of specimens A-30 and A-60. Moreover, the dependence of photocurrent on the anodizing time and the nanostructure of the oxide coating is consistent with that of the ratio of the dead/live cell quantity in Fig. 7.8d, where the ratio of the quantity of dead/live cells decreases with the anodizing time, suggesting that the attached bacteria are deactivated mainly due to the photocatalysis induced by the prepared nanostructured oxide coatings.
7.3.6 Mechanism for visible light enhanced anti-adhesion of bacteria on nanostructured iron oxide coatings

In this work, the nanostructure of the fabricated iron oxide coatings enables anti-adhesion of bacteria, and the photocatalytic property of iron oxides further enhances the anti-bacterial performance under visible light illumination. A conceptual mechanism is proposed, as shown in Fig. 7.14, to illustrate the photocatalytic anti-attachment of bacteria on the nanostructured iron oxide coating, as firmly demonstrated by the results presented herein. Under visible light illumination ($\lambda > 400 \text{ nm}$), reactive oxygen species, i.e., $\text{O}_2^-$ and $\cdot\text{OH}$, and holes are produced by photocatalysis of iron oxides on the surface of the nanocoatings. Due to the biological toxicity and the strong oxidizing ability of these species and holes, the attached *P. aeruginosa* bacteria become deactivated (killed) on the coating surface, while the bacteria existing in the bulk solution are not affected. The reactive oxygen species yielded by light illumination of the oxide coatings are not capable of deactivating bacteria in the solution due to the limited concentration of the species in liquid phase. Thus, the developed nanocoatings would not impact the aquatic ecosystem but deactivate the bacteria attached on the specimen surface only. Obviously, the iron oxide nanocoatings fabricated by anodization of steels in a concentrated alkaline solution in this work provide an effective, convenient and environment-friendly alternative for anti-bioadhesion on steel structures by sunlight illumination in practice.
Fig. 7.14 A conceptual mechanism illustrating the photocatalytic anti-adhesion of *P. aeruginosa* bacteria on the nanostructured iron oxide coating under sunlight.

7.4 Conclusions

Nanostructured iron oxide coatings, which are fabricated by anodization of a pipeline steel in a concentrated alkaline solution, enable anti-adhesion of *P. aeruginosa* bacteria on the steel surface, and the photocatalytic property of the iron oxide nanocoating further deactivate the bacteria remaining on the steel surface, enhancing the anti-bioadhesion performance under visible light illumination. For the nanostructured oxide coating formed by anodizing at 3.0 V for 10 min in 13 M NaOH solution, a 99.9 % anti-bioadhesion performance can be achieved, compared to bare steel, under visible light illumination. The formed iron oxide coating features of a homogenous nodular nanostructure, which,
compared to the nanocoatings formed at other anodizing times, has the largest surface roughness, the highest photocatalytic activity and the best performance for anti-adhesion of \textit{P. aeruginosa} bacteria. The formed iron oxides are a mixture of Fe$_2$O$_3$ and Fe$_3$O$_4$, which can deactivate the bacteria remaining on the coating surface by the oxidative holes and reactive oxygen species generated during light illumination. However, the bacteria existing in the aqueous phase is not affected. The iron oxide nanocoatings fabricated by anodization of pipeline steels in this work provide an effective, convenient and environment-friendly alternative for anti-bioadhesion on steel structures by sunlight illumination in practice.
Chapter Eight: Development of nanostructured oxide coatings on steel for anti-bioadhesion and self-cleaning of residual bacterial cells

8.1 Introduction

Biofouling and microbial corrosion under biofilm have been commonly observed in various industrial sectors, such as pipelines, water treatment equipment, ocean engineering structures, etc., resulting in a decrease in operating efficiency, material degradation and facility failure [122, 190, 191]. High-performance anti-biofouling coatings have been paid much attention due to their low-cost and easy application [9]. With increased environmental concerns, new generations of coatings are required to eliminate the contained toxic and non-degradative components during service [10, 192].

Deactivation (killing) of bacteria adhered to metals based on photocatalysis is considered as a promising strategy for coating design [13-15]. When irradiated with an energy greater than the band gap, electrons and holes separated in the photocatalysts exhibit high reducing and oxidizing activities, respectively. They react with water molecules or other scavengers to form ROSs, i.e., O$_2^-$-, and *OH, resulting in degradation of biological systems through either toxicity or strong oxidizability [130, 191]. As the photocatalytic efficiency and the yield rate of ROSs depend on the surface area of the photocatalyst, it is usually prepared by advanced manufacturing techniques to achieve a nanostructure [41, 193]. However, the nanostructure of oxide photocatalysts is easily attacked by halide ions that generally exist in natural waterbodies [82, 88]. Light irradiation is not sustainable in some service conditions especially either, when the photocatalytic coating is powered by

* This work has been submitted to Chemical Engineering Journal. It is under review.
cyclic sunlight. As a result, the anti-bioadhesion coating is subject to the formation of bacterial plaque which further compromises its function with a prolonging service period [11]. Thus, development of novel anti-bioadhesion coating techniques with a self-cleaning capability is of great importance to practical use and sustainability.

In this work, nanostructured coatings including Fe$_3$O$_4$, Fe$_2$O$_3$, Zn doped Fe$_2$O$_3$, and ZnFe$_2$O$_4$ were fabricated on a pipeline steel surface by anodization, Zn acetate (ZnAc) modification [194] and/or annealing [19], and their anti-bioadhesion performance and electrochemical stability were investigated. *P. aeruginosa* bacteria, a common type of bacteria in natural water [22], were chosen to evaluate the performance of the fabricated iron oxide nanocoatings. The surface morphology, composition and optical properties of the coatings were characterized by AFM, Raman spectroscopy and UV-visible diffuse reflectance spectroscopy, respectively. A CLSM was used to quantify the anti-bioadhesion of the coatings to *P. aeruginosa* bacteria under cyclic sunlight conditions. The deactivation of the pre-attached bacteria on the coatings was determined upon application of H$_2$O$_2$ and UV light, and the self-cleaning performance of the coatings to bacterial plaque was studied by FESEM and ROS measurements. At the same time, the electrochemical stability of the coated steel specimens was tested by OCP and linear polarization resistance (LPR) measurements in a chloride solution.

