6.1. Introduction

Industrially vital materials, especially stainless steels offer corrosion protection via the growth of passive (oxide) films. The mechanism by which these protective films grow and break (a process that leads to pitting corrosion in various environments) are still not clearly understood. It is known that non-metallic inclusions such as bacterial attachment played an important role in pitting and crevice corrosion; however it is unclear why certain inclusions produced pitting whilst others were innocuous. The active-passive transition had been attributed to the formation of either a monolayer of adsorbed oxygen on the surface or to the coverage of the surface by a three dimensional corrosion product film. In either case, the reactive metal was shielded from the aqueous environment and the current dropped penetratively to a low value that was determined by the movement of ions or vacancies across the film [1]. For most systems, particularly for diffuse metal-oxide junctions, it was assumed that the resistance to the movement of electrons across the interface was small compared with resistance to the movement of cations. The capacitance associated with this interface was probably due to the space charge layer within the oxide film. Nevertheless, it is now generally agreed that the barrier oxide layer is a highly defective structure, with the point defects being metal and oxygen vacancies and metal interstitials.
Stainless steel surface has Cr₂O₃ (p-type) inner layer with an outer precipitation layer of Fe₂O₃ (n-type). Nickel 200 has nickel oxide (p-type) film over the alloy surface [2] and alloy C276 has “stainless steel like” protective nature. Ennoblement is the term used to describe the shift of potential, which is associated also with an increase in cathodic current density. Ennoblement is considered a necessary first step in the initiation of corrosion in all passive metals and alloys [3].

Dexter and Maruthamuthu [4] found that the ennoblement is due to manganese oxidizing bacteria and the interaction between stainless steel oxide film (\(n/p\)-type semiconductors) and manganese biofilm. Maruthamuthu et al [5] noticed the concentration of manganese depositing heterotrophic bacteria in biofilm and suggested that the nature of cathodic curves were determined by nature of oxide film and biofilm on 6XN-SS and Titanium grade-2. Of the many mechanisms, the one which is popular is that which results in the relationship of deposition of manganese oxide (MnO₂) on the surface of the SS tubes [6]. Previous studies of bacterially mediated manganese biofilm, the acceleration on passive metals have been performed under a variety of conditions. The possible relationship between ennoblement and changes in surface oxide chemistry has not been well studied. In the present study, the electrochemical behaviour of ennobled passive alloys correlated with the Point Defect Model (PDM).

6.2. Materials and methods

6.2.1. Specimen preparation

The present study employs the specimen of AISI 316L stainless steel (UNS S31603), alloy C276 (UNS N10276) and Nickel 200 (UNS N02200). Table 6.1 shows the composition of these materials. Specimens of dimensions \(2 \times 2 \times 0.2\) cm in size were used for electrochemical studies. An electrical lead of nickel wire was spot-welded at the top end of each specimen. The
specimens were well polished with #600 grit sheets to provide a mirror finish surface sufficiently free of flaws for surface and electrochemical analysis. The specimens were sonicated, first in acetone, and then in trichloroethylene, each for five minutes, to remove any residual oil-based contamination. Then the specimens were kept in 3-4 minutes ultrasonicated process with Milli-Q water. Polished 316L SS and alloy C276 specimens were passivated with 10% nitric acid in 20 min at 60 °C. Polished Nickel 200 specimens were passivated with 15% hydrochloric acid in 3 min at 25 °C [7]. Passivated 316L SS, alloy C276 and Nickel 200 were used for all experimental purposes. All samples were prepared carefully, after polishing; the specimens were degreased with acetone, cleaned by Milli-Q water and dried in air. The samples were subjected to passivation treatment. The samples were prepared and kept in a desiccator for an hour prior to the time for the analysis.

6.2.2. Biofilm formation

The freshwater was collected from Ammon pond water, Karaikudi. The freshwater was sterilized with autoclave at 121°C and used as control. The specimens were exposed in to the natural freshwater and also allowed to form biofilm. The immersion of specimens was done carefully, so that the water line on the sample was sufficiently below the welded junction to ensure no galvanic interaction between the two metals. The natural freshwater was changed once for every 24 hrs interval, upto 60 days in order to make the biofilm active.

6.2.3. Experimental procedures

Freshwater samples were subjected to analysis for Physio-chemical characteristics as per the methods. After 60-days, the biofilm attached 316L SS, alloy C276 and Nickel 200 were removed from bioreactor and washed with sterilized water to remove unattached microorganisms on the passive alloy surface. Various types of bacteria viz heterotrophic bacteria (HB), acid producing
bacteria (APB) and manganese oxidizing bacteria (MOB) were enumerated by pour plate method [8]. The bacterial density was expressed as Colony Forming Units per cm² (CFU / cm²) of all biofilm sample. The morphology of the biofilm covered passive alloys were studied by Epifluorescent microscope (Nikon E200, Tokyo, Japan). Microscope images were captured by digital camera (Nikon COOLPIX5400). Scanning electron microscopy was used to image the surface topography after exposure. The specimens were searched for surface features with dimensions consistent with the size of the microorganisms colonizing the surface. A Hitachi model S-3000H, Scanning electron microscope with a beam voltage set to 20 KV. Carbohydrate and protein were estimated by phenol - sulphuric acid method [9] and Bradford’s method [10] respectively. Infrared (IR) spectrum, (Thermo Nicolet Nexus 670) was used for the analysis of the biochemical of characteristics of the biofilm samples. The open circuit potential (OCP) of 316L SS, alloy C276 and Nickel 200 specimens were measured using a high resistance Multimeter (Rish Multi 18S). OCP measurements were improvised in laboratory condition using natural freshwater. As soon as the samples were exposed in the freshwater, the initial potentials of the samples were recorded and monitored as a function of time until they reached a steady state value. Similar experiments were carried out using control. The average values for six specimens were plotted. All assessments were carried out at temperature of 30±1°C.

