CHAPTER V

Electrochemical characterization of biofilm on FTO glass substrate

5.1. Introduction

Marine and freshwater biofilm usually shift the open circuit potential (OCP) of stainless steels towards the electropositive direction (ennoblement) by +400 mV vs SCE. The ennoblement process on stainless steel was thought to be due to the presence of manganese oxide in the biofilm. The nature of oxide film and bacterial metabolism were also correlated with ennoblement process [1, 2]. The positive shift was explained with hydrogen peroxide, biologically produced siderophore and biogenic MnO₂ [1, 2-9]. The response of passive alloys with n and p -type passive films to manganese in biofilm, whereas the cathodic polarization curves were compared between n (6XN SS) and p (C4) -type semiconducting alloys [1]. The reason for the difference in cathodic polarization curves were explained with the structure of n / p -type semiconducting oxide film. The amount of ennoblement of the OCP of two alloys caused by MnO₂ was systematically higher on alloy N08367 with n-type passive film than on alloy N06455 with p-type semiconducting oxide film. It was explained why on the p-type passive film ennoblement was less and more slowly than those with n-type films. The range of positive shifts for various passive alloys may depend upon the donor concentration and anion available in the environment [2]. The concentration of manganese depositing heterotrophic bacteria in biofilm [6] and was seen that the nature of cathodic curves were determined by the nature of oxide film and the biofilm on 6XN SS and Titanium grade-2. It was assumed that the oxide film also plays an
important role on ennoblement process. Dickinson et al [5], Linhardt [7] and Olesen et al [8] demonstrated that the deposition of manganese oxides on stainless steels changed the electrochemical behaviour of stainless steels where the manganese oxidizing bacteria was active in the process of manganese dioxide biomineralization. Palanichamy et al [9] noticed manganese oxidizers on various metal surfaces viz stainless steel, brass and copper where ennoblement was noticed only on SS.

Transparent conducting oxide (TCO) semiconductors are widely used as transparent films in electronic devices such as thin film solar cells, electro-chromic displays, gas sensors and other areas [10]. Fluorine doped tin oxide (FTO) is a possible alternative to ITO because FTO is inexpensive as well as chemically and thermally stable [11]. FTO was to behave as n-type semiconductor with wide band gap within 3.0 to 3.6 eV [12, 13]. It has excellent electrical conductivity [14, 15], greater carrier mobility [16], and good mechanical stability [17]. The fluorine doping to the SnO$_2$ framework, can promote more numbers of charge carriers and therefore enhanced the electrical conductivity [18]. Addition of fluorine atom as oxygen substitute or at the interstitial into the SnO$_2$ structure causes a decrease in resistivity and gives rise to n-type conductivity.

The n-type semiconducting oxide film encourages the ennoblement process [1]. Hence we selected n-type semiconducting properties of FTO for ennoblement studies. In the present study, the electrochemical behaviour of biofilm observed on the FTO has been investigated.
5.2. Materials and methods

5.2.1. Biofilm formation on FTO

Fluorine-doped tin oxide (FTO) glass substrate was selected for all electrochemical experimental studies. FTO in aqueous solutions is considered to be an inert electrode like ITO. FTO ($R = 16\,\Omega/\text{sq.}$ and 2mm in thickness) was supplied by Pilkington, TEC Glass Products, Toledo, OH. All the substrates were cleaned by ultrasonic rinsing in pure ethanol, and subsequently dried under N$_2$ flow. When preparing an FTO electrode, a piece of copper tape, serving as the bus bar, was applied to the FTO coated surface of a glass substrate, and then an insulating tape was applied to the same surface to define an electrode area of 2.0 cm x 2.0 cm. FTOs were exposed in the freshwater, collected from Ammon pond water, Karaikudi and formed biofilm. The freshwater was sterilized with autoclave at 121°C and used as control. The freshwater was changed once in 24 hrs interval for test duration of 60-days.