### 8.2 Experimental

#### 8.2.1 Materials

Steel coupons used for preparation of iron oxide coatings was cut from an X100 steel pipe, with a chemical composition (wt. %): C 0.07, Mn 1.76, Si 0.1, Ni 0.154, Cr 0.016,
Mo 0.2, V 0.005, Al 0.027, S 0.005, P 0.018, and Fe balance. The coupon was machined into the dimension of 50 mm × 10 mm × 1 mm. Sodium hydroxide (≥ 98.5 % purity), sodium chloride (≥ 99.5 % purity), Zn acetate (ZnAc, ≥ 98 % purity), terephthalic acid (TA, ≥ 99 % purity), XTT (≥ 98 % purity), hydrogen peroxide (30 %), monopotassium phosphate, disodium hydrogen phosphate, tryptone and yeast extract were purchased from Sigma-Aldrich (Canada). High-purity nitrogen (99.999 %) was purchased from PRAXAIR (Calgary, Canada). All solutions were prepared with ultrapure water.

8.2.2 Preparation of nanostructured oxide coatings

Fig. 8.1 shows schematically the procedure to prepare three types of nanostructured oxide coatings, i.e., anodized coating, annealed coating, and ZnAc modified coating, on the steel surface. In the anodizing cell, both anode and cathode were made of X100 pipe steel, with a dimension of 50 mm × 10 mm × 1 mm. The exposed face, with an area of 500 mm², on each electrode was sequentially ground by emery papers up to 1,200 grits, then polished by 0.5 µm diamond paste, degreased in ethanol using an ultrasonic bath, rinsed with deionized water, and dried by high-purity nitrogen. The electrolyte of 13 M NaOH was stirred by a magnetic bar at 600 rad/min and maintained at 22 °C during anodization at 3 V for 15 min. The anodized specimen made at this stage was named “anodized coating”.
Fig. 8.1 Schematic diagram of the procedure to prepare three types of nanostructured oxide coatings, i.e., anodized coating, annealed coating and ZnAc modified coating, on the steel surface.

After anodization, the coated specimen was removed from the electrolyte, washed with deionized water and ethanol, and dried by high-purity nitrogen. The specimen was either heat-treated at 400 °C for 30 min to make the so-called “annealed coating”, or dip-wetted with 80 μL of 25 mM ZnAc/ethanol solution, and dried on a hotplate at 110 °C. After repeating the solution treatment and drying for 1 or 2 times, the specimen was annealed at 400 °C for 30 min. The obtained specimen was named “1-layer ZnAc coating” or “2-layer ZnAc coating”, respectively.

8.2.3 Characterization of the prepared oxide coatings

An AFM (Keysight 5500 scanning probe microscope system) was used for topographic characterization on the prepared nanostructured oxide coatings. Imaging was carried out in the tapping mode and a silicon beam shaped cantilever with a triangle pyramid tip
(Resonance Frequency, 190 kHz; Force Constant, 48 N/m; Tip radius, < 7 nm) was mounted on the scanner. The supplied software was used to create 3-D topographic profiles, and calculate the surface roughness of the coatings.

The composition of the prepared coatings was characterized by Raman spectroscopy, which was recorded through a Witec alpha 300 R Confocal Raman Microscope (WITec GmbH, Germany) using a 532 nm laser source.

The optical property of the prepared coatings was investigated by UV-visible diffuse reflectance spectroscopy over a wavelength ranging from 300 to 800 nm using a Cary 5000 spectrophotometer (Agilent, USA). The optical band gap of the coatings was evaluated form the UV-Vis spectra using Tauc’s relationship [184]:

\[(\alpha h \nu) = C(\nu - E_g)^{1/2}\]

(8.1)

where \(\alpha\) is the absorption coefficient, \(h\) is Planck’s constant, \(\nu\) is the frequency of incident light, \(C\) is a constant, \(E_g\) is the average band gap, and the exponent 1/2 is the direct transition value.

### 8.2.4 Bacterial culturing

The strain of *P. aeruginosa* (PAO1) bacteria used in this work was cultivated in LB medium, which was prepared by dissolving 10 g tryptone, 5 g yeast extract and 10 g sodium chloride in 1 L distilled water. The *P. aeruginosa* was inoculated in autoclaved LB and incubated with a shaking rate of 150 rpm at 37 °C. After cultivation for 40 h, the bacteria achieved a stable growth phase, as shown in Fig. 7.2a. The culturing medium with a cell
concentration of $7.5 \times 10^9$ CFU/mL was centrifuged ($4,000 \times g$) for 5 min and resuspended in 0.05 M PBS (pH=7.4) for 3 times to eliminate the metabolic products in the medium. The bacterial pellets were suspended in PBS to achieve a final concentration of $2.0 \times 10^7$ CFU/mL prior to further testing (Fig. 7.2b). The bacterial growth curves in both the LB medium and PBS solution were measured by the MPN method.

**8.2.5 Electrochemical measurements**

Electrochemical measurements were conducted on the coated steel in a three-electrode cell, where the coated steel specimen, a platinum sheet, and an SCE were used as WE, CE and RE, respectively. The OCP of the steel electrode was monitored at a sampling rate of 2 points/s in 3 wt. % NaCl-containing PBS solution until a steady state value was reached. The LPR was measured at the potential range of OCP $\pm 15$ mV at a potential sweep rate of 0.166 mV/s.

**8.2.6 Testing for anti-bioadhesion and self-cleaning of the prepared nano-oxide coatings**

To characterize the anti-bioadhesion performance of the prepared nanostructured oxide coatings under light irradiation, the coated steel specimens were immersed in 0.05 M PBS solution containing bacteria ($2.0 \times 10^7$ CFU/mL) at room temperature. A light source was applied on the specimens with an ON/OFF interval of 12 h to simulate the natural lighting period. Each test was performed in duplicate, with each one using three parallel specimens to ensure reproducibility of the results. The light source was a Xenon arc lamp (Ushio, Japan, 150 W) combining with a UV cut-off filter ($\lambda < 400$ nm) and an IR absorbed water
filter. The light intensity was measured by a light meter (LI-COR, USA), and the light intensity was fixed at 100 mW/cm² to simulate the local sunlight condition. Two lighting conditions were used in this work, i.e., light irradiation for 12 h (light-ON 12 h), and darkness for 12 h and light irradiation for 12 h (light-OFF 12 h + light-ON 12 h).