Cathodic polarization experiments were conducted in freshwater under air-saturated condition using a computer controlled Potentiostat DC105. (Gamry instruments Inc., Warminster, USA) in a one-liter corrosion cell. A three-electrode setup was used which consisting of SS 316L, stainless alloy C276 and Nickel 200 specimens as the working electrodes, SCE as the reference electrode and a platinum foil as the auxiliary electrode. The exposed specimen was first immersed in the corrosion cell for about ten minutes to allow equilibrium
with the electrolyte. Cathodic and anodic polarization were initiated at the specimen open circuit potential and polarized to -1.0 V and +1.2 V vs SCE at a sweep rate of 0.166 mV / s respectively. Polarizations were carried out on the specimen with and without natural biofilm. All measurements were also carried out at 30 °C for optimum bacterial growth. The Cyclic voltammetric experiments were carried out using the Gamry Potentiostat under the software control of Gamry Framework. Experimental data were recorded in the absence of stirring or gas bubbling into the electrolyte. In the AC impedance test, the stainless steel specimen was kept at the OCP and a sinusoidal potential with 5 mV amplitude and a frequency of 0.01 Hz to 30 kHz was applied by using a frequency response analyzer. Mott-Shottky plots are analyzed in a conventional three-electrode electrochemical cell equipped with Gamry potentiostat (EIS 300) measurements were made using lock-in-amplifier and adequate software. The frequency used as the very commonplace value 1580 Hz. The potential scanning range was -1V to +1V vs reference electrode. The amplitude of sinusoidal voltage perturbation signal was equal to 10 mV. The Mott-Shottky plots of the 316L SS, alloy C276 and Nickel 200 were measured at ambient temperature. All the potential were expressed with respect to SCE.

6.3. Results and discussion

6.3.1 Physico-chemical characteristics of pond water

The physico-chemical characteristics of freshwater are shown as Table 6.2. The pH of the water sample was 7.6, i.e., slightly alkaline. The total dissolved solid content was 288 mg l⁻¹. Chloride content was 80 mg l⁻¹ while sulphate content was 16 mg l⁻¹. Dissolved oxygen was 7.32 mg l⁻¹ at 30°C.
6.3.2. Enumeration of Bacteria

Table 6.3 presents the counts of viable bacterial population in natural biofilm on 316L SS, alloy C276 and Nickel 200. The bacterial count was in the range between $3.0 \times 10^5$ CFU/cm² and $3.7 \times 10^7$ CFU/cm² in natural biofilm on various passive alloys at 30 °C. The total viable bacterial count was $3.7 \times 10^7$ CFU/cm², which are obviously on the higher side and we can observe considerable build up of natural biofilm at 30 °C from freshwater. It indicated that the bacterial density was enormous in surface of various passive alloys exposed in freshwater environment.

6.3.3. Epi-Fluorescence Microscopy

Epi- Fluorescence images are presented as Fig. 6.1(a,b,c) The Epi-fluorescence microscopical review of the 316L SS, alloy C276 and Nickel 200 surface were with biofilm samples. It indicates the bacterial count was high at 30°C and also represent that the biofilm was heterogeneous surface.

6.3.4. Scanning electron microscopy

SEM images of natural biofilm on 316L SS, alloy C276 and Nickel 200 surface at 30 °C are shown as Figs. 6.2a to 6.2c. Microscopic observation indicated that biofilm contained different microbial populations. Images showed that 60-days mature biofilm were almost exclusively composed of rod-shaped bacteria, although certain diatoms also occurred at 30 °C. There were lots of rod-shaped bacteria with exo-polysaccharides deposit adsorbed on the 316L SS, alloy C276 and Nickel 200 surface. It was observed a patchy like accumulation of precipitate on all alloy surfaces. Besides, heterogeneity of biofilm forming clumps of cells heterogeneously distributed on passive alloys (Fig. 6.2a) was noticed at 30 °C. It may be inferred that higher number of bacteria at 30 °C contributed the ennoblement process significantly with higher
concentration of oxygen. In the control system (Fig. 6.2b), any matrix as biofilm on the alloy surface, was not noticed

6.3.5. Concentration of protein and carbohydrate in natural biofilm

Table 6.4 shows the concentration of carbohydrate and protein in natural biofilm on SS 316L, alloy C276 and Nickel 200 at 30 ºC. Carbohydrate and protein concentrations were higher on all passive alloys exposed in freshwater at 30 ºC. The rich content of protein and carbohydrate is due to the higher number of bacteria (biomass) at optimum temperature of 30 ºC.

6.3.6. FTIR

Fig. 6.3 shows FTIR spectrum of natural biofilm on 316L SS, alloy C276 and Nickel 200 and their control. FTIR spectrum of natural biofilm shows the peaks present in the range between 429-795 cm\(^{-1}\) indicated the MnO\(_x\) - stretching, bending and wagging vibrations [11]. FTIR spectroscopic study of biogenic Mn-oxide formation by *Pseudomonas putida* GB-1 that also identified, a peak at 433 cm\(^{-1}\) for MnO\(_x\) present in the biofilm [11]. Peak around 1383 cm\(^{-1}\) indicates CH\(_2\) vibrations of polysaccharides. A prominent band at 1035 cm\(^{-1}\) indicated the presence of polysaccharides in biofilm. A band at 1640.3 cm\(^{-1}\) was recorded and indicated the polysaccharide, which corresponded to amide being indicative of cell biomass. The peaks at around 1640.3 cm\(^{-1}\) and 3406.9 cm\(^{-1}\) were the OH-stretching vibration and bending respectively. These peaks stand for the presence of OH groups in the mineral structure of manganese oxide. FTIR analysis revealed that the MnO\(_x\), OH stretching and EPS fraction dominate the bulk of biofilm biomass. FTIR spectrum of biofilm collected from alloy C276 and Nickel 200 shows similar peaks. It may be observed that all the biofilm contains organic, polysaccharides and Inorganic, manganese oxides. IR spectrum of control (Figure 5.3), both organic and inorganic band region peak was not observed. There was no attachment of biofilm on all control system.
6.3.7. Open circuit potential measurements

Fig. 6.4 shows the variation of open circuit potential with time for five samples in each of the three freshwater exposed systems such as 316L SS, alloy C276 and Nickel 200 and their control. The initial potential was about -150 mV vs SCE while covered with the biofilm on SS 316L, the potential gradually became nobler about +380 mV vs SCE. It reveals that natural biofilm induced the ennoblement process, which supported the earlier observation [4, 5, 12-15]. Similar potential shifts were also observed that other passive alloys such as alloy C276 and Nickel 200. In control, slightly shift to nobler values was noticed from -200 mV vs SCE to -60 mV vs SCE.