5.2.2. Experimental procedures

Freshwater samples were subjected to analysis for Physico-chemical characteristics as per the methods [19, 20]. The morphology of the attached bacteria on FTO was studied by Epi-fluorescent microscope (Nikon E200, Tokyo, Japan). Microscope images were captured by digital camera (Nikon COOLPIX5400). In order to find out the nature of organic / Inorganic functional groups, 60-days old biofilm was scraped from polyvinyl chloride (PVC) sheet and used for FTIR analysis. The samples were mixed with spectroscopically pure KBr in the ratio of 1:100, pellets were fixed in the sample holder, and the analysis was carried out. FTO specimens were used for the isolation of bacteria. Enumerations of manganese oxidizing bacteria (MOB) from
freshwater biofilm were analyzed using standard pour plate technique [21]. Open circuit potential (OCP) measurements for FTO were made in the laboratory condition using the collected freshwater. OCP values were measured with time using a digital Multimeter (Rish, India) with saturated calomel electrode (SCE). Cathodic polarization experiments were conducted in freshwater under air-saturated condition using a computer controlled Potentiostat (Gamry DC105 using Echem Analyst version 5.3 software) in a one-liter electrochemical cell. Cathodic and anodic polarization were initiated at open circuit potential and polarized to -1.0 V and +1.2 V vs SCE at a scan rate of 0.166 mV / s respectively. The cathodic and anodic polarizations were carried out on FTO with and without biofilm and each experiment was done at least three times. All the cyclic voltammetry experiments were referenced to SCE. Potential cycling was carried out in the range of -0.5 V to 1.0 V vs SCE at a scan rate of 5 mV/s. In the AC impedance test, the FTO was kept at the OCP and a sinusoidal potential with 5 mV amplitude for the frequency region of 0.01 Hz to 30 KHz was applied using a frequency response analyzer.

5.3. Results and discussion

5.3.1 Physico-chemical analysis

Table 5.1 shows the Physico-chemical properties of freshwater. It was observed that the pH of the water sample was 7.6 and a dissolved oxygen concentration of 7.32 mg l⁻¹. The total dissolved solids content was 288 mg l⁻¹, which can be considered as quite normal. Chloride content was 112 mg l⁻¹, while sulphate content was 16 mg l⁻¹. The freshwater consists 0.74 mg l⁻¹ of manganese. The freshwater sample can be expected to be low salt content and high bacterial activity. The AAS results confirmed
that the manganese content in the biofilm increased with increasing exposure time. Hence, the study indicates that the FTO surfaces when exposed to freshwater were colonized by a large number of heterotrophic bacteria, which have the ability of bringing about biomineralisation of manganese.

5.3.2. Total viable count

The total viable counts of the culturable freshwater bacteria on the FTO surface increased with exposure time from $1.8 \times 10^3$ CFU cm$^{-2}$ after 1 month to about $5 \times 10^5$ CFU cm$^{-2}$ after 2-months exposure. The total viable count showed an increasing trend with exposure time and reached a value of $5 \times 10^5$ CFU cm$^{-2}$, close to the total viable count observed during the early report. The results showed that FTO was susceptible to biofouling. All these manganese oxidizing bacteria were heterotrophic.

5.3.3. Epi-Fluorescence Microscopy

The visual observation of the existence of brown colonies indicates that the bacteria possess the ability to oxidize Mn. Epi-Fluorescence image is presented as Fig. 5.1. The Epi-fluorescence microscopical review of the FTO surface was with biofilm samples. It indicates the bacterial count was high at 30°C and also represent that the biofilm was heterogeneous surface.