To evaluate the self-cleaning performance of the prepared nano-oxide coatings to remove pre-existing bacteria, the coated steel specimens were immersed in the inoculated LB culturing medium and cultivated for 40 h to form a biofilm on the coating surface. The specimens were then immersed in PBS solution or H₂O₂ (25 mM)-containing PBS solution, which was either under darkness or exposed to UV light for 1 h. The UV light was provided by a set of 8 W ultraviolet lamps (Hitachi, Japan) with an emission wavelength of 365 nm. The lighting intensity on the specimen surface was approximately 4.68 mW/cm². The tests were conducted under four conditions, i.e., PBS solution in darkness (Pre-formed biofilm), PBS solution with exposure to UV (UV-1 h), 0.2 mM H₂O₂-containing PBS solution in darkness (H₂O₂-addition), and H₂O₂-containing PBS solution with exposure to UV (UV-1 h + H₂O₂-addition).

After testing as mentioned above, the specimens were washed with sterile PBS solution, and stained with a fluorescent dye (Molecular Probes™ FilmTracer™ LIVE/DEAD® Biofilm Viability Kit) in darkness according to the manufacturer's procedure. Bacterial adhesion on the specimen surface was observed by a CLSM (Olympus FV-1000, Japan), where the live and dead cells were identified as green and red colors, respectively. At least five regions were selected randomly on each specimen for imaging. An image-processing software (Image J, National Institutes of Health, USA) was used to measure the coverage ratio of biofilm on the specimen.
At the same time, the morphology of the coated specimens after testing under various conditions as stated was characterized using a FESEM (FEI Quanta 250 FEG). The specimens were washed with PBS solution, immersed in 2.5% glutaraldehyde solution for 2 h, and then washed with PBS solution again. After that, the specimen was dehydrated with various concentrations of ethanol (30%, 50%, 70%, 90% and 100% for 15 min each), fully dried in high-purity nitrogen. The specimens were then coated with platinum for 4 min using an ion-sputter coater and visualized using FESEM at an emission voltage of 10 kV.

8.2.7 Measurements of ROS concentrations

The XTT and TA were used as indicators of the reductive oxygen species (i.e., O$_2^-$) and oxidative oxygen species (i.e., 'OH), respectively [188, 195, 196]. For measuring the concentration of O$_2^-$, the coated specimens were immersed in 5 mL PBS solution containing 100 μM XTT. After 1 h of UV irradiation, the reduction of XTT by O$_2^-$ forms XTT-formazan, which was measured by a UV1800 spectrophotometer (SHIMADZU, Japan) at 470 nm.

For measurements of the concentration of 'OH, 0.5 μM TA was used to react with 'OH to produce a highly fluorescent product, 2-hydroxy-TA(TA-'OH), which was measured by a Gary Eclipse fluorescence spectrophotometer (Varian, USA) at an emission wavelength of 425 nm and an excitation wavelength of 315 nm.
8.3 Results and discussion

8.3.1 Topography and surface roughness of the prepared nanostructured coatings

Figs. 8.2 and 8.3 show the AFM topographical views of bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating were characterized by AFM over a mapping area of 0.5 μm × 0.5 μm and area of 5 μm × 5 μm, respectively. As shown in Fig. 8.2a, the surface of the pre-treated bare steel is very smooth, with a few light scratches induced by surface finishing. The surface roughness is quite small, with an RMS roughness of about 2 nm (Fig. 8.3f). After anodizing at 3 V for 15 min (Fig. 8.2b), the specimen surface contains extensive, hollow cell-like nano-features, which have an average size of 150 nm, a depth of 60 nm and the wall thickness between adjacent cells of about 50 nm. The average surface roughness over small (0.5 μm × 0.5 μm) and big (5 μm × 5 μm in Fig. 8.3) areas is about 14 nm and 15 nm, respectively. Upon annealing, the nano-features are replaced with heterogeneous topographic fluctuations, as seen in Fig. 8.2c. The surface roughness over small and big areas is up to 18 nm and 110 nm, respectively. After using ZnAc solution to modify the anodized specimen prior to annealing, granular particles with an average size of 80 nm are present on the specimen surface, as shown in Figs. 9.2d and 9.2e. The surface roughness over the small and big areas is about 12 nm/28 nm and 11 nm/22 nm, respectively, for the 1-layer and 2-layer ZnAc modifications (Fig. 8.2f).
Fig. 8.2 AFM topographical images of (a) bare steel, (b) anodized coating, (c) annealed coating, (d) 1-layer ZnAc modified coating, and (e) 2-layer ZnAc modified coating. The measured area is 0.5 μm × 0.5 μm, and the range of the depth direction is normalized as 100 nm.

The surface roughness of bare steel and the anodized coating keeps is almost identical when the mapping area increases from 0.5 μm × 0.5 μm to 5 μm × 5 μm, suggesting the homogeneity of the specimen surfaces. Annealing treatment significantly increases the surface roughness of the coating, especially over the big mapping area. The ZnAc modification of the anodized coating also increases the surface roughness, but not as high as that upon annealing. The surface roughness of the coatings is associated with the topographic features, as shown in Fig. 8.4, where the cross-sections of all prepared coatings are present. The annealing process at 400 °C would destroy the surface homogeneity of the anodized coating, which potentially favors the bacterial attachment [197]. The ZnAc
modification of the coating prior to annealing increases the surface roughness slightly. Moreover, a dual layer of treatment causes a decrease of the surface roughness.

Fig. 8.3 AFM topographical images of (a) bare steel, (b) anodized coating, (c) annealed coating, (d) 1-layer ZnAc modified coating, and (e) 2-layer ZnAc modified coating. The measured area is 5 μm × 5 μm, and the range of the depth is indicated. (f) Surface roughness of all specimens derived from the AFM images taken from the area of 0.5 × 0.5 μm (a-e) in Fig. 8.2 and the area of 5 μm × 5 μm.
Fig. 8.4 SEM images of the cross-section of (a) anodized coating, (b) annealed coating, (c) 1-layer ZnAc modified coating and (d) 2-layer ZnAc modified coating. The measured thicknesses of the coatings are marked.