6.3.8. Polarization studies

6.3.8.1. Anodic polarization

Fig. 6.5a shows the anodic polarization curves for natural biofilm covered 316L SS and their control at 30 °C. The anodic Polarization on biofilm covered 316L SS started from +380 mV and the breakdown potential was +900 mV vs SCE. In control, anodic polarization curve started from OCP, the two peaks at +250 mV and +860 mV were observed. These two peaks are due to that the oxidation of ferric layer and inner layer of chromium. The breakdown potential was about +860 mV vs SCE. In the region of transpassivity, the oxidation of Cr$^{3+}$ to Cr$^{6+}$ occurred [16, 17]. As the film dissolved, cation vacancies were created in the oxide surface. The anodic polarization curve for biofilm covered alloy C276 was observed a peak at +600 mV with an increasing current density (Fig. 6.5b). These were due to the oxidations of species in the biofilm and oxide film. In the control system, the breakdown potential of alloy C276 at +475 mV was observed. The anodic polarization curve for biofilm covered Nickel 200 at 30 °C was started from +360 mV ($E_{corr}$) vs SCE. The breakdown potential was about + 850 mV vs SCE where
distinct passivation was noticed. In the control system, at +575 mV vs SCE the breakdown of nickel oxide film occurred. The biofilm improved the passivity on 316L SS and Nickel 200 when compared to C276 alloy.

6.3.8.2. Cathodic polarization

Cathodic polarization curves for natural biofilm covered 316L SS, alloy C276 and Nickel 200 and their control systems (without biofilm) are shown as Fig. 6.6. Cathodic polarization curves for natural biofilm covered 316L SS is shown as Fig. 6.6a. Natural biofilm covered 316L SS exhibited two reduction peaks. The first peak at +200 mV was due to as ferric reduction peak, which supports with the earlier observation [4]. The second peak at 0 mV vs SCE was due to manganese dioxide reduction [4, 18]. The presence of manganese in natural biofilm encouraged the reduction whereas the high oxygen reduction current was due to the interaction between H₂O₂ and manganese in the biofilm. In the presence of biofouling, metabolic production of active oxygen species (H₂O₂) raised the contribution to the cathodic current [18]. It was observed that limiting current of biofilm covered stainless steel increased when compared to control. Cathodic polarization of biofilm covered alloy C276 did not exhibit significant changes. In the potential range of -600 mV to -650 mV, another broad peak corresponding to chromium reduction was observed in 316L SS and alloy C276 [19], but not in Nickel 200. The MnO₂ reduction reaction did not take place readily on the chromium rich (alloy C276), p-type passive film [4]. The chromium reduction peak current (Fig. 6.6a) was higher at alloy C276 when compared to 316L SS. This may be due to an enhanced ability of the chromium oxide (p-type) to accept electrons from the MnO₂ (n-type) in the presence of natural biofilm. Besides, cathodic polarization of Nickel 200 was observed in similar hysteresis of 316L SS curves at above -600 mV. Current densities of curves were more or less the same for 316L SS and alloy
C276, whereas for Nickel 200, the reduction of current density at 0 mV. Corrosion potentials of passive alloys were correlated with the cathodic reduction of oxygen. In the present study, the rate of reduction of oxygen was favored on 316L SS and alloy C276 surface, whereas Nickel 200 did not favor the reduction of oxygen reaction (Fig. 6.6c). Cathodic behaviour of passive alloys changed not only because of the presence of biofilm and also the composition of oxide film. In control system of all passive alloys, peaks were not observed.

6.3.9. Cyclic voltammetry studies

Cyclic voltammogram (CV) of natural biofilm on 316L SS, alloy C276 and Nickel 200 is shown as Fig. 6.7 (a, b and c). On natural biofilm on 316L SS, three peaks were seen; was two peaks at anodic region (oxidation) followed by a peak at cathodic region (reduction). Spectroelectrochemical characterization of nanostructured, mesoporous MnO_x in aqueous electrolytes [20] indicated an oxidation and a reduction peak at +240 mV and -15 mV vs Ag/AgCl which were similar to those observed in the present study. In the present study, the cyclic voltammetric peak currents increased in both oxidation and reduction scans (Fig. 6. 7c). During the redox behaviour of MnO_x, an oxidation peak (I) and a reduction peak (III) were observed at +248 mV and 0 mV vs SCE respectively. The potential difference of redox peaks (E_p) was approximately +248 mV observed from CV response of natural biofilm suggesting the MnO_x redox couple was quasi-reversible. Another peak (II) in anodic scan was noticed due to the oxidation of H_2O_2. The presence of H_2O_2 in the native biofilm is due to marine and freshwater micro organisms [21, 22]. Mn-peroxidase needed the presence of H_2O_2 to catalyse the oxidation of manganese oxide. Dexter et al [23] proposed that microbially catalyzed peroxidatic Mn oxidation as an important mechanism for manganese redox cycling. Natural biofilm had
attachment of enriched cations such as manganese oxides. The biogenic manganese oxide was deposited on biofilm covered 316L SS by manganese oxidizing bacteria [3, 24].

