Chapter 7

Summary

Fang HHP, Xu LC, Chan KY (2002). Effects of toxic metals and chemicals on biofilm and biocorrosion. Water Research 36: 4709-4716


Van der Aa BC, Dufrene YF (2002). In situ characterization of bacterial extracellular polymeric substances by AFM. Colloid and surfaces B: Biointerfaces 23: 173-182


355
Summary

➢ In this thesis, studies on the development of biofilm on the stainless steel panels immersed in the surface waters of the Dona Paula Bay, west coast of India, was carried out over a period of 15 days during monsoon (September), post-monsoon (January) and pre-monsoon (April/May). Various physical, chemical and biological parameters of the biofilm and suspended particulate material (SPM) of the surface waters were analysed. Moreover, a novel approach based on molecular biomarkers such as monosaccharide, amino acid, fatty acid and hydrocarbon was used to assess development of the biofilm on stainless steel panels.

➢ Biofilm biomass (measured as DW, OC, ON, Chl-a, protein and the abundance of bacteria (TBC) and diatoms) generally increased over the period of immersion. On the other hand, the physical, chemical and biological parameters of the surface seawater showed small variation during all the sampling periods. Therefore the observed increase in the biofilm biomass is due to increase in growth of attached microorganisms. This is further supported by the decreasing trends observed for the OC/ON and OC/Chl-a ratios over the period of immersion. Biofilm biomass parameters showed significant temporal differences, however, the seasonal and annual variations were not significant. Significant relationships were observed between various biomass parameters indicating their common origin.

The OC/ON and OC/Chl-a ratios were initially high for 1 and 3 day period
of immersion indicating the presence of degraded and/or terrestrial material. These ratios decreased over the period of immersion indicating the presence of fresh biogenic material. In contrast, the OC/ON and OC/Chl-a ratios in the SPM was high throughout the period of sampling indicating the presence of degraded and/or terrestrial material in the SPM samples.

Capillary gas chromatography analysis of both the biofilm material and the SPM of the surface seawater samples revealed the presence of eight individual sugars, namely rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose. The absolute concentration of these monosaccharides in the biofilm material generally increased, suggesting their production by the fouling microorganisms over the period of immersion. However, the weight percent contribution of individual monosaccharides to the total carbohydrates (sum of all the identified monosaccharides) varied over the period of immersion. This observed temporal variability in carbohydrate composition may be due to changes in the composition of the biofilm organisms. At day 1 and 3, the monosaccharides glucose, arabinose and xylose were relatively more abundant in the biofilm samples indicating the presence of degraded biogenic material and/or the presence of some vascular plant debris in the biofilm material. In SPM, glucose was also the abundant monosaccharide, while, the wt % contribution of arabinose and xylose was relatively low suggesting less contribution of terrestrial material to the SPM.
The weight % abundance of rhamnose, fucose, ribose and galactose increased while that of arabinose, xylose and glucose decreased between days 5 to 15 of the immersion period. The decreasing trend observed for arabinose and xylose for the subsequent period of immersion indicates the decrease in the contribution of degraded material and/or terrestrial material to the biofilm organic matter. The abundance of rhamnose, fucose, ribose and galactose indicates the presence of biogenic material derived from bacteria and diatoms at during 5 to 15 period of immersion.

➢ In comparison to SPM, the THAA concentration and THAA-C contribution to OC of the biofilm increased several folds over the period of immersion. At days 1 and 3 of the immersion the THAA-C contribution to OC was \(-3.3 \pm 1.1\) and \(-5.7 \pm 3.7\) %, respectively. These values resemble to that of degraded material or terrestrial plant material. As the period of immersion increased THAA-C contribution to the OC also increased and reached a highest value of \(-22.3 \pm 3\) % at day 15 following immersion. This implies increasing contribution of labile biogenic matter to the biofilm over the period of immersion.

Fourteen protein amino acids and four non-protein amino acids were quantified by HPLC upon acid hydrolysis. The amino acid glycine, aspartic acid, alanine, lysine, glutamic acid and serine were abundant in the biofilm samples. Glycine and the hydroxyl amino acids serine and threonine are enriched in biofilm at day 1 of immersion period suggesting the presence of degraded organic matter in biofilm. As the period of
samples ranged from C_{11} - C_{30} (Table 3). The distribution followed a bimodal pattern with the first mode ranged from C_{12}-C_{22} and accounted for ~77 \% and ~80 \% and the second mode ranged from C_{23}-C_{30} and accounted for ~15 \% and 10 \% of the total n-alkanes in the biofilm and SPM, respectively. The CPI values were generally < 1, with some exception, indicating even carbon predominance in both the ranges. The possible explanation for this even carbon predominance at day 1 following immersion could be the presence of the degraded material derived from diatom and bacteria. Subsequently, the even carbon predominance in short chain length n-alkanes (C_{12}-C_{22}) over the period of immersion clearly suggests the increasing abundance of bacteria and diatom.

➢ Bacterial cultures isolated from the biofilm material were screened for EPS production. One of the cultures identified as Bacillus sp. (SS-15) produced highest amount of EPS. The various aspects of microbial EPS were studied using Bacillus sp. (SS-15). The kinetic of growth and exopolysaccharide production by Bacillus sp. (SS-15) showed that EPS was produced during both exponential and stationary phases of growth. The amount of EPS produced by Bacillus sp. (SS-15) was influenced by the nutrient concentrations in the medium. The pH of the growth medium also influenced the production of EPS by Bacillus sp. (SS-15). EPS production by Bacillus sp. (SS-15) was also observed at high temperature (50 °C) and high pH (10). EPS was eluted as a single peak after passing through the sephadex G-200 and DEAE-sepharose.
indicating that EPS contain only one type of polymer. The average molecular weight of the EPS produced by Bacillus sp. (SS-15) was \(1.63 \times 10^7\) Da. Chemical characterization of the polymer showed that carbohydrate was the major fraction. EPS is a heteropolysaccharide containing glucose, mannose and galactose as major monosaccharides. The presence of uronic acid, pyruvate and sulphate indicates the acidic nature of polysaccharides.