8.3.2 Chemical composition of the prepared nanostructured coatings

Fig. 8.5 shows the Raman spectra of the prepared anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating, respectively. It is seen that the anodized coating is mainly composed of $\alpha$-Fe$_2$O$_3$, as indicated by the Raman shifts at 220, 282 and 397 cm$^{-1}$ [14, 198]. The peaks at 670, 704 and 1360 cm$^{-1}$ confirm the presence of Fe$_2$O$_4$ and $\gamma$-Fe$_2$O$_3$, which are intermediate products produced during
anodization [186]. For the annealed coating, the peaks representing the mixture of Fe$_3$O$_4$ and γ-Fe$_2$O$_3$ are replaced with two weak peaks of α-Fe$_2$O$_3$ at 604 and 1310 cm$^{-1}$, suggesting the composition of pure α-Fe$_2$O$_3$ in the annealed coating. At the annealing temperature of 400 °C, Fe$_3$O$_4$ is oxidized into α-Fe$_2$O$_3$, while γ-Fe$_2$O$_3$ transforms into the more stable α-Fe$_2$O$_3$ [199]. When the anodized coating is under one layer of ZnAc treatment, i.e. 1-layer ZnAc modified coating, similar peaks to those of the annealed coating are observed, demonstrating that there is no additional phase introduced by the ZnAc pre-treatment prior to annealing. At the same time, a broad shoulder is observed at 648 cm$^{-1}$, and the peak at 220 cm$^{-1}$ becomes weaker while those at 397 and 604 cm$^{-1}$ are stronger. All of these changes are attributed to the doping of Zn in the coating and the resulting distortion of the structure [200]. When the ZnAc pre-treatment is applied twice on the anodized coating prior to annealing, the obtained coating, i.e., 2-layer ZnAc modified coating, possesses a single phase of ZnFe$_2$O$_4$, as identified by the peaks at 354, 648 and 1135 cm$^{-1}$ [201, 202], which is an interesting phenomenon.
Fig. 8.5 Raman spectra of (a) anodized coating, (b) annealed coating, (c) 1-layer ZnAc modified coating and (d) 2-Layer ZnAc modified coating, respectively. The arrow indicates the shoulder caused by Zn doped in Fe$_2$O$_3$.

### 8.3.3 Semiconducting properties of the prepared nanocoatings

Fig. 8.6 shows the UV-Vis diffuse reflectance spectra and the derived Tauc’s plots of the prepared anodized, annealed, 1-layer ZnAc modified and 2-layer ZnAc modified coatings. It is seen that both the anodized and annealed coatings exhibit preferential light
absorption at the wavelength range below 600 nm, but the annealed one shows the enhanced light absorption over the measured wavelength range. This is ascribed to the compositional change and structural transformation, as shown in Fig. 8.5, caused by the annealing treatment at 400 °C [194, 203]. With ZnAc modification, the spectra shift right, indicating broadened absorption spectrum when Zn is doped in the coatings. The band gaps of the coatings are evaluated by extrapolation based on Tauc’s relationship in Eq. 9.1 as the main components of Fe₂O₃ and ZnFe₂O₄ in the coatings are direct band gap materials [40, 184]. As seen in Fig. 8.6b, the derived band gap of the anodized and annealed coatings are 2.21 eV and 2.17 eV, respectively, which are similar to the published results [204]. The annealing and ZnAc modification are able to narrow the band gap of the coatings, with a low value of 2.12 eV reached for the 2-layer ZnAc modified coating, where the ZnFe₂O₄ is formed.

Fig. 8.6 (a) UV-Vis diffuse reflectance spectra and (b) the derived Tauc’s plots of the prepared anodized, annealed, 1-layer ZnAc modified and 2-layer ZnAc modified coatings.
8.3.4 Electrochemical stability of the prepared nanocoatings

Fig. 8.7 shows the time dependence of OCP measured on bare steel, anodized coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating immediately after immersion and after 24 h of immersion in the PBS solution containing 3 wt. % NaCl, as well as the polarization resistance measured after the OCP achieves a steady state value for bare steel and the prepared coatings. It is noted that chloride ions are added to the solution in order to degrade the prepared coatings within a reasonable time period for a quick screening of the electrochemical stability of the coatings. It is seen that, immediately after immersion in the test solution, the coated steel specimens show much less negative OCP, which are within -0.2 V ~ 0 V (SCE), than the bare steel, which has an OCP value of approximately -0.75 V (SCE), the typical corrosion potential of pipeline steels in chloride solutions [205]. The results show that the prepared coatings are intact and possess a higher electrochemical stability than the steel in the solution. After 24 h of immersion, the OCP of the anodized coating drops to -0.73 V (SCE), indicating that the anodized coating degrades and exposes the steel substrate to solution directly. While the 1-layer ZnAc modified coating specimen shifts its OCP negatively to about -0.48 V (SCE), the annealed and 2-layer ZnAc modified coating still maintain at less negative levels, i.e., between -0.3 V and -0.2 V (SCE). Of all the prepared coatings, the 2-layer ZnAc modified coating shows the least negative OCP and the smallest OCP drop after 24 h of immersion in the solution, showing its highest electrochemical stability in the testing environment.
Fig. 8.7 Time dependence of OCP measured on bare steel, anodized coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating (a) immediately after immersion, and (b) after 24 h of immersion in the PBS solution containing 3 wt. % NaCl.

(c) Polarization resistance measured after 24 h of immersion in the solution.

The $R_p$ values are derived from the LPR measurements conducted on bare steel and prepared nanocoatings after 24 h of immersion in the solution. The deposit of corrosion products on bare steel raises the $R_p$ to about 1300 $\Omega$ cm$^2$, but the estimated corrosion rate is still up to 0.2 mm/y [52]. The anodized coating has a $R_p$ with the same order of magnitude as that of the bare steel, indicating that the anodized coating is not able to protect the steel
from corrosion in the solution even after 24 h of immersion. The annealed and 2-layer ZnAc modified coatings have $R_p$ of two orders of magnitude higher than those of the bare steel and anodized coating, showing a higher corrosion resistance in the solution. Thus, the annealed and 2-layer ZnAc modified coatings are suitable for applications in natural waterbodies.