On natural biofilm on alloy C276 and Nickel 200 redox peaks were not seen; during the anodic scan currents slightly increased at +600 mV vs SCE. It may be the synergetic effect of oxidation of biofilm and oxide film occurred. The CV response of biofilm on alloy C276 and Nickel 200 revealed that no significant redox behaviour of manganese oxide (MOB). There was no electron flow between passive film and manganese biofilm. The deposition of manganese oxide on all passive alloys by MOB (Table 6.3) was observed. Deposited manganese biofilm was electrochemically active only on 316L SS. In all control systems, no peaks in cyclic voltammogram curves were seen.

6.3.10. AC impedance studies

Based on the physical and chemical properties of oxide film / solution interface, the impedance of the oxide film / solution interface consists of impedances of metal / passive film interface ($Z_{m/f}$), passive film ($Z_f$), passive film / solution interface ($Z_{f/s}$) and solution ($R_s$). As the elements are connected in series, the largest impedance elements are frequency dependent, so that each of the elements may dominate over different frequency ranges. The total impedance ($Z_T$) of passivated alloys in a solution is expressed in equation (1).

$$Z_T = R_s + Z_{m/f} + Z_f + Z_{f/s}$$

Fig. 6.8 and Fig. 6.9 are the Nyquist and Bode plots for biofilm covered 316L SS, alloy C276 and Nickel 200. Performing Kramers-Kronig transformations of the imaginary and real components of impedance validated the measured impedance. The experimental data were analyzed and fitted to circuit parameters using the non-linear least square method with chi-square ($\chi^2$) values are around $10^{-5}$. Data for 316L SS in the presence of natural biofilm containing
biogenic MnO\textsubscript{x} was analyzed using the equivalent circuit shown in Fig. 6.10a wherein Warburg impedance (Z\textsubscript{w}) was introduced to account for the diffusion process within the biogenic MnO\textsubscript{x} deposited layer. The 45° phase angle shift in the Bode plot and the slope of Z\textsubscript{mod} value was 0.656 nearer to the 0.5 at low frequency region confirmed the diffusion process. This result suggests that bacterial factors are related to the Helmholtz double layer capacitance associated to the biofilm / solution interface. The distribution of bacteria on the surface was non uniform. Diffusivity at the interface with biomolecules and bacteria indicated that dissolved metal ions were accumulated at the interface leading to an accumulation of MnO\textsubscript{x}. The heterogeneity of the surface was due to the bacteria attachment. A lower value of n was observed in the presence of biofilm. The heterogeneous distribution of the biofilm may increase the passive film heterogeneity. The presence of a natural biofilm led to a decrease in the polarization resistance (R\textsubscript{p}) of 316L stainless steel. The Warburg component in the equivalent circuit explains the diffusion of divalent Mn\textsuperscript{2+} and its cycling within the biofilm by MOB [25, 26] and the production of H\textsubscript{2}O\textsubscript{2} on the biofilm covered 316L SS was a combination of kinetic and diffusion processes. In the low frequencies, it was the diffusion process, and the redox process gave rise to the pseudo-capacitance behaviour, H\textsuperscript{+} diffused into the pores formed by MnO\textsubscript{2} to achieve the electrochemical reaction [27]. The redox couple of MnO\textsubscript{x} i.e., MnO\textsubscript{2} + H\textsuperscript{+} + e\textsuperscript{-} $\rightleftharpoons$ MnOOH, with H\textsubscript{2}O\textsubscript{2} determined the electron flow between manganese ions and reduction of oxygen in the biofilm. In the biofilm covered alloy C276, Nyquist plot exhibited a semicircle with a diffusion process in freshwater (Fig. 6.8b). It was due to changes with outer oxide (Fe\textsubscript{2}O\textsubscript{3}) film in presence of biofilm. Polarization resistance of biofilm covered Nickel 200 was low in presence of biofilm. The composition of the oxide film would have changed in the presence of biofilm environment.
For Control systems of 316L SS, alloy C276 and Nickel 200, increase the polarization resistance was seen (Table 6.5) compared with natural biofilm system. This observation was seen from the low frequency Nyquist plot (Fig. 6.8). In the control system, oxide film is represented by constant phase element (CPE) impedance. \( Z_{\text{CPE}} \) is considered parallel with passive film resistance (Fig. 6.10b). It can be seen for sterile (control) condition, the AC impedance response did not exhibit diffusion [28]; there was no bacterial attachment in control system and the ennoblement was not also noticed. Protective nature of passive (oxide) film caused higher polarization resistance. An increase in polarization resistance in the control system exhibited enhanced-corrosion resistance. There was no electron transfer between films.

The impedance results showed that the biofilm attached on stainless steel surface, \( C_{\text{dl}} \) values had an increasing tendency. The increase of \( C_{\text{dl}} \) was due to the adsorption of negatively charged anions (bacteria) on the metal surface. There was donor and acceptor’s interactions between the electrons of oxygen compound and the vacant d-orbital of stainless steel surface atoms. According to equation (2), the thickness of passive film (\( L \)) at a given passive film formation potential can be obtained by the measurement of impedance plots [29].

\[
L = \frac{\varepsilon \varepsilon_0 A}{C}
\]

where \( C \) is the capacitance of passive film of 316L SS at 30 °C, \( \varepsilon \) is the dielectric constant of the oxide film, \( \varepsilon_0 \) is the vacuum permittivity of the free space (8.8542 × 10^{-14} \text{ Fcm}^{-1}) and \( A \) is the effective surface area. The calculated thickness of passive films about 6.32 × 10^{-10} m, 8.85 × 10^{-11} m and 15.71 × 10^{-9} m were observed the biofilm 316L SS, alloy C276 and Nickel 200 at 30 °C respectively. The thickness of passive films at control about 9.32 × 10^{-9} m, 29.27 × 10^{-9} m and 28.71 × 10^{-9} m were observed on 316L SS, alloy C276 and Nickel 200 at 30 °C respectively. Reduction in the passive film thickness in presence of biofilm compared to passive film at
control. The electric field is no longer uniformly distributed in the space and concentrates itself by biofilm with redox species in the polarized ionic species so-called shield effect [30]. The oxide film thickness decreased when exposed in natural freshwater. The reduction of thickness was due to the redox behaviour of biofilm, which was seen in the corrosion behaviour of 316L SS.