5.3.4. Fourier Transform Infrared spectroscopy (FTIR)

Fig. 5.2 shows the FTIR response of biofilm sample. The peaks present in the range between 429 –795 cm$^{-1}$ indicate the MnO$_x$ - stretching, bending and wagging vibrations [22]. Parikh and Chorover [22] carried out the FTIR spectroscopic study of biogenic Mn-oxide formation by *Pseudomonas putida* GB-1 and identified a peak at
433 cm⁻¹ for the MnOₓ present in the biofilm. The peak at 582.5 cm⁻¹ suggested that the main crystal type of MnO₂ [23] which was observed in biofilm. The peak around 1383 cm⁻¹ indicates CH₂ vibrations of polysaccharides. A prominent peak at 1035 cm⁻¹ indicates the presence of polysaccharides in biofilm. Record peak at 1640.3 cm⁻¹ was recorded indicates the polysaccharide, which corresponds 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 suggest the presence of OH groups in the mineral structure of manganese oxide. FTIR analysis reveals that the MnOₓ, OH stretching and EPS fraction dominate the bulk of biofilm biomass. Hence, it may be concluded that the electrochemical behaviour of natural biofilm is due to the combination of MnOₓ and organic molecules of biofilm.

5.3.5. Potential measurements

Fig. 5.3 shows potential measurements with time for four samples with and without biofilm covered FTO. The initial potential of FTO was about -100 mV. After the immersion of electrodes in pond water, the potential for FTO gradually shifted in the positive direction to about +400 mV within 360 hrs. The potential for FTO without biofilm did not shift to positive direction. This suggest that biofilm encourages the positive shifts like other metals viz., 316 SS, 316L SS, C276, C4, 6XN SS obtained from various places viz., Delaware water[1], Tuticorin water[4], Montana water[5] and Baltic seawater[24] etc. Angappan et al [2] suggested that the range of positive shifts for various alloys may depend upon the donor concentration (excess cations) and anions available in the environment or biofilm. However in the present study, ennoblement was noticed from biofilm covered FTO electrode with n- type oxide film.
5.3.6. Cathodic polarization

Fig. 5.4a and Fig. 5.4b show the cathodic polarization curves for FTO with and without biofilm. There are no peaks noticed in the control FTO electrode whereas biofilm covered FTO curve exhibited a reduction peak. The peak at 0 mV vs SCE was noticed. It was observed that the electrochemical behaviour of the biofilm on FTO was similar to biofilm covered 6XN SS [1]. In the earlier studies on the cathodic polarization of biofilm covered stainless steel, two peaks were identified by Dexter and Maruthamuthu [1], ferric reduction peak at +220 mV and manganese dioxide reduction peak at 0 mV. Dickinson and Lewandowski [5] also identified the peak at 0 mV vs SCE as manganese dioxide reduction. As FTO does not contain any ferric, the appearance of ferric reduction peak is debatable. The cathodic polarization of biofilm covered FTO showed only MnO₂ reduction peak. In the presence of biofilm, due to ennoblement process distinct reduction peak at 0 mV vs SCE can be noticed. In the present study, the peak was identified from FTO in the presence of biofilm. Hence, to confirm the reduction peak CV experiments by employing biofilm covered FTO were done.

5.3.7. Anodic polarization

Fig. 5.5 shows the anodic polarization for natural biofilm covered FTO and control. In the control system, the breakdown potential for FTO was 1.0 V vs SCE. The breakdown potential for natural biofilm covered FTO was 0.8 V vs SCE. \( I_p \) was higher in natural biofilm covered FTO compared to control. It indicated that the oxide film of the electrode may be due to the inclusion of protein during ennoblement
process in freshwater medium. Cachet et al [25] observed that protein immobilization at the SnO\textsubscript{2} surface.