8.3.5 Anti-bioadhesion performance of the prepared nanocoatings under cyclic sunlight irradiation

The biofilm of P. aeruginosa bacteria formed on the prepared coatings under light irradiation conditions of light-ON 12 h, and light-OFF 12h + light-ON 12 h was characterized by CLSM, and the results are shown in Fig. 8.8. While the bare steel suffers from extensive bacterial attachment, the coated specimens are generally free from formation of a biofilm under light irradiations. The main components of the prepared coating such as Fe$_2$O$_3$ and ZnFe$_2$O$_4$ are photocatalysts that can be excited by light and release ROS into the solution. The toxic ROS react with bacterial cells to prevent their attachment or even deactivate the bacteria present on the coating surface by damaging their structural integrity [191].
Fig. 8.8 Confocal fluorescent images of live and dead *P. aeruginosa* cells (2.0 × 10⁷ CFU/mL in PBS solution) on bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating under light-ON 12 h, and light-OFF 12h + light-ON 12 h, respectively. The green and red colors indicate live and dead cells, respectively. The yellow color results from the overlay of live and dead cells. The scale bar is 40 μm.

It is further found that the coatings under light-OFF 12h + light-ON 12 h contain more bacterial cells on their surfaces than those under light-ON 12 h, suggesting that the coatings can inhibit the bacterial adhesion more effectively under sustained light irradiation than cyclic lighting. During the light-OFF period, the photocatalytic components in the coatings stop releasing ROS to allow adhesion and accumulation of bacteria on the coating surface. When the light is available again, the attached bacteria can be killed to leave dead cells and released organic matters on the coating surface. As indicated by the red color in Fig. 8.8, the coatings under light-OFF 12h + light-ON 12 h possess much more dead cells than those
under light-ON 12 h. The biofilm residual will insulate the photocatalysts from the solution, and reduce the yield rate of ROS. As a result, the anti-bioadhesion performance of the coatings is compromised.

Fig. 8.9 shows the bacterial coverage on bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating after light-ON 12 h and light-OFF 12h + light-ON 12 h in the test solution. It is seen that the coverage of bacteria on bare steel under light-OFF 12 h is about 51.5 %, which is close to that under light-ON 12 h, i.e., 46.7 %, indicating that the bacterial attachment to the steel achieves an equilibrium after 12 h of immersion (Fig. 7.2).

Fig. 8.9 Bacterial coverage on bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating after (a) light-ON 12 h, and (b) light-OFF 12h + light-ON 12 h in the test solution. The insets show the zoom-in of each diagram. Asterisks indicate the statistical significance ($p < 0.05$).

The anti-bioadhesion performance ($A$) of the surface coatings can be calculated by:
where $S_n$ and $S_B$ are the bacterial coverages on the coated specimen and bare steel, respectively. Under light-ON 12 h treatment, all coated specimens show a quite high anti-bioadhesion performance with the $A$ more than 99.6 %. However, when treated under darkness for 12 h, the anti-bioadhesion of coated samples drops. For the annealed coating, it drops to 91.6 %. The anodized and 2-layer ZnAc modified coatings show the best anti-bioadhesion performance, which still maintains up to 99.2 %. It is expected that, as the irradiation cycles increase, the anti-bioadhesion performance of the prepared nanocoatings will drop. The main reason is that the residuals of bacteria and the resulting biofilm can block the photocatalytic reaction. Moreover, the surface roughness of the coatings would increase with the accumulation of biofilm, which favors the bacterial attachment under darkness [197]. The exact service life of the coatings for anti-bioadhesion is being determined in authors’ laboratory.

8.3.6 Self-cleaning performance of the prepared nanocoatings

While the prepared nanocoatings are able to resist the bacterial adhesion under light irradiation, bacterial cells or organic matters could accumulate on the coating surface and develop biofilm during the period of cyclic lighting. The biofilm formation compromises the function of the coatings for anti-bioadhesion. Thus, a technique capable of restoring the coating integrity without damaging the surface nanostructure is expected. Fig. 8.10 shows the CLSM views of the bacterial cell attachment on bare steel and the prepared
nanocoatings after 40 h of immersion in LB culturing medium, followed by treatment under UV-1 h, H₂O₂-addition, and UV-1 h + H₂O₂-addition, respectively. It is generally believed that a dose of H₂O₂ and UV light is effective to enhance the photocatalytic activity and yield more ROS to deactivate and remove organic contaminations [191]. Like the bacterial attachment in PBS solution (Fig. 8.8), the formed biofilm covers more area on bare steel than the coated specimens. While the cells adhere to the whole surface of the bare steel specimen, the bacterial cells tend to aggregate to form bacterial plaques on the coated specimens. Under UV-1 h, there is a slight decrease of the attached cells on bare steel, as seen from the change of green color area, most bacterial cells are deactivated on the coated specimens, as the reduced green color area and presence of red color in the images. This indicates the photo-excited anti-bacterial adhesion of the prepared nanocoatings. As a comparison, the H₂O₂-addition is more effective than the UV irradiation to deactivate bacterial cells, as shown by the red colored areas present on the specimen surface. However, the dead cells still remain on the specimen surface. Under the combined treatment of UV-1 h + H₂O₂-addition, all bacterial cells are killed, and the coverage of the biofilm reduces remarkably. In particular, there is the best performance for the 2-layer ZnAc modified and anodized coatings, where all bacterial plaques are almost removed from the coating surface, with a few isolated cells present. It is noted that the anodized coating is not electrochemically stable in actual environments, as seen in Fig. 8.7.
Fig. 8.10 Confocal fluorescent images of the bacterial cell attachment on bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating after 40 h of immersion in LB culturing medium, followed by treatment under UV-1 h, H₂O₂-addition, and UV-1 h + H₂O₂-addition, respectively. The green and red colors indicate live and dead cells, respectively. The yellow color results from the overlay of live and dead cells. The scale bar is 40 μm.