6.3.11. Electronic properties of passive films

The capacitance of the passive film ($C_T$) is considered to be the combination of two series capacitances: the space charge capacitance ($C_{SC}$) at the film / solution interface and the Helmholtz capacitance ($C_H$) [31, 32].

$$\frac{1}{C_T} = \frac{1}{C_{SC}} + \frac{1}{C_H}$$  \hspace{1cm} (3)

Assuming that the space charge capacitance is much smaller than the Helmholtz capacitance and hence the measured $C_T$ is equal to $C_{SC}$. Therefore, the data points in $C_{SC}^{-2}$ versus E plots can describe the semiconducting behaviour of passive film. The semiconductor properties of passive film could be described by Mott-Schottky theory as follows (equations 4 and 5) [33].

n-type: $$\frac{1}{C^2} = \frac{2}{\varepsilon\varepsilon_0 eN_D} \left( E - E_{fb} - \frac{K_B T}{e} \right)$$  \hspace{1cm} (4)

p-type: $$\frac{1}{C^2} = -\frac{2}{\varepsilon\varepsilon_0 eN_A} \left( E - E_{fb} - \frac{K_B T}{e} \right)$$  \hspace{1cm} (5)

where $C$ is the capacitance of the semiconductive passive layer, $\varepsilon$ the layer permittivity, $\varepsilon_0$ the vacuum permittivity, $e$ the electronic charge ($1.602 \times 10^{-19}$ C), $N_D$ and $N_A$ stands for the donor and acceptor electron density, $E_{fb}$ the flat-band potential (V vs. SCE), $K_B$ the Boltzmann
constant, and $T$ the absolute temperature. The permittivity of the passive layer of stainless steel varies between 12 and 15.6. In this work $\varepsilon$ is fixed at 12 [22].

The plot of $C^{-2}$ versus the applied potential $V$ is called Mott–Schottky plot. The slope of this plot is associated with the semiconducting behaviour of the passive film. Positive slope is typical of n-type semiconductors while negative slope is typical of p-type semiconductors. Fig. 6.11 shows the Mott-schottky plots of 316L SS, alloy C276 and Nickel 200 for natural biofilm and their control. For stainless steel passive films, the capacitance measurements revealed the existence of a straight line with a positive slope for potentials higher than 0.1 V and with a negative slope for potentials lower than 0.1 V. This means that the oxide films behaved as n-type semiconductor in the more noble potential region correlated with the presence of iron oxides and as a p-type semiconductor in the lower potential region attributed to the response of chromium oxide. The duplex character of the passive film formed on the surface of 316L stainless steel in exposed in freshwater was well characterized. Electronic structure of oxide film changed with environments. In alloy C276 biofilm disturbed the outer iron oxide layer end exposed inner chromium oxide layer which showed negative slope in the more noble potential region where as in nickel alloy biofilm attachment was insignificant in the semiconducting properties. Linear fit was obtained from the data in this region to determine the flat-band potential of the oxide. The X-axis intercept of the linear fit to this curve indicated that the flat-band potential was about -1.15 V vs SCE for natural biofilm covered 316L SS and -0.15 V vs SCE for the 316L SS control. In thermodynamic point of view, the negative shift in $E_{fb}$ potential resulted in decrease in free energy change for the charge transfer from oxide film which elevated the concentration of $\text{H}_2\text{O}_2$ in mature biofilm. Such variability of $E_{fb}$ is ascribed to the change of metal oxide / solution interfaces in freshwater. The Nernstian expression for the $E_{fb}$ of a semiconductor is:
\[ E_{fb} = -\frac{E_f^0}{q} + \Delta \Phi_H \]  \hspace{1cm} (6)

\[ \Delta \Phi_H = 0.059 (pzc - pH) \]  \hspace{1cm} (7)

where \( pzc \) is the point of zero charge at which the surface excesses of \( H^+ \) and \( OH^- \) are equal under the condition of no other specifically adsorbed species except for \( H^+ \) and \( OH^- \), \( E_f^0 \) the Fermi level of a semiconductor material at \( pzc \); and \( \Delta \Phi_H \) the potential drop in Helmholtz layer.

The dissolved oxygen content had little influences on the dominate components in the passive films of 316L SS and alloy C276. Thus, the variation of \( E_{fb} \) was attributed to the change of \( \Delta \Phi_H \) in the system. The value of \( \Delta \Phi_H \) is defined as the difference between the potential at the electrode surface which is determined by the surface charge \( (q_s) \) and the potential at the outer Helmholtz plate [32]. Specially adsorbed anions on the passive film were mainly negatively charged bacteria. More dissolved oxygen favored reduction reaction; the specific adsorptions of bacteria were partly utilized by \( O^{2-} \) and \( OH^- \). As sizes of \( O^{2-} \) and \( OH^- \) were small, more negative charges accumulated on the surface, caused an increase of residual negative charges in the surface causing potential drop in Helmholtz layer. This clearly explained why the flat-band potentials became more negative with dissolved oxygen in freshwater. Furthermore, the adsorbed anion \( O^{2-} \) or \( OH^- \) on the passive film was beneficial to the corrosion behaviour as they played a more important role in the reaction of accelerated passive film dissolution. Hence the flat-band potential of passive film decreased (more cathodic side) when higher concentration of dissolved oxygen was in freshwater at 30 °C.