5.3.8. Cyclic voltammetry studies

Fig. 5.6 and Fig. 5.7 show the cyclic voltammogram for FTO electrode with and without biofilm. There was no oxidation and reduction peaks noticed in control FTO electrode. Electrochemically active biofilm was observed on FTO where exposed in freshwater. The oxidation peak at +330mV vs SCE and another reduction peak in the range from +100mV to 0 mV vs SCE were noticed in the presence of 15-days, 30-days and 60-days old biofilm of FTO electrode. Long et al [26] investigated the spectroelectrochemical characterization of nanostructured, mesoporous MnO\textsubscript{x} in aqueous electrolytes. They noticed an oxidation and reduction peak at +240 mV and -15 mV vs Ag / AgCl respectively which are similar to the present study. In the present study, it is also noticed that the cyclic voltammetric peak currents increased in both oxidation and reduction directions with time, indicating the growth of MnO\textsubscript{x} redox-active biofilm on the surface of the FTO. Torresi and Gorenstein [27] studied the electrochromic behaviour of manganese dioxide on SnO\textsubscript{2} electrodes. They have carried out the CV by employing MnO\textsubscript{2} film on glass / SnO\textsubscript{2} conducting substrates in 0.1M Na\textsubscript{2}B\textsubscript{2}O\textsubscript{7} solutions where the pH was 9.2. An oxidation peak at 0.33 V vs SCE and a reduction peak at -0.1 V were noticed by them. They concluded that the formation of a lattice structure was different from that of the MnO\textsubscript{2} + MnOOH single phase. The natural 60-days old biofilm has both acidophilic and alkalophilic bacteria where the count was 5.2 x 10\textsuperscript{4} and 6.8 x 10\textsuperscript{5} CFU / cm\textsuperscript{2} on the surface of FTO. Hence,
it may be concluded that the electrochemical behaviour reflects the wide pH tolerating manganese-accumulating bacteria on FTO as follows (equation 1)

\[
\text{MnO}_2 + \text{H}_2\text{O} + e^- \rightleftharpoons \text{MnOOH} + \text{OH}^- \quad (1)
\]

It should be considered that the biofilm has a broad range of pH between 2 and 9.2 [28, 29]. The observed oxidation and reduction of CV in the present study is due to the mixed effect of MnO\(_2\) / MnOOH. A broad peak at +675 mV may be assumed as peroxide peak produced by rich 60 days of old biofilm. This observation supports with the observation made by Yao et al [30] who noticed peroxide peak at +700 mV vs SCE by using GCE in phosphate buffer at pH 7.4. In the present study, during N\(_2\) purged system in the presence of biofilm, peroxide peak could not be noticed. The peroxide peak in biofilm covered FTO may be due to the high oxygen evolution due to the production of high quantity of H\(_2\)O\(_2\) in biofilm [31, 32]. Cyclic voltammogram reveals that the oxidation and reduction potential of biofilm are similar to the peaks noticed at anodic and cathodic polarization scans respectively. The potential shift is due to the presence of microorganism on the surface which induces localized changes in the chemistry of the electrolyte (e.g. pH and dissolved oxygen concentration). Cyclic voltammetric measurements carried out in aqueous solution are likely to be pH dependent since the addition or removal of an electron from an organic molecule in particular may induce the uptake or loss of a proton [33]. Redox behaviour of biofilm covered FTO is due to the faradaic nature of the pseudo capacitance behaviour of MnO\(_x\) [26, 27] where as in the same potential (+200 mV) a distinct peak may be noticed in CV with natural biofilm. It may be assumed that MnO\(_x\) formed in the biofilm is due to the interaction between biogenic manganese and H\(_2\)O\(_2\). It is
concluded that uncovered biofilm of FTO does not carry the electrons, however biofilm covered FTO flow the electrons between biofilm and oxide film.