The bacterial coverage on bare steel and coated specimens before and after UV, H₂O₂, and UV+H₂O₂ treatments are derived from the fluorescent images and the results are shown in Fig. 8.11. It is seen in Fig. 8.11a that the formed biofilm covers up to 90% of the bare
steel specimen, while the bacterial coverages on the coated specimens are at least 4 times less, demonstrating the improved capability of the nanostructured nanocoatings for anti-bioadhesion even the photocatalytic performance of the coatings does not apply [90].

The bacterial removal efficiency ($E$) can be calculated by:

\[ E = 1 - \left( \frac{S_n}{S_{Pre}} \right) \]  

(8.3)

where $S_n$ is the coverage of residual bacterial cells on the specimen surface upon treatment, and $S_{Pre}$ is the coverage of pre-formed biofilm on the specimen. Under UV-1h, the coverage of live cells on the coated specimens decreases, but the coverage of dead cells increases (Fig. 8.11b), as compared with the results in Fig. 8.11a. This causes a negative bacterial removal efficiency due to the increased number of dead cells from disassembled bacterial plaques. After 1 h of immersion in 0.2 mM H$_2$O$_2$, the coverage of live cells reaches to 0, as shown in Fig. 8.11c, and the counts of dead cells decrease for all specimens. The estimated removal efficiency of all specimens ranges from 41.8 % to 52.4 %, suggesting that the removal of bacterial cells by H$_2$O$_2$ is irrelevant to the surface condition and composition of the specimen. Under the combined treatment of UV-1 h + H$_2$O$_2$-addition, the bacterial removal efficiencies of bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating are 66.7 %, 96.1 %, 87.4 %, 97.7 % and 99.3 %, respectively, showing a much higher performance for anti-bioadhesion and removal by the combination of UV and H$_2$O$_2$ treatments. Particularly, the prepared nanocoatings improve significantly the performance compared to bare steel, with the 2-layer ZnAc modified coating giving the best performance.
Fig. 8.11 Bacterial coverage of (a) formed biofilm in LB culturing medium, and those of residual biofilm under (b) UV-1 h, (c) H$_2$O$_2$-addition, and (d) UV-1 h + H$_2$O$_2$-addition of bare steel, anodized coating, annealed coating, 1-layer ZnAc modified coating and 2-layer ZnAc modified coating respectively. The inset is the zoom-in of the diagram.

Asterisks indicate the statistical significance ($p < 0.05$).
Fig. 8.12 SEM images (2,000 ×) of *P. aeruginosa* bacterium cells present in (a) pre-formed biofilm, and residual biofilms under (b) UV-1 h, (c) H$_2$O$_2$-addition and (d) UV-1 h + H$_2$O$_2$-addition on the 2-layer ZnAc modified coating. The insets are the magnified photos (50,000 ×).

Since the 2-layer ZnAc modified coating gives the best bacterial removal performance of all the prepared nanocoatings, the bacterial cells present on the coating surface are characterized in the pre-formed biofilm and the residual biofilms after UV-1 h, H$_2$O$_2$-addition, and UV-1 h + H$_2$O$_2$-addition, as shown in Fig. 8.12. It is seen that the shape of
bacterial cells in the pre-formed biofilm shows an intact state (Fig. 8.12a). After exposure to UV for 1 h, the cells deform somewhat (Fig. 8.12b) as compared to those in Fig. 8.12a. The bacterial cells further deform after 1 h of treatment by 0.2 mM H₂O₂ (Fig. 8.12c). When the biofilm is treated by the combined UV-1 h + H₂O₂-addition, the bacterial cells are fully killed, as seen in Fig. 8.12d. The visualization of the bacterial cells on the coating clearly demonstrates that the treatments of UV-1 h, H₂O₂-addition, and UV-1 h + H₂O₂-addition are able to destroy the structural integrity of the bacteria and decompose the biofilm attached to the coating surface. Particularly, there is the strongest ability to kill bacteria and remove the biofilm under the combined UV-1 h + H₂O₂ treatment.

### 8.3.7 Measurements of ROS concentrations

The yield rates of reductive O₂⁻ and oxidative 'OH are estimated by the contents of reacted XTT and TA indicators, i.e. XTT-formazan and TA-'OH respectively. Fig. 8.13 shows the kinetics for production of ROS on variously prepared nanocoatings under UV-1 h + H₂O₂-addition. From Fig. 8.13a, the linear increase in absorbance intensity at 470 nm vs. time until all XTT transformed into XTT-formazan (i.e., the absorbance reaches 2.0) demonstrates the constant production of O₂⁻ on the prepared nanocoatings. The yield rate, \( r \), can be calculated by:

\[
\frac{An}{laSt} = r
\]

(8.4)
where $A$ is the absorbance of XTT-formazan measured at 470 nm, $n$ is the reaction coefficient [187], $l$ is the light path of cuvette, i.e., 1 cm, $\alpha$ is absorbance coefficient of XTT-formazan, i.e., 21600 M$^{-1}$ cm$^{-1}$ [188], $S$ is the surface area of the coated specimen exposed to solution, and $t$ is light irradiation time. The yield rates of O$_2^-$ on the anodized, annealed, 1-layer ZnAc modified and 2-layer ZnAc modified coatings under UV light and H$_2$O$_2$ addition are 82.94, 63.73, 144.91 and 160.65 μM/h cm$^2$, respectively.
Fig. 8.13 Kinetics for production of ROS on variously prepared nanocoatings under UV-1 h + H$_2$O$_2$-addition (a) O$_2^-$, and (b) 'OH. The insets are the UV-Vis spectra and fluorescent spectra measured on 2-layer ZnAc modified coating.
By comparison of the fluorescence intensity measured on the specific specimen with the known concentration of TA-'OH, the content of produced TA-'OH can be determined [206]. It is seen in Fig. 8.13b that the time dependence of 'OH production features of two stages, i.e., the initial exponential relationship between the fluorescence intensity and time lasting about 20 min, and the linear relationship between the fluorescence intensity and time. The yield rates of 'OH in the first stage for anodized, annealed, 1-layer ZnAc modified and 2-layer ZnAc modified coatings are 3.12, 2.02, 2.47 and 2.82 μM/h cm², respectively, while those at the second stage are 0.18, 0.14, 0.29 and 0.36 μM/h cm², respectively. Of all prepared coatings, the 2-layer ZnAc modified coating achieves an estimated amount of 'OH of 0.68 μM/cm² in 1 h, ranking the highest level to represent the strongest oxidative ability.