M-S plots revealed that the presence of two regions (II, III). In the anodic region \((V > +0.15 \text{ V vs SCE})\) the capacitance represented the electrochemical behaviour of an n-type semiconductor, on the other hand, in the more cathodic region \((V < +0.15 \text{ V vs SCE})\) the
capacitance described the behaviour of a p-type semiconductor (Fig. 6.11). In the present study, donor concentrations were measured from Mott-Schottky plots for the natural biofilm covered 316L SS and control. The increase in donor concentration with time in the freshwater system was due to the presence of a biofilm [4, 34, 35] because, manganese oxidizing bacteria were deposited biogenic MnO$_x$ and produced some extracellular polymers. Hence, the biologically produced negative charges were neutralized by manganese cations in the oxide film. Generally, n-type passive film carrying metals was induced ennoblement process exposed in freshwater. The presence of biogenic MnO$_x$ in natural biofilm decreased the donor density. The present study confirmed that n-type (region IV) semiconducting property of biofilm on 316L SS. The donor concentration on the oxide film in presence of natural biofilm was higher compared to control system (Table 6.6), n / p-type semiconducting oxide film on control was seen on 316L SS surface. The semiconductor property of the oxide film was altered due to the change of the composition of oxide film [2]. Drastic change in the semiconducting behaviour of oxide film on 316L SS exposed in freshwater environment was seen. Biofilm covered C276 and its semiconductor property changed the n-type to p-type transition (Fig. 6.11b). Presence of tungsten content enhanced the growth of a chromium-rich region at the alloy/oxide interface under oxidizing condition [36]. According to equations (4 and 5), the slope of the linear portion of $C_{SC}^{-2}$ versus E plot, gave the charge carrier density ($N_d$) from the following equation (8).

$$N_d = \frac{2}{q \varepsilon \varepsilon_0 \cdot s}$$

(8)

where $s$ is the slope of the Mott-Schottky plot in the liner-region of interest. As seen from this equation [8] the slope’s of the linear region of the Mott–Schottky plot is inversely proportional to the doping density. Hence, if the slope diminishes with effect of biofilm attachment, the
doping density should increase, i.e., the passive film would become more defective. The donors in the outer iron rich layer have been correlated with oxygen vacancies. The higher $N_d$ caused the possibilities of film breakdown and pitting initiation, as the defects in the oxide film (Fig. 6.12) were active sites where corrosion processes were more likely to occur. It seems that interaction between manganese biofilm, freshwater and inclusion materials determined semiconducting properties of passive layers. The heavy doping reduced the depletion width to such an extent that the electrons can transfer / tunnel through the spiked barrier easily in either direction.

6.3.12. Point Defect Model (PDM) of the passive state

PDM provides the microscopic description of the growth and breakdown of the passive film under steady-state and transient conditions. This model is based on the migration of the point defects under the influence of the electrostatic field in the passive film. A passive film is envisaged to grow into the alloy by the generation of the oxygen ion vacancies at the alloy / passive film interface and by their annihilation at the passive film / solution interface. Fig. 6.13 shows the dynamic and steady state properties of a passive film expressed by five electrochemical reactions (equation 9-13) basing on the PDM [37].

(1) Metal vacancies are annihilated and produced at the alloy / passive film interface:

$$m + V^{3-}_m \rightarrow \overset{\gamma}{\rightarrow} M_m + 3e^-$$  \hspace{1cm} (9)

$$M_m \rightarrow \overset{\gamma}{\rightarrow} M^{3+} + V^{3-}_m$$  \hspace{1cm} (10)

(2) Oxygen ion vacancies are produced and annihilated at the passive film / solution interface:

$$m \rightarrow \overset{\gamma}{\rightarrow} M_m + V^{2+}_o + 2e^-$$  \hspace{1cm} (11)
\[ V_{o}^{2+} + H_2O \xrightarrow{\gamma} O_o + 2H^+ \]  \hspace{1cm} (12)

(3) The film dissolves chemically at the passive film / solution interface:
\[ M_2O_3 + 6H^+ \xrightarrow{\gamma} 3H_2O + 2M^{3+} \]  \hspace{1cm} (13)

Where \( m \) represents metal atom, \( M_m \) is metal cations in film, \( O_o \) is oxygen anions in film, \( V_{m}^{3-} \) metal vacancy, \( V_{o}^{2+} \) oxygen ion vacancy, \( M^{3+} \) metal ion in solution, and \( M_2O_3 \) chemical expression of the passive film. Matured biofilm attached on passive films show n-type semiconductive behaviour, the donor density (oxygen ion vacancy) was higher than that of the acceptor (metal vacancy). Passivity breakdown was described analytically by Point Defect Model claims that aggressive anions absorb into surface oxygen vacancy generation.

### 6.4. Conclusions

Electrochemical behaviour of natural biofilm on 316L SS, alloy C276 and Nickel 200 at 30 °C was studied. The potential shifts towards nobler direction were noticed on 316L SS, alloy C276 and Nickel 200 when exposed to freshwater. Electrochemical behaviour of passive alloys were, especially 316L SS was entirely different from other passive alloys. Cyclic Voltammetric response of biofilm covered 316L SS revealed a redox behaviour of \( \text{MnO}_x \), whereas alloy C276 and Nickel 200 did not. The redox behaviour was due to free flow of electron between biogenic \( \text{MnO}_x \) biofilm and passive film. FTIR spectrum of natural biofilm on passive alloy indicated the presence of \( \text{MnO}_x \) - stretching, bending and wagging vibrations. It may be concluded that the shifting of open circuit potential (ennoblement) of passive alloys observed was due to the interaction between manganese biofilm and bacterially produced peroxide. This process was also influenced by the electronic structure of the passive (oxide) film. The biofilm covered 316L SS was due to the ease of electron flow between the passive film (\( \text{Fe}_2\text{O}_3 \)) and the \( \text{MnO}_x \) biofilm,
which are both n-type (Fig. 6.14a). In contrast, on alloys such as alloy C276 and Nickel 200 with passive films having a low concentration of free electrons, MnOₙ reduction was more difficult, resulting in a reduced amount of ennoblement in the presence of manganese biofilm. When manganese biofilm was present, it supplied electrons to the p-type oxide film for chromium / nickel ion reduction (Fig. 6.14b). There was a good agreement with cyclic voltammograms and Mott-Schottky plots. The natural biofilm encouraged the ennoblement process ($E_{\text{max}}$) on passive alloy where changes the behaviour of surface oxide film were seen.
Fig. 6.1. Epi-fluorescence microscopy images for biofilm a) 316L SS; b) alloy C276 and c) Nickel 200.
Fig. 6.2. SEM images for natural biofilm and control on 316L SS, alloy C276 and Nickel 200 at 30°C.