5.3.9. Electrochemical Impedance studies

Fig. 5.8, Fig. 5.9 and Fig. 5.10 show the Nyquist plot, Bode plot and proposed equivalent circuit respectively for biofilm and control FTO, together with their numerical fittings. The equivalent circuit consisted of parameters characterizing the electrolyte resistance ($R_s$), high frequency resistance ($R_{ct}$) and capacitance ($C_{dl}$), Warburg diffusion impedance ($W$). The results are tabulated as Table 5.2. According to AC circuit theory an impedance plot obtained for a given electrochemical system can be correlated with one or more equivalent circuits. Fig. 5.8 shows the Bode plot for the control and biofilm covered FTO. This indicates in “phase” angle plot that the time constant around 0.095 Hz for biofilm attachment on FTO electrode. Data for FTO in sterile control condition and exposed in freshwater (biofilm) were analyzed using the equivalent circuit illustrated in Fig. 5.9. Fig. 5.9a, is represented by the resistance $R_{ct}$ parallel with $C_{dl}$ component account for the FTO film with a value were 844 kΩ cm$^2$ and 46.4 µF / cm$^2$ respectively. Fig. 5.8a shows the low frequency spectrum in control and represented by constant phase element (CPE) parallel with $R_p$. It may be seen for the control, the AC impedance response shows no diffusional effect [34], which can vary between 1 (perfect capacitor) and 0 (perfect resistor). A value of $n$, 0.68, which is less than one would represent a somewhat imperfect capacitor and is generally thought to arise from the presence of surface heterogeneities.

Data for the FTO in the presence of manganese biofilm were analyzed using the equivalent circuit shown in Fig. 5.9b where the high frequency spectrum was
described by a resistance in parallel with CPE network (Fig. 5.8b) representing FTO film. The semicircle appearing at the high frequencies should be related to a electrochemical reaction, the semicircle was explained by Ding [35, 36] that the reaction involving MnO₂ i.e., MnO₂ + H⁺ + e⁻ ⇌ MnOOH, resulted in a semicircle in the high frequencies. The biofilm had the deposition of biogenic MnOₓ on the FTO, which is a conducting material [37] and it decreased the Rₓ (2.2 kΩcm²) and CPE values, Y₀ was 18.5 x 10⁻¹² and n was 0.98. Most of the pure oxide crystals, undoped MnO₂ was normally an electrical insulator. But its electrical conductivity as practically observed is due to the presence of many crystal defects such as a stoichiometric excess or deficiency of vacancies introduced on the surface. MnO₂ was a transparent semiconductor with an n-type carrier in conduction [38].

The low frequency spectrum in biofilm covered FTO is represented by CPE parallel with Rₚ and Warburg impedance. The Warburg component in the equivalent circuit explains the diffusion of divalent Mn²⁺ and its cycling within the biofilm by MOB [39] and the production of H₂O₂ which clearly indicates the biofilm covered FTO is a combination of kinetic and diffusion process. In the low frequencies, it is thought to be related to the diffusion process, since the redox process giving rise to the pseudo-capacitance behaviour is a surface process, H⁺ diffused into the pores formed by MnO₂ to achieve the electrochemical reaction [35, 36]. It can be explained that the redox couple of MnOₓ i.e., MnO₂ + H⁺ + e⁻ ⇌ MnOOH, with H₂O₂ determine the electron flow between manganese ions and reduction of oxygen in the biofilm. The Y₀ associated with CPE was found to be 2.15 x 10⁻⁴ (Ω⁻¹) and n is 0.39. This result suggests that the bacteria is related to the Helmholtz double layer capacitance.
associated to the biofilm / solution interface, nevertheless, the capacitance is higher than the value for the sterile control, CPE value of \(2.83 \times 10^{-8} \) (\(\Omega^{-1}\)) and \(n\) is 0.68. It is due to the accumulation of rich anions and cations by bacteria in the biofilm. Some investigators [2, 5] reported that the biofilm has lower capacitance on 316 SS when compared to control. It may be explained that it is due to the interaction of biogenic MnO\(_x\) (n-type) and n-type semiconducting oxide film. In the present study, since FTO has n-type oxide film, the capacitance value is lower when attachment of biofilm. On the other hand, the distribution of bacteria on the surface is not uniformly distributed. Diffusivity at the interface with biomolecules and bacteria indicate that dissolved metal ions are accumulated at the interface leading to an accumulation of manganese biofilm. This suggests that the surface has become heterogeneity due to the bacterial activity.