In the absence of H₂O₂ in the solution, the photocatalytic reactions include [206]:

\[
\begin{align*}
\text{Fe}_2\text{O}_3/\text{ZnFe}_2\text{O}_4 + h\nu & \rightarrow e^- + h^+ \quad (8.5) \\
\text{O}_2 + e^- & \rightarrow \text{O}_2^- \quad (8.6) \\
\text{O}_2^- + h^+ & \rightarrow \text{O}_2 \quad (8.7)
\end{align*}
\]

where e⁻ and h⁺ are photo-excited electrons and holes in the conduction band and valence band, respectively. The 2-layer ZnAc modified coating shows the highest yield rate of \(\text{O}_2^-\), which is attributed to the main component of ZnFe₂O₄ possessing a more negative conductive band than Fe₂O₃, the primary component of other prepared coatings, as seen in Fig. 3. The presence of ZnFe₂O₄ favors the oxygen reduction [39]. It is noted that the
oxidative reaction induced by $h^+$ does not produce $'OH$ because the components of the coatings have a more negative valence band energy in relative to the equilibrium potential of $OH^-/OH$ [207]. As a result, there is no $OH^-$ detected by TA indicator when the coated specimens are exposed to UV without the $H_2O_2$ addition. When $H_2O_2$ is added, it participates in both reductive and oxidative reactions [208]:

$$H_2O_2 + O_2^- \rightarrow 'OH + OH^- + O_2$$  \hspace{1cm} (8.8)

$$H_2O_2 + 2h^+ \rightarrow O_2 + 2H^+$$  \hspace{1cm} (8.9)

Moreover, $H_2O_2$ prevents the recombination of electrons and holes by replacing the reaction (8.7) with reaction (8.9), enhancing reaction (8.6) to cause an increased yield rate of $O_2^-$. Previous work demonstrated that $H_2O_2$ was able to absorb on the surface of oxide coatings, and was reduced by photo-excited electrons directly [206]:

$$H_2O_2 (\text{adsorbed}) + e^- \rightarrow 'OH + OH^-$$  \hspace{1cm} (8.10)

Since the anodized coating possesses a cell-like nanostructure, favoring molecular absorption, the largest yield rate of $'OH$ at the first stage is thus obtained (Fig. 8.13b) by reaction (8.10). After that, $'OH$ is produced mainly due to reaction (8.8), where the reaction rate is linearly proportional to the concentration of $O_2^-$. 
8.3.8 Mechanism for anti-bioadhesion and self-cleaning of residual bacterial cells by the nanocoatings

This work demonstrates that the inclusion of photocatalytic components, i.e., Fe$_2$O$_3$ and ZnFe$_2$O$_4$, in the prepared oxide nanocoatings achieves high-performance anti-bioadhesion and self-cleaning of residual bacterial cells under sustained light irradiation. The performance is further enhanced with additional H$_2$O$_2$ treatment. The mechanism for the photocatalytic anti-bioadhesion of nanostructured oxide coatings is illustrated schematically in Fig. 8.14a. Under light irradiation, O$_2^-$ and holes are generated by photocatalytic components contained in the coating, accumulating on the coating surface. Due to the biological toxicity and oxidative capability of the ROS and holes, *P. aeruginosa* bacteria are deactivated, preventing the formation of biofilm on the coating surface. However, the sunlight is not capable of removing residual dead cells and biofilm that form in the absence of light irradiation. Therefore, an enhanced photocatalytic activity is achieved to improve the decomposing efficiency of the residual biofilm by combining UV light with H$_2$O$_2$ treatment, as shown mechanistically in Fig. 8.14b. Compared with the sunlight irradiation, the UV-1 h + H$_2$O$_2$-addition increases the yield rate of O$_2^-$ and enables the nanocoating to produce more oxidative ‘OH, which is capable of decomposing almost all organic matters [191]. As a result, the residual cells and biofilm are removed.
Fig. 8.14 Schematic diagrams for illustrating the mechanisms for (a) anti-bioadhesion by the prepared 2-layer ZnAc modified nanocoating under light irradiation, and (b) the self-cleaning for residual bacterial cells under UV light combined with H$_2$O$_2$ treatment on the nanocoating.
8.4 Conclusions

A 2-layer ZnAc modified nanocoating containing photocatalytic ZnFe$_2$O$_4$ is successfully prepared by anodization of pipeline steel followed by ZnAc modification and annealing, enabling high-performance anti-bioadhesion and self-cleaning of residual bacterial cells and biofilm under light irradiation combined with H$_2$O$_2$ addition, while maintaining a high electrochemical stability in a simulated aqueous solution. Of various nanostructured oxide nanocoatings prepared in this work, the 2-layer ZnAc modified nanocoating achieves 99.6% of anti-bioadhesion under light irradiation relative to bare steel. The treatment of 1 h UV irradiation + 0.02 mM H$_2$O$_2$ allows 99.3% of removal of bacterial cells and biofilm plaque which could be formed in the absence of sunlight. The prepared nanocoating on the steel and the following treatment ensure a reliable, highly effective anti-bioadhesion and integrity maintenance of steel structures in practical applications.

The high-performance anti-bioadhesion of the 2-layer ZnAc modified nanocoating is attributed to the production of high concentration of O$_2^\cdot$ induced by photocatalytic activity on the ZnFe$_2$O$_4$ component contained in the coating. ZnFe$_2$O$_4$ possesses a higher yield rate of O$_2^\cdot$ than Fe$_2$O$_3$ (i.e., the main component in the prepared anodized, annealed and 1-layer ZnAc modified nanocoatings) due to its more negative conductive band position. Under combined UV irradiation and the addition of H$_2$O$_2$, the 'OH is produced due to the photocatalytic reaction of H$_2$O$_2$ with O$_2^\cdot$ or photo-electrons on the coating. The self-cleaning of residual bacterial cells and biofilm through the combined treatment of H$_2$O$_2$ and UV light provides an economic and risk-free method for routine maintenance of steel structures in reality.