Fig. 6.2a) Control 316L SS; 6.2b) biofilm covered 316L SS; 6.2c) Control alloy C276; 6.2d) biofilm covered alloy C276; 6.2e) Control Nickel 200; 6.2f) biofilm covered Nickel 200.
Fig. 6.3a

Fig. 6.3b

Fig. 6.3c

Fig. 6.3. FTIR spectrum for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm.

Fig. 6.4. Variation open circuit potential with time for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm.
Fig. 6.5. Anodic polarization curves (E-logi) for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm. Fig. 6.5a) 316L SS; Fig.6.5b) alloy C276; Fig.6.5c) Nickel 200.

Fig. 6.6. Cathodic polarization curves (E-logi) for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm. Fig. 6.6a) 316L SS; Fig. 6.6b) alloy C276; Fig. 6.6c) Nickel 200.
Fig. 6.7a-Fig. 6.7c
Fig. 6.7. Cyclic voltammograms for with and without biofilm. Fig.6.7a) 316L SS; Fig.6.7b) alloy C276; Fig.6.7c) Nickel 200.

Fig. 6.8a-Fig. 6.8c
Fig. 6.8. Nyquist plots for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm. Fig.6.8a) 316L SS; Fig.6.8b) alloy C276; Fig.6.8c) Nickel 200.
Fig. 6.9a  
Fig. 6.9. Bode plots for 316L SS, C276 and Nickel 200 in natural freshwater with and without biofilm. Fig. 6.9a) 316L SS; Fig. 6.9b) alloy C276; Fig. 6.9c) Nickel 200.

Fig. 6.10a)  
Fig. 6.10 a) Equivalent circuits for biofilm covered 316L SS and alloy C276; b) biofilm covered Nickel 200 and control for 316L SS, alloy C276 and Nickel 200.
Fig. 6.11. Mott-Schottky plots obtained for natural biofilm and control. Fig. 6.11a) 316L SS; Fig. 6.11b) alloy C276 and Fig. 6.11c) Nickel 200

Fig. 6.12. Schematic of the structure of a passive film on a metal (gray), comprising a defective barrier layer (orange) and a porous outer layer with biofilm (green)
Fig. 6.13. Schematic representation of physicochemical processes that occur within a passive film according to the point defect model.

Where, m = metal atom, MM = metal cation in cation site, OO = oxygen ion in anion site, VMX’ = cation vacancy, Vo = anion vacancy.

1. \( m + V_M X' \leftrightarrow M_M + Xe' \)
2. \( M_M \leftrightarrow M^{X'}_{\text{aq}} + V_M X' \)
3. \( m \leftrightarrow M_M + (X/2) V_O + Xe' \)
4. \( V_O + H_2O \leftrightarrow O_O + 2H^+ \)

\[ V_M X' \]
\[ V_O \]
Fig. 6.14. (a) n-n-type biofilm covered 316L SS; (b) p-n-type biofilm covered alloy C276 / Nickel 200
### Table 6.1. Chemical composition (%) of 316L SS, alloy C276 & Nickel 200.

<table>
<thead>
<tr>
<th>Elementwt.(%)</th>
<th>C</th>
<th>Mn</th>
<th>P</th>
<th>S</th>
<th>Si</th>
<th>Cr</th>
<th>Ni</th>
<th>Mo</th>
<th>Fe</th>
<th>Co</th>
<th>V</th>
<th>W</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 316L</td>
<td>0.03</td>
<td>1.98</td>
<td>0.034</td>
<td>0.02</td>
<td>0.84</td>
<td>17.85</td>
<td>12.11</td>
<td>2.12</td>
<td>Balance</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alloy C276</td>
<td>0.01</td>
<td>1.0</td>
<td>0.04</td>
<td>0.03</td>
<td>0.08</td>
<td>16.5</td>
<td>Balance</td>
<td>17.0</td>
<td>4.0</td>
<td>2.5</td>
<td>0.35</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>Nickel 200</td>
<td>0.12</td>
<td>0.305</td>
<td>-</td>
<td>0.005</td>
<td>0.11</td>
<td>-</td>
<td>99.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.24</td>
</tr>
</tbody>
</table>

### Table 6.2. Physico-chemical characteristics of freshwater

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>30.2°C</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>7.32</td>
</tr>
<tr>
<td>Total solids</td>
<td>337 mg l⁻¹</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>288 mg l⁻¹</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>49 mg l⁻¹</td>
</tr>
<tr>
<td>Conductivity</td>
<td>525μ s/cm</td>
</tr>
<tr>
<td>Chloride ions</td>
<td>80 mg l⁻¹</td>
</tr>
<tr>
<td>Total hardness</td>
<td>130 mg l⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>Total alkalinity</strong> (bicarbonate)</td>
<td>140 mg l⁻¹</td>
</tr>
<tr>
<td><strong>Sulphate ions</strong></td>
<td>16 mg l⁻¹</td>
</tr>
<tr>
<td><strong>Calcium ions</strong></td>
<td>50 mg l⁻¹</td>
</tr>
<tr>
<td><strong>Magnesium ions</strong></td>
<td>78.67 mg l⁻¹</td>
</tr>
<tr>
<td><strong>Sodium ions</strong></td>
<td>104.63 mg l⁻¹</td>
</tr>
</tbody>
</table>