5.4. Conclusions

The electrochemical behaviour of biofilm was investigated by employing FTO. The cathodic polarization curve of biofilm was similar to the cathodic polarization curve of biofilm covered stainless steel alloys which noticed earlier. The electrochemical behaviour of biofilm is determined by accumulated biogenic MnO\(_x\) where MnO\(_2\) reduction only is the contributor for a reduction peak at 0 mV vs SCE. Cyclic voltammogram explained the redox behaviour of biogenic MnO\(_x\) and H\(_2\)O\(_2\) on ennoblement process. It may be claimed that biofouling is due to biomineralization of MnO\(_x\) at the surface of the FTO. MOB is able to cause the increase of the free corrosion potential. It indicates that biofilm contributed to ennoblement process with n-type semiconducting oxide film. Impedance revealed that the biofilm increased the
kinetic and diffusion process on FTO. FTIR studies suggested that the biofilm contained organic molecules as EPS and inorganic metal oxides such as MnO\textsubscript{x}. In the present study, it was observed that an electron flow was between n (FTO) -type and n (MnO\textsubscript{x} biofilm) -type semiconducting behaviour.
Fig. 5.1. Epi-Fluorescence Microscopy image for biofilm covered FTO

Fig. 5.2. FTIR spectrum for biofilm
Fig. 5.3. OCP measurements for biofilm covered FTO and control

Fig. 5.4. Cathodic polarization curves for biofilm covered FTO and control
Fig. 5.5. Anodic polarization curves for biofilm covered FTO and control

Fig. 5.6. Cyclic voltammogram curves for FTO (a) Control; (b) 15-days biofilm
Fig. 5.7. Cyclic voltammogram curves for FTO (a) Control; (b) 30-days biofilm (c) 60-days biofilm (d) N₂ purged 60-days biofilm.

Fig. 5.8. Nyquist plot for biofilm covered FTO and control
Fig. 5.9. Bode plot for biofilm covered FTO and control

![Bode plot for biofilm covered FTO and control](image)

Fig. 5.10a. Equivalent circuit for control (Sterile) FTO

![Equivalent circuit for control (Sterile) FTO](image)

Fig. 5.10b. Equivalent circuit for biofilm covered FTO

![Equivalent circuit for biofilm covered FTO](image)
Table 5.1
Physico-chemical parameters of freshwater

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
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<tr>
<td>Temperature</td>
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<td>Turbidity</td>
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<tr>
<td>pH</td>
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<tr>
<td>Total solids</td>
<td>337 mg l⁻¹</td>
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<tr>
<td>Total dissolved solids</td>
<td>288 mg l⁻¹</td>
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<td>Total suspended solids</td>
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<tr>
<td>Dissolved oxygen content</td>
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<tr>
<td>Conductivity</td>
<td>525 µS m⁻³</td>
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<tr>
<td>Chloride</td>
<td>112 mg l⁻¹</td>
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<tr>
<td>Total hardness</td>
<td>130 mg l⁻¹</td>
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<td>Sulphate</td>
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<tr>
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<tr>
<td>Zinc</td>
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Table 5.2
Impedance parameters of equivalent circuits representing, biofilm modified FTO and control at 30°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>OCP (Vs SCE)</th>
<th>R_s (Ω)</th>
<th>C dl(Fcm⁻²)</th>
<th>Y_o (Ω⁻¹) n</th>
<th>R_a(Ωcm⁻²)</th>
<th>R_p(Ωcm⁻²)</th>
<th>Y_a(Ω⁻¹) n</th>
<th>B</th>
<th>Z_CPE</th>
<th>Z_W</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTO (control)</td>
<td>+50mV</td>
<td>112</td>
<td>46.4 x 10⁻⁶</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.84 x 10⁸</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FTO (biofilm)</td>
<td>+400mV</td>
<td>101</td>
<td>-</td>
<td>1.81 x 10⁻¹¹</td>
<td>0.98</td>
<td>2.21 x 10³</td>
<td>2.17 x 10³</td>
<td>2.15 x 10⁻⁴</td>
<td>0.39</td>
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References