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Chapter Nine: Conclusions and recommendations

9.1 Conclusions

When X100 pipeline steel corrodes in aqueous environments at either an active dissolution or a passive state, the nanoscale structure can develop on the steel surface, depending on the early-stage corrosion behavior. The conditions such as surface finish of the steel, electrolyte concentration, and electrochemical potential, affect the thermodynamics and kinetics of corrosion process, making the convenient control of the nanostructure on steel substrate possible. The nanostructure of steel surface shows a quick-response to changes of the conditions, by either exposure to corrosive electrolytes, or altered electrochemical potentials. The surface nanofeatures are under a non-equilibrium state and last from hundreds to thousands of seconds, during which the corrosion processes of the steel can be successfully “visualized” by in-situ ECAFM. In this time period, the development of nanoscale topography results in the nanostructures on the steel surface if ending with an equilibrium stage of corrosion.

The nanoscale features produced by surface polishing affect the early-stage corrosion of the steel. Two stages of dissolution are identified through the ECAFM in-situ measurement of topographic evolution. The first stage features a rapid dissolution of the steel, and the second stage achieves an approximately constant corrosion rate. The steel corrosion increases with the surface roughness. A longer time is required to achieve the second stage for the steels with a finer surface finish. Moreover, a coarser surface finish causes rougher corrosion features, since local preferential dissolution occurs at irregular patterns such as scratches with a high local stress concentration, resulting in corrosion deepening.
The steel corrodes actively in 0.01 M NaHCO$_3$ solution, while it is passivated in solutions with higher concentrations such as 0.1 and 0.5 M, in which the passive ability of the bicarbonate solution improves with increased concentration. The corrosion of the steel corrosion in 0.01 M NaHCO$_3$ solution includes three stages indicated by the development of nanofeatures on the surface. Upon immersion of the steel in the solution, the dissolution of air-formed oxide film on the steel surface results in a negligible topographic evolution (i.e., stage I). Corrosion of the steel then occurs, indicated by the rapid increase of surface roughness (stage II). As the steel corrosion achieves a steady state, the surface roughness maintains an approximately stable value (stage III). On contrary, once the steel is passivated, the formed passive film can eliminate surface features and reduces the surface roughness in 0.1 and 0.5 M NaHCO$_3$ solutions. The higher the passive ability, the smoother the surface of the steel is.

The X100 pipeline steel can be passivated in the concentrated carbonate-bicarbonate solution over a wide passive potential from -0.1 V (SCE) to 0.8 V (SCE). As the passive potential shifts from -0.1 V (SCE) to 0.5 V (SCE), the sub-structures of passive film become finer, attributing to the more compact and protective passive film. However, when the passive potential exceeds 0.5 V (SCE), the electrochemical properties of the passive film degrades but the compositions of iron oxides and sub-structures remain unchanged. Especially, when the film-forming potential is up to 0.7 V (SCE), the content of iron hydroxides in the film increases and form an irregular structure of the passive film. The development of nanostructure on steel surface stops when the corrosion system reaches equilibrium at a potential within the passive range such as -0.1 and 0.5 V (SCE), while the
nanostructure develops continuously at a potential closed to the transpassive region, i.e., 0.7 V (SCE).

Anodically polarizing a pipeline steel in a passivation-favored solution at proper potential can develop nanostructured iron oxide coatings on the steel surface. The obtained nanostructures have common properties, i.e., reproducible structures at nanoscale, chemical and physical homogeneity, good adhesion to steel substrate, main composition of iron oxides, and high electrochemical stability in aqueous environments. The nanostructures can reduce the surface energy and interactive force between microorganisms and the steel, and thus enable anti-bioadhesion of pipeline steel. Under an optimal anodizing potential of 2.0 V, the nanocoating is prepared through anodizing pipeline steel in a concentrated sodium hydroxide solution. The coating possesses the best hydrophobicity (with a water contact angle of 118.53°) and anti-adhesion performance to SRB (a 23.5 time of reduction of SRB adhesion compared to bare steel). Furthermore, the photocatalytic property of nano-iron oxides in the coatings releases toxic and oxidative ROSs to deactivate (kill) bacteria under light illumination. The anti-bioadhesion performance of nanocoating is enhanced under visible light illumination, achieving a 99.9 % anti-bioadhesion of P. aeruginosa compared to bare steel. Further treatment by ZnAc modification and annealing allows the formation of ZnFe₂O₄ in the nanocoating, which enhancing the electrochemical stability of the nanocoating in corrosive environments while maintaining the high performance of anti-bioadhesion (99.6 % compared to bare metal) and self-cleaning of residual attached bacteria (up to 99.3 % of total coverage).
9.2 Recommendations

This research enhances our understanding of the electrochemical interaction between nanostructure and environmental conditions, and advances the electrochemical development of anti-bioadhesion coatings on steels. Further work should be conducted in order to improve the applicability of the research outcomes in practice.

1) The fabricating method should be refined to produce the nanocoatings in large scale to meet the industrial use. The fabrication method is very reproducible at a lab scale. Industrial using focuses on a large scale of production of the intelligent coating to meet the actual needs.

2) The mechanical property of nanocoating, such as erosion resistance, adhesive force, and tensile strength should be evaluated as per industrial standards. The failure mechanism and lift prediction of nanocoating are also important for industrial application.

3) The electrochemical method of fabricating nanostructured iron oxide coatings should be modified to meet newly emerging requirements of various applications. The ingredient of electrolyte is relatively simple compared with other well-developed electrolytes for anodizing, which means there is a potential to improve the controllability, complexity, and homogeneity of nanostructures through adding more beneficial components.

4) It is meaningful to explore other applications of the nanostructured iron oxide coatings. The porosity of iron oxides leaves considerable capacity to load inhibitors and biocides which prevent the corrosion of structure and microbial
infestation. A sustained release mechanism can balance the expense and performance of fillers, i.e., inhibitors and biocides.
References