**Table 6.3.** Total viable bacterial counts of natural biofilm on the SS 316L, alloy C276 and Nickel 200 (60 days old) at 30 °C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>HB (x10⁶)</th>
<th>MOB (x10⁷)</th>
<th>IOB (x10⁵)</th>
<th>APB (x10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 316L</td>
<td>4.5</td>
<td>3.7</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Alloy C276</td>
<td>3.1</td>
<td>7.2</td>
<td>3.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Nickel 200</td>
<td>4.5</td>
<td>2.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
</tbody>
</table>

HB - Heterotrophic Bacteria; MOB - Manganese Oxidizing Bacteria; IOB - Iron Oxidizing Bacteria; APB - Acid producing Bacteria; CFU - Colony Forming Units
**Table 6.4.** Concentration of protein and carbohydrate in natural biofilm at 30 ºC

<table>
<thead>
<tr>
<th>Various passive alloy at 30ºC</th>
<th>Carbohydrate (µg / cm²)</th>
<th>Protein (µg / cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 316L</td>
<td>252</td>
<td>561</td>
</tr>
<tr>
<td>Alloy C276</td>
<td>305</td>
<td>588</td>
</tr>
<tr>
<td>Nickel 200</td>
<td>238</td>
<td>553</td>
</tr>
</tbody>
</table>
Table 6.5 Parameters derived from impedance plots for natural biofilm covered 316L SS, alloy C276 and Nickel 200 at 30 °C.

<table>
<thead>
<tr>
<th>Substrate (SS 316L)</th>
<th>OCP (mV vs. SCE)</th>
<th>$R_s$ (Ω)</th>
<th>$C_{dl}$ (F cm$^{-2}$)</th>
<th>$R_{dl}$ (Ω cm$^{-2}$)</th>
<th>$\frac{Y_{dl}(\Omega^{-1})}{Z_{CPE}}$</th>
<th>$n_1$</th>
<th>$R_p$ (Ω cm$^{-2}$)</th>
<th>$W$(Ω$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-50 mV</td>
<td>313</td>
<td>$1.14 \times 10^{-4}$</td>
<td>967</td>
<td>$3.67 \times 10^{-4}$</td>
<td>0.80</td>
<td>$2.39 \times 10^6$</td>
<td>-</td>
</tr>
<tr>
<td>Biofilm</td>
<td>+380 mV</td>
<td>655</td>
<td>$1.68 \times 10^{-3}$</td>
<td>2348</td>
<td>$1.21 \times 10^{-3}$</td>
<td>0.65</td>
<td>$1.16 \times 10^4$</td>
<td>$2.39 \times 10^{-5}$</td>
</tr>
<tr>
<td>Control (alloy C276)</td>
<td>-60 mV</td>
<td>119</td>
<td>$3.63 \times 10^{-5}$</td>
<td>734</td>
<td>$1.45 \times 10^{-4}$</td>
<td>0.86</td>
<td>$1.93 \times 10^{16}$</td>
<td>-</td>
</tr>
<tr>
<td>Biofilm</td>
<td>+370 mV</td>
<td>277</td>
<td>$1.2 \times 10^{-2}$</td>
<td>1639</td>
<td>$7.14 \times 10^{-4}$</td>
<td>0.75</td>
<td>$2.14 \times 10^5$</td>
<td>$2.39 \times 10^{-4}$</td>
</tr>
<tr>
<td>Control (Nickel 200)</td>
<td>-40 mV</td>
<td>287</td>
<td>$3.7 \times 10^{-5}$</td>
<td>919</td>
<td>$1.21 \times 10^{-4}$</td>
<td>0.90</td>
<td>$2.15 \times 10^{14}$</td>
<td>-</td>
</tr>
<tr>
<td>Biofilm</td>
<td>+372 mV</td>
<td>922</td>
<td>$6.76 \times 10^{-5}$</td>
<td>6579</td>
<td>$4.27 \times 10^{-4}$</td>
<td>0.83</td>
<td>$5.67 \times 10^5$</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 6.6. Values of the donor density $N_d$ and flatband potential (M-S plot) for the n-type behavior.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Corrosion potential (mV vs. SCE)</th>
<th>Flat band potential (V vs. SCE)</th>
<th>Correlation coefficient ($R^2$)</th>
<th>Donor density ($N_d$) (cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS 316L(biofilm)</td>
<td>+380 mV</td>
<td>-1.51 V</td>
<td>0.996</td>
<td>$2.74 \times 10^{21}$</td>
</tr>
<tr>
<td>SS 316L(control)</td>
<td>-50 mV</td>
<td>-0.11 V</td>
<td>0.999</td>
<td>$2.26 \times 10^{20}$</td>
</tr>
<tr>
<td>Alloy C276(biofilm)</td>
<td>+370 mV</td>
<td>-1.12 V</td>
<td>0.998</td>
<td>$2.21 \times 10^{21}$</td>
</tr>
<tr>
<td>Alloy C276(control)</td>
<td>-60 mV</td>
<td>-0.85 V</td>
<td>0.992</td>
<td>$6.91 \times 10^{20}$</td>
</tr>
<tr>
<td>Nickel 200(biofilm)</td>
<td>+372 mV</td>
<td>-0.67 V</td>
<td>0.999</td>
<td>$7.41 \times 10^{22}$</td>
</tr>
<tr>
<td>Nickel 200(control)</td>
<td>-40 mV</td>
<td>-0.52 V</td>
<td>0.997</td>
<td>$1.82 \times 10^{20}$</td>
</tr>
</tbody>
</table>
References


society, 150 (2003) B120.