Bacterial cultures were screened for EPS production and their potential to inhibit the corrosion of mild steel. Bacteria produced EPS at various level and also influence the corrosion at different level. The concentration of EPS appears to have a direct role in corrosion inhibition. This was evident from a significant inverse relationship observed between the concentrations of EPS and the rate of corrosion. Four of the cultures showed good corrosion inhibition of mild steel and also produced high EPS, however, the extent of corrosion inhibition varied between these cultures. They were tentatively identified as Pseudomonas sp. (CE-2), Pseudomonas sp. (CE-7), Bacillus sp. (CE-10) and Bacillus sp. (SS-15). Electrochemical potentiodynamic polarization studies also confirm that presence of these bacteria inhibited the corrosion of mild steel. the Icorr and Vcorr were low as compared to the control indicating the inhibition of corrosion of mild steel. The resistance of polarization increased over the period of immersion, which means that there is formation of protective film on the surface of the mild steel panel. EPS isolated from Pseudomonas sp. (CE-2), Pseudomonas sp (CE-7), and Bacillus sp.
(SS-15), inhibited the corrosion of mild steel and the inhibition increased with increasing concentration of EPS. While, in the presence of EPS isolated from *Bacillus* sp. (CE-10) the corrosion inhibition rate decreased with increasing concentration of EPS. Chemical characterization of these bacterial polymers indicates that the chemistry of the EPS influences the kinetic of corrosion reaction. In addition, molecular weight fraction of the EPS isolated from the *Pseudomonas* sp. (CE-2) influenced the corrosion of mild steel. The high molecular weight EPS showed good corrosion inhibition as compared to low molecular weight.

➢ Spectrofluorometeric technique has been developed and used to assess the bacterial adhesion to stainless steel panels. The method is based on the fluorescent dye 4',6-diamidino-2-phenylindole (DAPI), which complexes with DNA molecules and when measured by the fluorescence spectrophotometer, the fluorescence intensity increased with the increase in the cell numbers. This implies that the method can be utilized for the estimation of bacterial numbers. Three bacterial cultures *Bacillus* sp. (SS-15), *Pseudomonas* sp. (CE-2) and *Pseudomonas stutzeri* were used to assesses their adhesion to stainless panels. The attachment of these bacterial cultures to the stainless steel surface was influenced by cell density and the incubation period. Conditioning the stainless steel surface with the EPS affected the attachment of the bacterial cultures and the attachment of bacterial cells to stainless steel increased with increasing EPS concentrations. Conditioning the stainless surface with different molecular weight fractions of the EPS of *Pseudomonas* sp. (CE-
2) resulted in variable degree of bacterial adhesion. In the case of *Pseudomonas* sp. (CE-2) the adhesion is increased with increasing molecular weight. While, the adhesion of *Bacillus* sp. (SS-15) and *Pseudomonas stutzeri* to stainless decreased with increasing molecular weight of EPS.

Treatment of the bacterial cells with enzymes proteinase K, lipase and α-amylase altered the attachment of the bacterium to stainless steel. All three enzymes decreased the adhesion of *Pseudomonas* sp. (CE-2) cells to stainless steel and the effect was more pronounced with lipase treated cells. In the case of *Bacillus* sp. (SS-15), only protease treated cells showed decrease in adhesion. On the other hand, *Pseudomonas stutzeri* adhesion was affected by lipase and α-amylase. This suggested that each bacterial culture adopts different biopolymers component in adhesion to the surfaces. Moreover, the adhesion of bacterial cultures decreased when sodium periodate treated cells were used indicating the role of carbohydrates in adhesion. The lectin Concanavalin A did not influence the adhesion of all the three bacteria cultures, however, lectin from *Triticum Vulgaris* significantly altered the attachment of all the three bacterial cultures suggesting that N-acety glucosamine moiety may be involved in adhesion process.

➢ The EPS concentration in the biofilm increased over the period of immersion. The EPS-C contribution to the total carbohydrate carbon (TCHO\textsubscript{SP}-C) was high at day 1 followed by a decrease at day 3 following immersion. The high contribution of EPS-C to TCHO\textsubscript{SP}-C at day 1
following immersion indicates induced production of EPS by biofilm organisms during their initial attachment. The EPS/Chl-a ratio and the EPS per diatom cell in the biofilm were also high at day 1 following immersion and decreased over the period of immersion. Laboratory grown *Pseudomonas* sp. (CE-2) culture showed increased EPS<sub>c</sub> production in the presence of stainless steel, fiberglass and acrylic surfaces. Whereas, in the presence of copper the EPS<sub>c</sub> production decreased as compared to control. On the other hand, the EPS<sub>p</sub> production was enhanced in the presence of copper. EPS produced by *Pseudomonas* sp. (CE-2) revealed the presence of three monosaccharides galactose, glucose and mannose and the relative percentages of these monosaccharides varied with the surfaces over the period of incubation. Certain amino acids concentrations were also induced in the presence of different surfaces.

Based on the above research work the Following conclusions could be drawned

1) Molecular biomarkers useful to understand biofilm development.
2) Fouling bacteriae produce acidic heteropolysaccharides.
3) Production of EPS induced during initial microbial attachment.
4) Surfaces influenced bacterial EPS production and composition.
5) The EPS can inhibit the corrosion of mild steel.
6) The different polymeric components in exopolymers are involved in bacterial adhesion.